CONTROL AND MANIPULATION OF THE IMMUNE RESPONSE

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Control and Manipulation of the Immune Response

Keynote Address

C2-001 LESSONS FROM PARASITES ON THE INITIATION AND REGULATION OF CELLULAR IMMUNE FUNCTION, Alan Sher, Immunobiology Section, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892.

During the past decade, studies on parasite infection models have provided immunologists with important paradigms concerning the selection and regulation of cellular immune reponses. The power of these systems and the insights they have yielded stem in large part from several unusual features of the host-parasite interaction: (1) the highly evolved relationship between parasites and host resistance mechanisms typically resulting in prolonged survival of both the pathogen and infected individual, (2) the prominent immunoregulatory consequences of chronic parastic infection, and (3) the often polarized nature of the immune responses induced by different parasites or by the same parasite in hosts with different genetic backgrounds. Of particular significance to cellular immunogy has been the information gained from studies with murine parasite infection models on the regulation of CD4+ subset selection and function. Here the triggering by many parasites of highly polarized Th1 or Th2 cytokine responses associated with either host resistance or disease progression has enabled systematic analyses in vivo of the factors responsible for the selective differentiation of these subsets as well as their role in the regulation of effector function. In this talk, I will introduce three major murine models of human parasitic disease (leishmaniasis, toxoplasmosis and schistosomiasis) and use them to illustrate the nature of parasite intitiated subset selection and regulation as well as its influence on the outcome of infection. Finally I will present several examples of how immunoparasitologists are attempting to utilize the information gained from these experimental models in the design of new strategies for immunologic intervention in parasitic disease.

Deletion & Receptor Modification in Central Tolerance Induction

C2-002 THE EXTREME SENSITIVITY OF B CELL TOLERANCE, David Nemazee[1,2], Julie Lang[1,2], Jennifer Kench[1,2], Kevin Kane[3], [1]Division of Basic Sciences, Department of Pediatrics, National Jewish Center for Immunology and Respiratory Medicine, Denver, Colorado, [2] Departrment of Immunology, University of Colorado Health Sciences Center, Denver, Colorado and, [3] Department of Immunology, University of Alberta, Edmonton, Alberta.

We have extended our use of anti-MHC class I alloantibody transgenic mice to determine the affinity limits to B-cell tolerance. In the absence of autoantigens to which 3-83 antibody reacts, mice transgenic for heavy and light chain genes encoding the 3-83 antibody specificity generate large numbers of B-cells of transgene-defined specificity. In the presence of H-2K^k or K^b antigens these B-cells fail to develop because of central or peripheral deletion, depending on the presence of autoantigen in the bone marrow or exclusively in the periphery, respectively. 3-83 binds with moderate affinity to K^k, low affinity to K^b, and extremely low affinity to D^k. We measured the binding affinity of 3-83 antibody to purified K^k, K^b and D^k antigens using surface plasmon resonance techniques and compared their effects in vivo in crosses with the appropriate mouse strains. Significantly, even antigens with a single site association constant weaker than 10^5 M^{-1} were capable of inducing deletion in the bone marrow. Under these conditions evidence for induced secondary rearrangements of light chain genes (receptor editing) was apparent. RAG mRNA levels were elevated in the bone marrows and an excess of 3-83-idiotype negative, lambda light chain bearing B-cells was produced. To determine the density of MHC class I antigen required for this tolerance we assessed tolerance in heterozygous class I antigen-bearing mice and in such F1 mice which were in addition heterozygous deficient in β_2-microglobulin. Complete deletional tolerance was again found. Homozygous β_2-microglobulin deficent mice failed to delete, however. We conclude that only a few thousand molecules of membrane autoantigen/cell is capable of deleting even low affinity self-reactive B-cells during their development. These results will be discussed in terms of their relevance to B-cell tolerance and receptor editing in non-transgenic immune systems.

The Route, Dose and Form of Antigen in Controlling Immunity

C2-003 OCULAR REGULATION OF SYSTEMIC IMMUNITY, J. Wayne Streilein, Schepens Eye Research Institute, Harvard Medical School, Boston, MA 02114

Immunity engendered by antigens/pathogens introduced into the eye is unconventional, which is the basis for its status as an immune privileged site. Privilege has been implicated in the success of corneal allografts, the growth of ocular tumors, and susceptibility to autoimmune uveoretinitis. Mechanistically, immune privilege involves important modifications in both the induction and the expression of immunity: (a) injection of antigenic material into the eye of naive individuals evokes an antigen-specific, systemic immune response that is deviant, *i.e.* devoid of effector modalities that elicit intense immunogenic inflammation; (b) expression of delayed hypersensitivity (DH) and antibody-dependent complement fixation in the eye of pre-immunized individuals is profoundly impaired. Induction of systemic immunity to ocular antigens occurs when the injected material is endocytosed by intraocular antigen presenting cells. The cells then migrate via the blood to the spleen, and present peptide fragments on MHC molecules. Eventually, regulatory CD8$^+$ T cells are produced which interfere with expression of DH and with production of complement fixing antibodies. In vitro, dendritic cells from non-ocular sites can be directed to present exogenous antigens in this unique fashion by pulsing the cells with antigen in the presence of transforming growth factor-beta (TGF-β), a normal constituent of intraocular fluids. TGFβ and other factors in aqueous humor inhibit (a) antigen-driven T cell activation, (b) conventional processing and presentation of antigens by local APC, (c) lymphokine-directed activation of macrophages, and (d) activation of complement. Thus, the nature of the immune response to ocular antigens is dictated by the eye's microenvironment which contains immunomodulatory factors secreted by parenchymal cells of the eye. The eye may be a specific example of a general principle wherein differentiated tissues and organs can modify the afferent and efferent limbs of the immune response so that protection afforded by the immune system does not jeopardize physiologic function.

C2-004 ORAL TOLERANCE; ROUTE, DOSE, AND FORM OF ANTIGEN IN CONTROLLING IMMUNITY, Howard L. Weiner, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02215.

Oral tolerance is a long recognized method to introduce peripheral immune tolerance. The primary mechanisms by which orally-administered antigen induces tolerance is via the generation of active suppression or clonal anergy. Low doses of orally-administered antigen favor active suppression, whereas higher doses favor clonal anergy. The regulatory cells which mediate active suppression act via the secretion of suppressive cytokines such as TGF-beta and IL-4 after being triggered by the oral toleragen. Furthermore, antigen that stimulates the gut-associated lymphoid tissue preferentially generates a Th2-type response. Because the regulatory cells generated following oral tolerization are triggered in an antigen-specific fashion, but suppress in an antigen non-specific fashion, they mediate "bystander suppression" when they encounter the fed autoantigen at the target organ. Thus, it may not be necessary to identify the target autoantigen to suppress an organ-specific autoimmune disease via oral tolerance, only to orally administer a protein capable of inducing regulatory cells that secrete suppressive cytokines. We also used ovalbumin specific T cell receptor (TcR)- transgenic mice to investigate oral tolerance and found that orally administered antigen can directly delete antigen-specific T cells in Peyer's patches following their activation. The deletion was mediated by apoptosis and was dependent on dosage and frequency of feeding. At lower doses of antigen apoptosis was not observed; to the contrary there was induction of antigen specific cells that produced TGF-β and Th2 (IL-4 and IL-10) cytokines. At higher doses, both Th1 and Th2 cells were deleted following their initial activation whereas TGF-β secreting cells were resistant to deletion. These findings demonstrate that in addition to anergy and active suppression, orally administered antigens can induce tolerance by extrathymic deletion of both Th1 and Th2 antigen-reactive T cells.

Orally-administered autoantigens suppress several experimental autoimmune models in a disease-and antigen-specific fashion, including experimental autoimmune encephalomyelitis, uveitis, and myasthenia; collagen-and adjuvant-induced arthritis; and diabetes in the NOD mouse. In addition, orally-administered alloantigen suppresses alloreactivity and prolongs graft survival. Pilot clinical trials of oral tolerance in multiple sclerosis, rheumatoid arthritis, and uveitis have demonstrated positive clinical effects with no apparent toxicity and decreases in T-cell autoreactivity. Multicenter trials in multiple sclerosis and rheumatoid arthritis are underway. Clinical trials are planned in Type I diabetes with oral insulin.

Acquired Immunologic Unresponsiveness

C2-005 PERIPHERAL TOLERANCE AND SYSTEMIC BREAKING OF TOLERANCE BY IL-2, Günter J. Hämmerling[1], Judith Alferink[1], Iris Ferber[2], Andreas Limmer[1], Anna Tafuri[1], and Bernd Arnold[1], [1]German Cancer Research Center, Division of Molecular Immunology, Tumor Immunology Program, Im Neuenheimer Feld 280, 69120 Heidelberg, [2]Stanford University Medical Center, Stanford.

Recent work has demonstrated that tolerance can be induced not only to thymic but also to extrathymic antigens. There exist multiple levels of peripheral tolerance including peripheral deletion of T cells, indifference of the potentially autoreactive T cells towards the self-antigen, and various stages of energy which are characterized by phenotypic changes of the tolerant T cells, e.g. downregulation of TCR and CD8 molecules and their distinct ability to become activated under certain in vitro conditions. These many levels of peripheral tolerance raised the question whether they were the result of qualitatively distinct signals transmitted by single interaction with the tolerizing tissue or whether repeated contact with the tolerogen could push tolerant cells onto a different level of tolerance. We have established H-2^{dxk} transgenic mice coexpressing an anti-K^b specific TCR (Des-TCR) and K^b molecules controlled by the inducible CRP (C-reactive protein) promoter which leads to hepatocyte-specific expression. Without induction low levels of K^b expression were observed on hepatocytes resulting in peripheral tolerance as demonstrated by the inability of these mice to reject K^b-positive skin graft. Thymus chimeras ruled out a contribution of the thymus to tolerance induction. The tolerant T cells displayed a partial downregulation of their Des-TCR. Induction of the CRP promoter resulted in strong K^b expression on hepatocytes. The subsequent encounter of the already tolerant T cells with the increased amount of K^b on the hepatocytes rapidly led to a complete downregulation of the TCR. These observations demonstrate that tolerant T cells are still susceptible to further tolerogenic signal driving them into a deeper stage of unresponsiveness.

We have also investigated the reverse situation, namely whether tolerant peripheral T cells can be activated in vivo. For this purpose, transgenic mice displaying peripheral tolerance towards K^b were injected s.c. with K^b-positive tumor cells. As expected these tumor cells were not rejected by the K^b-tolerant mice. However, when the tumor cells coexpressed the IL-2 gene, rejection was observed. The IL-2 secretion led to a systemic breaking of tolerance in vivo because 14 days later, the mice were still able to reject K^b-positive skin transplanted onto the other flank. Although these mice contained activated T cells which could mediate skin graft rejection, no infiltration of the K^b-reactive cells into the K^b-positive liver or autoreactivity against hepatocytes was observed. Coexpression of B7 on the tumor cells did not break tolerance. These observations are of relevance for autoimmunity and demonstrate that the presence of activated autoreactive cells does not necessarily lead to autoimmune destruction.

C2-006 FATE OF SELF REACTIVE T LYMPHOCYTES, J.F.A.P. Miller, The Walter and Eliza Hall Institute of Medical Research, Post Office, Royal Melbourne Hospital, Victoria 3050, Australia

The dominant tolerogenic mechanism for T lymphocytes is intrathymic negative selection of differentiating T cells with self reactivities. Nevertheless, there are in the normal T cell pool, potentially autoaggressive T cells that have escaped thymus censorship. What then are the mechanisms that prevent autoimmunity? The autoreactive T cells could ignore their autoantigen, if unable to penetrate endothelial barriers, or unable to be activated solely as a result of autoantigen recognition. Alternatively, they could be anergized or deleted peripherally , or silenced by immunoregulatory T cells. To explore these possibilities, transgenic mice have been produced expressing a known gene in a given site and in most of their T cells a receptor specific for the transgene product and identifiable by a clonotypic antibody. The results obtained in two distinct models will show that potentially autoaggressive T cells can either ignore their target antigen under normal circumstances or be deleted after activation in situations when the target antigen persists.

Control and Manipulation of the Immune Response

Cell and Molecular Signals for Lymphocyte Activation

C2-007 **COSTIMULATORY SIGNALS THAT PREVENT DEATH DURING T CELL ACTIVATION** Craig B. Thompson[1,2,3,4], Andrew Minn[3,4], John Pena[3,4], Carl June[6], Tullia Lindsten[2,3] Gabriel Nuñez[7], Maribel González-García[7], José Quintáns[4,5], Alex Gottschalk[4], and Lawrence H. Boise[2,3]; [1]Howard Hughes Medical Institute, Department of Molecular Genetics and Cell Biology; [2]Department of Medicine;[3]The Gwen Knapp Center for Lupus and Immunology Research, and [4]Committee on Immunology; [5]Department of Pathology and Cancer Research Center, University of Chicago, Chicago, IL 60637; [6]Immune Cell Biology Program, Naval Medical Research Institute, and the Department of Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD 20889; [7]Department of Pathology, University of Michigan Medical School, Ann Arbor, MI 48109.

The immune system provides a model system in which to study the signal transduction events that regulate programmed cell death. Mature lymphocytes have the capacity to survive for prolonged periods of time. During an immune response, cells of the appropriate antigenic specificity must undergo clonal amplification to mount a protective response. In addition, cells participating in inflammatory immune responses need to have the capacity to survive at sites of inflammation. Upon completion of a successful inflammatory response, the majority of cells produced must die off in order to maintain the homeostasis of the organism. Recently we have begun to learn a great deal about how mature lymphocytes regulate their susceptibility to death. Three types of information appear to be used by the lymphocyte to control its susceptibility to undergo programmed cell death. The intrinsic susceptibility of a cell to undergo programmed cell death is determined by members of the Bcl-2 gene family. Recent evidence suggest Bcl-2 regulates the basal apoptotic threshold of a T cell. In contrast, the Bcl-2-related gene, Bcl-x_L, provides additional protection from cell death when induced in response to costimulatory signals. In addition, extrinsic survival factors, such as IL-2, can initiate signal transduction events that can prevent a cell from initiating apoptosis. Finally, lymphocytes appear to have specific receptors, such as FAS, which can directly induce programmed cell death upon ligand binding. The integration of these three systems will be discussed.

C2-008 MOLECULAR AND GENETIC STUDIES OF T CELL ANTIGEN RECEPTOR SIGNAL TRANSDUCTION, Arthur Weiss, Howard Hughes Medical Inst., Depts. of Medicine, Univ. of California, San Francisco, Ca. 94143-0724

The T cell antigen receptor (TCR) initiates cell activation by regulating cellular protein tyrosine phosphorylation. The TCR CD3 and ζ chains interact with members of two families of cytoplasmic PTKs via sequence motifs termed ARAMs (for antigen recognition activation motifs), containing the consensus sequence $YXXLX_{(6-8)}YXXL$. PTKs of the Src family, Lck or Fyn, and of the Syk/ZAP-70 family interact with the TCR ARAMs sequentially. Studies of Lck or CD45 deficient mutant T cell lines and as well as a heterologous Cos cell system in which wild-type or mutant Lck and ZAP-70 proteins were expressed suggest the following model by which the TCR initiates PTK activity: Stimulation of the TCR on T cell lines or hybridomas results in oligomerization of ARAMs and their tyrosine phosphorylation in an Lck-dependent manner. ZAP-70, a cytoplasmic protein, is then recruited via both of its SH2 domains to a doubly phosphorylated ARAM. In resting thymocytes or lymph node T cells, the ζ chain is already constitutively phosphorylated and associated with nonphosphorylated ZAP-70. This suggests differences in the regulation of these interactions in distinct cell types. Subsequent oligomerization of the TCR alone, or with the CD4 or CD8 coreceptors, facilitates the tyrosine phosphorylation of ZAP-70 by Lck leading to a synergistic activation of PTK activity. This synergistic induction of PTK activity depends upon ZAP-70 catalytic function, suggesting an important role for ZAP-70 kinase activity in cellular protein tyrosine phosphorylation.

Important roles for ZAP-70 in TCR signal transduction leading to thymocyte selection and T cell activation is further supported by recent observations of a severe combined immunodeficiency disease syndrome which results from mutations in the ZAP-70 coding sequence. In patients with this syndrome, $CD4^+$ peripheral T cells have a TCR-mediated signal transduction defect. There is also a paucity of $CD8^+$ peripheral T cells in these patients. This appears to result from a developmental arrest since the thymuses of patients with this syndrome have been reported to contain an abundance of $CD4^+/CD8^+$ and $CD4^+/CD8^-$ thymocytes but only very few $CD4^-/CD8^+$ thymocytes. Thus, it appears that the absence of ZAP-70 results in a selective failure in the positive selection of the $CD8^+$ lineage. More recent studies on one such patient has provided important insights into the paradoxical phenotype of the cells in these patients. These studies provide strong biochemical and genetic evidence for important roles of two distinct cytoplasmic PTKs, Lck and ZAP-70, in TCR signal transduction function during T cell development and activation.

Lymphocyte Subset Interaction in the Control of the Immune Response

C2-009 CONTROL OF $CD4^+$ CELL DIFFERENTIATION AND TOLERANCE, Abul K. Abbas, Victor L. Perez, Michael P. Sethna and Luk van Parijs, Immunology Research Division, Department of Pathology, Brigham & Women's Hospital and Harvard Medical School, Boston.

The magnitude and nature of helper T cell-dependent immune responses are controlled by a balance between T cell activation and tolerance, and by the phenotypes of effector T cells that develop as a consequence of immunization. In vitro and in vivo experiments using transgenic mice have been used to define the roles of antigen, costimulators, and regulatory cytokines in $CD4^+$ T cell differentiation and tolerance. Cytokines present during antigenic stimulation are the major determinants of the patterns of T cell differentiation and the functional phenotypes of differentiated effector cells. Differentiated T cells expressing a transgenic antigen receptor retain their cytokine profiles and functional phenotypes for prolonged periods following adoptive transfer into T cell-deficient recipients. In population assays, T_H1 effectors can be converted into IL-4 producers but the T_H2 phenotype is stable and cannot be reversed by antigenic stimulation in the presence of cytokines or cytokine antagonists. Cells of the T_H1 subset are more dependent on costimulation, and more sensitive to tolerance induction, than are T_H2 cells. This accounts for the finding that tolerogenic protein antigens tend to induce T_H2-dominant responses. Costimulators play a role not only in initiating the process of T cell activation, but also in regulating the magnitude and persistence of T cell-dependent immune responses. The deregulated expression of one costimulator, B7-1, in transgenic mice has a net inhibitory effect on immune responses to T-dependent antigens. Thus, manipulation of costimulatory signals and cytokines is an important approach for regulating immunity, and transgenic mice provide valuable tools for analyzing the functional effects and mechanisms of action of these regulatory stimuli.

C2-010 REGULATION OF T HELPER CELL RESPONSES, Anne O'Garra, DNAX Research Institute, Palo Alto.

The selective induction of CD4+ T helper cells into T cells with distinct cytokine profiles may be dictated by factors that include: cytokines produced by APC/accessory cells or T cells in the microenvironment, the nature and dose of the antigen, or the APC. To study this we have used an OVA-peptide specific, ab T cell receptor (TCR) transgenic mouse as a source of naive T cells. We have recently shown that dendritic cells were potent stimulators of antigen specific proliferation of OVA-specific CD4+ T cells, but addition of IL12 or IL-4 were required for development of a Th1 (IFNg-producing) or a Th2 (IL-4-producing) phenotype respectively. Dendritic cells could drive IL-12-dependent Th1 development in the absence of endogenous IL-4.

We have now further dissected the signals required for the development of Th0, Th1 and Th2 phenotypes from a single naive CD4+ T cell. Furthermore, we show that we can redirect a Th1 to a Th2 phenotype and vice versa at a population level. However, Th cell cytokine production at a clonal level can only be transiently modulated by costimulatory or inhibitory cytokines.

Modification of the Immune Response in Infection

C2-011 CHARACTERISTICS OF VIRUS-SPECIFIC $CD4^+$ AND $CD8^+$ T CELL MEMORY, Peter C. Doherty, David Topham and Ralph Tripp, Department of Immunology, St. Jude Children's Research Hospital, Memphis TN 38105.

Quantitative analysis of the host response in mice infected with an H3N2 influenza A virus or a parainfluenza type 1 virus (Sendai) indicates that the development of virus-specific $CD8^+$ T cells, determined by analysing CTLp frequencies, and the establishment phase of $CD8^+$ T cell memory are essentially a continuum. With these respiratory viruses which, in the main, cause productive infection only in the respiratory tract, there is no suggestion that the responding $CD8^+$ T cells are "exhausted" by the need to provide terminally differentiated effector CTL at any stage of the infectious process. In fact, CTLp that can be expanded by limiting dilution analysis (LDA) are present from day 7-8 after infection in the lung, the only site where fully-functional CTL are found with the virus isolates used in these experiments. Comparison with other virus models indicates that, with the exception of massive doses of LCMV isolates that have the capacity to grow in $CD4^+$ T cells, this is the normal course of events in virus infections. Diminishing the $CD8^+$ CTLp response 10-fold by giving a single dose (10 mg/Kg) of cyclophosphamide at the time of peak proliferation (day 5) does not prevent either virus clearance or the establishment of memory. The $CD8^+$ CTLp are then maintained for life in laboratory mice held under conditions of strict isolation. Neither virus is known to persist at the genome level, and adoptive transfer experiments indicate that the $CD8^+$ CTLp survive in the absence of antigen. Furthermore, the majority of the CTLp progressively lose evidence of constitutive activation, switching (for influenza) from the acutely stimulated/memory L-selectin (L-sel)-lo to the "naive" L-sel-hi phenotype after 12-14 months and earlier with Sendai virus. This means that the CTLp would again traffic through lymph node HEVs, increasing the possibility of "bystander" or cross-reactive stimulation during the course of (for example) intercurrent virus infections. We have preliminary evidence, both from cell-cycle analysis and changes in L-sel phenotype, that this does occur and may be an important factor in the maintenance of effective T cell memory. We have now developed a reproducible LDA for $CD4^+$ T cells showing, at the peak of the acute response, Thp frequencies as high as 1:100 for the $CD4^+CD44$-hi set. This is being used to analyze the characteristics of long-term Thp memory, and for adoptive transfer (parking) experiments with uninfected class II MHC (-/-) recipients. We are intrigued to know whether there is indeed a need for persistence of the inducing antigen to maintain $CD4^+$ T cell memory. Our thinking is very much constrained by the view that there is a fundamental difference between B cell and $CD8^+$ T cell memory, in the sense that the need is to maintain effectors (plasma cells) in the former and activated precursors (but not effectors) subject to rapid recall in the latter.

C2-012 DEVELOPMENT OF CD4+ EFFECTOR CELLS IN EXPERIMENTAL MURINE LEISHMANIASIS. Richard M. Locksley[1], Steven L. Reiner[2], David B. Corry1, Adil E. Wakil[1], and Deborah J. Fowell[1]. [1]University of California San Francisco, San Francisco, CA 93143, and [2]University of Chicago, Chicago, IL.

Infection of inbred strains of mice with *Leishmania major* comprises an exceptionally well-studied model for the development of Th1 and Th2 effector cell subsets in the CD4+ lineage. Most strains respond to *Leishmania* with the appearance of parasite-specific Th1 cells that generate IFN-γ required for macrophage activation and control of infection. Mice with disruption of MHC class II genes or IFN-γ are unable to generate protective Th1 cells and suffer fatal disseminated disease. In contrast, mice with disruption of the β2-microglobulin gene readily control infection, demonstrating that CD8+ T cells are not required for Th1 responses. In contrast to other strains of mice, animals on a BALB background respond inappropriately to *L. major* infection with the appearance of parasite-specific Th2 cells that are unable to mediate macrophage activation and that generate cytokines, particularly IL-4, that inhibit the macrophage-activating capacity of Th1-derived cytokines. Neutralization of IL-4 at the time of infection results in redirection of the underlying genetic response, such that treated mice develop Th1 cells and successfully control disease. Recombinant IL-12 administered at the time of infection is similarly successful at redirecting CD4+ subset development, principally due to its capacity to powerfully suppress IL-4 production. Suppression of IL-4 transcription and protein production by IL-12 was comparable in mice with a disrupted IFN-γ gene, or in mice given anti-IL-10 antibodies, suggesting a direct effect of IL-12. Delay in administration of IL-12 resulted in loss of the protective effect and was related to loss of the capacity of IL-12 to down-modulate IL-4 expression as assessed by quantitation of transcripts and the frequency of IL-4-secreting cells measured using ELISPOT assays. Similar results occurred using TCR transgenic mice with T cells reactive to an immunodominant *L. major* antigen that were bred onto susceptible and resistant background, suggesting that the antigen/TCR/MHC class II complex does not itself mediate Th development.

C2-013 HIV EPITOPE VARIATION AND T CELL RECOGNITION, Andrew McMichael[1], Sarah Rowland-Jones[1], Paul Klenerman[1], Steve McAdam[1], Frances Gotch[1], Rodney Phillips[1] and Martin Nowak[2].
[1]Institute of Molecular Medicine and Department of Zoology, [2]University of Oxford.

Infection with HIV normally elicits a potent specific cytotoxic T lymphocyte (CTL) response. There is evidence that this reduces the initial viraemia and controls the infection during the asymptomatic stage. In the late phase of infection, the CTL response breaks down and viraemia results. It has not been clear why the CTL response fails. Variation in the epitopes recognised by CTL may be important in subverting this immune response. We have found that many of the epitopes in HIV proteins recognised by CTL of patients vary within the patient's viral quasispecies and these are often not recognised by the predominant CTL. The variant epitopes rarely fail to bind to the presenting HLA molecule; the available CTL clones fail to react or are antagonised. This results in a change in the dominant CTL clones, switching the response to previously minor epitopes. These events occur when more than one epitope is recognised by CTL giving a complex pattern of changing immunodominance rather than fixation of a single antigenic variant.
Although it might be expected that HLA-binding variants would in due course elicit new specific CTL responses, in *the late* stages of HIV infection they do not. Furthermore, CTL clones reacting to the original epitopes can decay and not be replaced. Thus there is a failure to prime new CTL responses.
These findings offer an explanation of why the HIV-specific CTL response fails to control the infection indefinitely, unlike the response to an invariant persisting virus such as Epstein Barr virus. The continuous bursts of escaping virus ensures eventual damage to the CD4 positive cells necessary for initiating and maintaining the CTL response. When it becomes impossible to prime CTL to new variants and replace CTL clones, that responded to old variants but died, the total response may fail.

C2-014 MODIFICATION OF THE IMMUNE RESPONSE IN HIV INFECTION BY TYPE 1/TYPE 2 CYTOKINE REGULATION, Mario Clerici[1], Apurva Sarin[2], Daniel R. Lucey[2], Ligia A. Pinto[2], Thomas A. Wynn[3], Stephen P. Blatt[4], Craig W. Hendrix[4], Italy, Matthew. J. Dolan[4], Stanley F. Wolf[5], Jay A. Berzofsky[2], Pierre A. Henkart[2], and Gene M. Shearer[2], [1]Universita degli Studi, Milano, [2]National Cancer Institute, Bethesda, [3]National Institute of Allergy and Infectious Diseases, Bethesda, [4]Wilford Hall Medical Center, San Antoino, [5]Genetics Institute, Cambridge.

Peripheral blood mononuclear cells (PBMC) from approximately 60% of HIV-infected, asymptomatic (HIV+) individuals are unresponsive by in vitro interleukin-2 (IL-2) production (T helper function) to recall antigens such as influenza A virus and HIV envelope peptides. Elevated programmed cell death (PCD) is also seen in PBMC from HIV+ individuals when stimulated via T cell receptor cross-linking (1-to-2-day cultures), or with HIV gp120 peptides (7-day cultures). Addition of the type 1 cytokine IL-12 or antibody specific for the type 2 cytokine IL-10 to cultures of PBMC from HIV+ individuals restores T helper function. Furthermore, IL-2 and IL-12, as well as antibodies against IL-4 and IL-10, prevent PCD. In contrast, anti-IL-12 blocks in vitro T helper responses of PBMC from HIV- individuals, and this antibody promotes PCD, as do the type 2 cytokines IL-4 and IL-10. These results indicate that the two fundamental immunologic problems observed in progression of HIV+ individuals to AIDS, loss of T helper function and CD4+ T cell depletion, are related, and that both can be suppressed by type 1 cytokines and enhanced by type 2 cytokines in vitro. In vivo modification of responses in this syndrome might be best effected by multiple strategies involving a combination of type 1 cytokines and antibodies against type 2 cytokines. Our findings raise the possibility of using cytokine-based therapy for blocking progression to AIDS by enhancing cellular immune responses, thereby protecting against advancing HIV disease by preventing more extensive HIV infection and T cell loss by PCD, as well as stopping the opportunistic infections that are characteristic of AIDS.

Autoimmunity and Its Regulation

C2-015 INDUCTION, REGULATION AND DIVERSIFICATION IN AUTOIMMUNITY
Charles A. Janeway, Jr., Mark J. Mamula, Takuma Kato, A. Nicola Schweitzer, and Kim Bottomly
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We have studied two autoimmune disease models in our laboratories, using TCR transgenic mice in one instance and rat insulin promoter driven islet expression of the co-stimulatory molecule in the islets of Langerhans in the other. Using this set up, we have made a number of findings about the regulation and *induction* of autoimmune disease in these models.
In the EAE model, we have used TCR transgenes to follow the expression of the TCR on a cloned T cell line that transfers this disease. We find that there is no central control on this disease, and that it takes place solely in the periphery. Here, we find fully activatable cells, and the degree to which they can be activated is not determined by their microenvironment, but solely by their receptor. These mice have now been bred to all available MHC alleles, and we have found that only I-Au can positively select this TCR. This selection is very intense, suggesting that the self peptide itself may be driving positive selection in this case. We will be testing this, along with the role of B cells in the diversification of the response, in the coming months.
In the RIP-B7.1 model, we have observed that the islets stimulate a very potent diabetes that is fully regulated by the genotype at the MHC. We have derived cloned T cell lines from normal NOD mice by growing them on F1 hybrid islets, and these make IFNγ, TNFβ, and the perforin protein P1. They do not make IL-5, iNOS, IL-2, or TGFβ. These CD8 positive cloned T cell lines will transfer very rapid onset diabetes to NOD mice. These will be used as donors for TCR sequences that will examine the role of CD8 T cells in this disease process.

C2-016 THE ROLE OF MHC ALLELES IN SUSCEPTIBILITY AND RESISTANCE TO IDDM IN THE NOD MOUSE. Hugh McDevitt, Steven Singer, Diane Wherrett, and Xiao-Dong Yang, and Roland Tisch. Department of Microbiology and Immunnology, Stanford University School of Medicine, Fairchild Bldg.D345, Stanford, CA 94305.

During the past 3 years we have produced 8 transgenic lines of NOD mice expressing diabetes susceptible or resistant I-Aβ alleles. Of these 8 lines, 6 manifest mild to severe forms of a previously described syndrome seen in mice expressing high levels of Aβ chains. In the extreme form mice have nearly absent splenic B cells, extramedullary hematopoiesis, eosinophilic infiltrations, and elevated levels of IgE and IgG_1 with decreased levels of IgG_{2a}. (This phenotype suggests over-expression of IL4 but persists in the presence of the IL4 knockout). The mild form is manifested by a progressive decrease in splenic B cells from 40% at 6 wks to 15% at 25 wks.

Of 2 $A\beta^{g7}$ transgenic lines, 1 shows no change in transgenics and non -transgenics. The second line has decreased spleen B cells and a significant reduction in incidence of diabetes (45% at 28 wks in females versus 70% in non-transgenics).

In the other 6 transgenic lines, all but 1 have varying reduction of spleen B cells after 12 wks. This includes 1 $A\beta^{g7}$.PD transgenic line which is phenotypically normal at 8 wks but has decreased spleen B cells of 15 to 20% at 12 wks. This line does not develop IDDM.

One of the 2 $A\beta^{d}$ transgenic lines has 35% spleen B cells in both transgenics and non-transgenics at 21 wks., decreased diabetes incidence, from 50% to 10% at 30 wks., and had insulitis and islet cell autoantibodies to the same extent as non-transgenics. Cell transfer studies added to spleen T cells from normal, recently diabetic NOD females suggests the decrease in diabetes in $A\beta^{d}$ transgenics is due to induction of a predominant T_{h2} response which is capable of partially suppressing transfer of diabetes. Experiments to confirm this hypothesis through the use of cell transfer combined with anti-IL4 and anti-IL10 are in progress.

C2-017 THE SELF-DIRECTED T CELL REPERTOIRE AND ITS REGULATION. E Sercarz, L Brossay, H-K.Deng, V Kumar, M Melo, A Miller, K Moudgil, N Nanda, D Stevens and J Ohmen. UCLA. Dept. of Microbiol & Molec. Genetics, Los Angeles, CA 90024-1489.

The T cell repertoires that are available to become engaged in self-reactivity evade negative selection in the thymus and are directed against a variety of cryptic and subdominant determinants on self antigens. Determinants are cryptic for several reasons, among them that they do not compete successfully either for inducing an immune response or for inducing tolerance. This premption of tolerance induction by dominant determinants can lead to the preservation of the repertoire directed against cryptic determinants, and can also account for cases in which extra transgenic MHC molecules protect against disease. We will describe same of the components of the very broadly directed autoreactive repertoire.

Hindrance-related repertoire. Examples will be described of two types of hindrance which account for crypticity in the lysozyme (HEL) system: in the first, an example of hindered access by antigen to the MHC, R61 on HEL is shown to obstruct binding of 46-61, a dominant peptide in the H-2^k haplotype to the A^b molecule. 46-60 binds strongly to A^b and furthermore induces a potent T cell proliferative response. Of special interest is the fact that C3H.SW mice (H-2^b) can respond to 46-61 while the C57BL/6 (H-2^b) strain cannot. It could be shown that the former strain can process 46-61 into the acceptable 46-60. The bm-12 mutation in I-A^b permits binding of the peptide with R61. Another type of cryptic T cell repertoire that exists because of epitypic hindrance occurs when the MHC-Ag complex is prevented from interacting effectively to the TcR. We have evidence for mouse lysozyme (ML) peptide 46-62 binding effectively to A^k but not being able to activate a set of T cells which can readily be induced with 46-59 or 49-62.

Regulatory repertoire. There are other repertoires which are self-reactive and regulatory that do not seem to be rendered tolerant during development. One of these under study is a regulatory Vβ14, CD4+ T cell (Treg) which seems to act by recruiting CD8+ T cells to down-regulate a Vβ8.2, CD4+ T effector cell that causes EAE. This Treg can be ablated by treatment of the animal with anti-Vβ14 mAb, and such treatment greatly prolongs the myelin basic protein-induced disease, and in many cases induces recurrences and cycling of the disease pattern. This Treg represents a stable and necessary member of a hard-wired circuit that seems to function to permit only brief outbreaks of Vβ8.2-mediated EAE.

Changing the self. Since the self can best be defined as those self-determinants that effectively exert negative selection, it is of great interest that the processing machinery can be adjusted to permit certain determinants that ordinarily are invisible, to induce tolerance. Experiments with site-directed mutants in determinant flanking regions will be shown that exemplify this view of the aleatory self. Supp. by grants from NIH, ACS, NMSS, and JDFI.

Discussion: Modeling of Immunological Phenomena

C2-018 HYPERMUTATION AND AFFINITY MATURATION IN THE B-CELL RESPONSE. Philip E. Seiden, IBM Research Center, Yorktown Heights, NY 10598 and Franco Celada, Hospital for Joint Diseases, New York, NY 10003

We have used a cellular automaton immune system simulation to investigate the behavior of hypermutation and affinity maturation in evolving the B-cell response. Hypermutation and affinity mutation are often referred to interchangeably, however, they actually refer to two quite different aspects of the evolution of the response. We find that our model system can exhibit affinity maturation quite naturally with no mutation at all. Affinity maturation is a case of natural selection among cells of differing affinities. The higher-affinity cells win this competition by virtue of their higher affinity which makes them fitter. The difference between the primary response, which is not dominated by high-affinity cells, and the secondary, which is, is due to the rarity of any B cells capable of responding at all to a new antigen. In order to have competition leading to natural selection there must be a high enough density to force the cells to compete. This affinity maturation without mutation arises when we have all B-cell receptor types available in our system, i.e., there are no holes in the possible diversity. However, the competitive mechanism can only multiply an existing population. If there are no cells to start with it cannot work. In the real animal there are many holes in the diversity so that there is a low probability for having a high-affinity cell for a random new antigen. Hypermutation allows the system to eliminate these holes. We have carried out a number of *in machina* experiments to investigate the effect of affinity maturation and hypermutation on the immune response. We will present results showing conditions necessary to get good affinity maturation in a time short enough to provide an effective response. In particular we discuss how strongly the affinity must increase, how much mutation is necessary, and whether cycling is needed between periods of cell division with and without mutation.

Overview Lecture

C2-019 TUMOR ANTIGENS RECOGNIZED BY CYTOLYTIC T LYMPHOCYTES, Thierry Boon, Pierre van der Bruggen, Benoit Van den Eynde, Bernard Lethé, Pierre Coulie, Vincent Brichard, Aline Van Pel, Etienne De Plaen, and Christophe Lurquin. Ludwig Institute for Cancer Research, Brussels Branch, 74 avenue Hippocrate - UCL 7459, B-1200 Brussels, Belgium and Cellular Genetics Unit, Université Catholique de Louvain - B1200 Brussels, Belgium.

Antigens recognized on mouse tumor cells by cytolytic T lymphocytes can result from point mutations affecting the region coding for the antigenic peptides. These point mutations either render a peptide capable of binding to a MHC class I molecule or they confer a new epitope to a peptide which was already capable of binding. Another type of antigen recognized on mouse tumors P815 by syngeneic CTL results from the activation of gene P1A which is completely or nearly completely silent in normal cells with the exception of testis. The tumor-specific expression of this gene is due at least in part to demethylation. Unlike the antigens induced by point mutations, this antigen is shared by different tumors. Recently, a third type of mouse tumor antigen has been identified : it results from the transposition of a defective endogenous retroviral sequence. It is likely that this transposition activates the transcription of the gag-like region which codes for the antigenic peptide.

In human tumors, several antigens recognized by autologous CTL have been identified. A first class results from the activation of genes such as MAGE-1, MAGE-3, BAGE and GAGE, which are not expressed in normal tissues with the exception of testis. MAGE derived peptides binding to HLA-A1, Cw16 and A2 have been identified. The MAGE family comprises 6 genes that are expressed in a significant fraction of tumors of several different types. PCR analysis indicates that MAGE-3 for instance is expressed by 64% of melanomas, 48% of head and neck tumors, 31% of non small cell lung tumors, 33% of bladder tumors. No expression has been found in renal carcinomas or leukemias. Considering that each of these MAGE proteins is about 300 amino acids long, it is likely that many more MAGE-encoded antigens presented by the products of various HLA alleles will be identified. A second type of antigen identified in melanoma consists of differentiation antigens derived from proteins such as tyrosinase, and Melan-A, which are specific for melanocytes and melanomas. Recently, we have identified a melanoma antigen which results from a point mutation in an intron. The antigenic peptide is encoded by the end of an exon and the initial part of intron. Another antigen recognized on a large fraction of HLA-A2 melanomas involves an antigenic peptide encoded by an intron.

A major observation made both on mouse tumors and on human tumors is that a considerable number of antigens (>6) is recognized on these tumors by autologous CTL. Accordingly, the identification of new antigens will not only extend the range of patients eligible for specific immunotherapy, but will also make it possible to immunize against several antigens borne by the same tumor. This may be a critical condition for therapeutic success.

Brichard, V. et al. *J. Exp. Med.*, **178**, 489-495 (1993).
Gaugler, B.et al. *J. Exp. Med.*, **179**, 921-930 (1994).
van der Bruggen, P.et al. *Eur. J. Immunol.*, in press (1994).
De Plaen, E.et al. *Immunogenetics*, in press. (1994).
Coulie, P. G.et al. *J. Exp. Med.*, **180**, 35-42 (1994).
van der Bruggen, P.et al. *Eur. J. Immunol.*, in press. (1994).

Signals for Lymphocyte Activation and Inactivation

C2-100 INTERACTION OF SYK AND ZAP-70 WITH p56^{lck}/CD4, Oreste Acuto[1], Margot Thome[1], Pascale Duplay[1] and Maria Guttinger[2], [1]Department of Immunology, Institut Pasteur, 75724 Paris Cedex 15, France, [2]DIBIT, San Raffaele Scientific Institute, 20132 Milano, Italy.

During antigen recognition by T-cells, CD4 and the T-cell receptor TCR/CD3 complex are thought to interact with the same MHC II molecule in a stable ternary complex. The association of CD4 with the TCR/CD3 complex requires the interaction of p56^{lck} with CD4. We have taken a biochemical approach to understand the mechanisms by which p56^{lck} and, in particular, its src homology (SH) 2 domain contributes to the association of CD4 with the TCR/CD3/ζ-complex during activation. We have previously shown that the p56^{lck} SH2 domain binds to tyrosine phosphorylated ZAP-70. We have now formally demonstrated by immunoprecipitation experiments that p56^{lck} associates in vivo with ZAP-70 and the homologous tyrosine kinases Syk after CD3 stimulation of Jurkat cells. A tyrosine phosphorylated peptide (YEEI) containing the sequence predicted to be optimal for binding to the SH2 domain of src family kinases specifically competes for this association (and not the ZAP/Syk -ζ association) suggesting that tyrosine phosphorylated ZAP-70 and Syk bind to p56^{lck} by an SH2-mediated interaction. In these complexes, the CD3/ζ chains are present, indicating an activation-dependent recruitment of p56^{lck} to the CD3/ζ-ZAP or Syk complex. We could show that after activation of Jurkat cells, CD4 can be co-immunoprecipitated with the TCR (and vice-versa). This activation-dependent association could be disrupted by the phosphorylated YEEI peptide. Moreover, ZAP-70 and CD4 co-cap only after CD3 stimulation in human T lymphoblasts. We propose that the interaction of the p56^{lck} SH2 domain with ζ-associated tyrosine phosphorylated ZAP-70 and Syk enables CD4 to associate with the TCR/CD3/ζ complex.

C2-101 INTERACTION OF PHOSPHOTYROSINE PHOSPHATASE 1D WITH THE T-CELL ANTIGEN RECEPTOR

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The early signalling events mediated by the T-cell antigen receptor (TCR) involve the phosphorylation of Tyr residues within TAM ('tyrosine-based activation motif') sequences located in the TCR polypeptide cytoplasmic tails. Upon phosphorylation TAMs form high affinity binding sites for proteins containing src homology type 2 (SH2) domains. We have investigated the possible recruitment of phosphotyrosine phosphatases (PTPase) to the TCR and have found PTPase activity associated with the TCR in Jurkat T-cells following receptor aggregation induced using a CD3 mAb. A TCR-ζ chain TAM motif peptide was used to characterise this PTPase further. PTP1D (Syp), which contains two SH2 domains, was found to bind *in vitro* to the phosphorylated, but not to the non-phosphorylated, TCR-ζ TAM peptide. Another PTPase which contains two SH2 domains, PTP1C, did not bind to the TCR-ζ TAM peptide. Interestingly, more PTP1D protein bound to the phosphorylated TAM peptide following TCR aggregation, and this correlated with an increase in associated PTPase activity. We have been unable to detect a TCR-mediated increase in PTP1D Tyr phosphorylation. However, the PTP1D associated with the ζ-TAM peptide had higher PTPase activity (2-5 fold) than an equivalent amount of PTP1D immunoprecipitated from cell lysates. The precise mechanism(s) whereby PTP1D interacts with the ζ-TAM peptide have therefore yet to be elucidated, but it is clear that this interaction leads to an increase in its PTPase activity. Further work is being directed to investigating the possible role of PTP1D in TCR signal transduction coupling and the search for physiologically relevant substrates.

C2-102 **Transgenic mice expressing a dominant negative ZAP-70 effect specific peripheral T-cell populations.**

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Two transgenes that express cDNA sequences for the 70kD. murine T-cell Receptor Zeta-Associated Protein (ZAP-70) under the control of the T-cell specific *lck* promoter were introduced into FVB/N germline (H-2q). One transgene expresses the full-length cDNA (ZAP-FL, ~2.0kb), the second truncated transgene (ZAP-NT) expresses the 5' 800bp of coding sequence corresponding to the two ZAP-70 SH2 domains only. Transgenic mice demonstrated a copy-number dependent, T-cell specific expression of the transgenes. As expected, we observed no changes in the ZAP-FL mice FACS profiles using antibodies for many white blood cell sub-populations. However, in the ZAP-NT transgenic lines, we observed a slight reduction in T-cell surface markers as early as 2 months followed by a precipitous loss of T-cells at 6 months of age versus control and ZAP-FL mice. A decrease of approximately 50% of lymphocytes has been confirmed for different ZAP-NT transgenic lines by FACS using antibodies to different CD3 chains and whole blood cell counts. Interestingly, the peripheral CD4 cell population is specifically reduced in the ZAP-NT mice, with no changes observed in the thymocytes. No change in PMN's or monocytes is observed in the 6 month ZAP-NT mice. We have begun assessment of the allogeneic response using sponge matrix allografts and H-2^d spleen cells. Preliminary results indicate a specific reduction in CD4 helper cells and no changes in NK cell populations. We will present further functional analysis of the ZAP-70 dominant negative transgenic mice.

C2-103 A mAb FIB-39 defines a novel costimulation molecule COST.1 on CD8 Lymphocytes.

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To identify cell signalling molecules on CD8 T cells we have raised mAbs to a CD8 T cell lymphoma, TK1 and searched for mAbs which exhibited costimulatory activity with suboptimal doses of anti-CD3 mAb. One mAb, FIB-39, recognizes two glycoproteins species (COST.1) of 90 and 150 kD molecular weight in Western Blots of TK1 lysates. The epitope recognized by FIB-39 is removed by the proteolytic enzyme pronase as well as by neuraminidase indicating that FIB-39 may recognize a carbohydrate epitope on a glycoprotein. FACS studies show that in the peripheral lymphoid organs COST.1 is mainly expressed on CD8 lymphocytes, while in the thymus the majority of thymocytes are COST.1^{+ve}. COST.1 is expressed on 64% of bone marrow cells. All bone marrow neutrophils and monocytes express COST.1, while subsets of small bone marrow cells are COST.1^{+ve}.

On immobilization with anti-CD3, FIB-39 augments the proliferation of purified T cells to low doses of anti-CD3. Surprisingly, FIB-39 also shows some costimulatory activity when in solution. Therefore, we hypothesize that cross-linking of COST.1 results in a signalling event in the T cell and that FIB-39 costimulatory activity does not simply result from an increase in the adhesion of T cells to anti-CD3 coated plate. Cross-linking of COST.1 also augments allo-proliferation of T cells in a Mixed Lymphocyte Reaction as well as the proliferation of spleen lymphocytes to ConA.

C2-104 MIF IS AN ESSENTIAL MEDIATOR OF T-CELL ACTIVATION, IS RELEASED FROM T-CELLS BY GLUCOCORTICOIDS, AND OVERRIDES GLUCOCORTICOID SUPPRESSION OF T-CELL PROLIFERATION.

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The cytokine MIF was described more than 25 years ago to be a T-cell product and to be associated with delayed-type hypersensitivity reactions. In more recent studies, MIF has been identified to exist pre-formed within anterior pituitary cells and in resting macrophages. MIF appears in the circulation after LPS administration, and anti-MIF antibodies have been shown to fully protect animals from lethal endotoxemia [*Nature* 1993;365:756, *JEM* 1994;179:1895].

Using recently cloned recombinant MIF (rMIF) together with neutralizing anti-MIF antibodies, we examined the release of MIF by T-cells and the role of this cytokine in T cell responses. MIF protein was found by Western blotting to be present, pre-formed, in resting primary T cells and in T-cell lines (ASL-1, Jurkat) and to be released by activating stimuli such as ConA, αCD3, and TSST-1. Neutralizing anti-MIF IgG was observed to inhibit T-cell proliferation in response to antigen-dependent stimuli. Anti-MIF antibody blocked the antigen-dependent proliferative response of RNase- or PPD-primed murine T-cells, as well as tetanus-primed human T-cells. By contrast, the addition of rMIF (0.1 - 1000 ng/ml) to T-cell cultures had little appreciable effect on either mitogen- or antigen-driven T-cell proliferation, indicating that T-cells under these conditions may be stimulated maximally by endogenously-released MIF.

In direct analogy to the situation which has been described for monocytes/macrophages, T-cells were observed to release MIF after stimulation with low concentrations of glucocorticoids (10^{-10}-10^{-14} M dexamethasone). Of note, rMIF (0.1-10 ng/ml) was found to override dexamethasone suppression (10^{-8} M) of both mitogen- and antigen-driven human T-cell proliferation.

These data indicate that MIF is an essential mediator of T-cell proliferation and, as for macrophages, T-cell MIF is induced by glucocorticoids and acts to counter-regulate that immunosuppressive effect of steroids.

C2-105 DOMINANT AND CASUAL INTERACTIONS THAT DRIVE T- DEPENDENT B CELL ACTIVATION.

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We have established two culture systems using human PBMNCs. In both systems we quantify IgG, IgM and IgA production. The first, driven by PWM, depends on CD40-CD40L interactions and is completely inhibited by mAb 5C8 (intact or Fab) anti-CD40L (TBAM). The mAb may be added briefly, but must be added early in the culture period. The mAb, known to block the CD40L-CD40 interaction, appears to function by un-coupling events that have critical kinetic components. The second culture system examines Ig production at the end of an 8 day culture without any added stimulus. Ig production in the second system is inhibited by mAbs that block the CD2-LFA3 pathway, but not by 5C8. MAbs to other cell interaction antigens (ICAM, CD18, CD28, VLA4) have intermediate or no effect in both systems. A model for the variable participation of these cognate interactions will be presented.

C2-106 THE MACROPHAGE IS THE PRIMARY SOURCE OF MACROPHAGE MIGRATION INHIBITORY FACTOR (MIF) IN DELAYED-TYPE HYPERSENSITIVITY (DTH) REACTIONS.

Jürgen Bernhagen, Michael Bacher, Thierry Calandra, & Richard Bucala. The Picower Institute for Medical Research, 350 Community Drive, Manhasset, NY 11030.

MIF was one of the first cytokine activities to be discovered and was characterized initially by its ability to inhibit the migration of macrophages. Historically, this function was associated with the infiltration and activation of T-cells in DTH sites. More recent investigations have identified MIF to be present, pre-formed, within the corticotrophic cells of the pituitary and to be released into the circulation during endotoxic (LPS) shock. Monocytes/macrophages also contain abundant stores of MIF which is released by LPS, TSST-1, TNFα, IFNγ, and physiological levels of glucocorticoids [*Nature* 1993; 365:756, *JEM* 1994; 179:1895, *unpublished data*].

Using recently obtained molecular sequence data for mouse MIF, we re-examined the role of MIF in the classical footpad model of the DTH response. Mice (n=4 per group) were immunized with Freund's complete adjuvant in one hind footpad and challenged 12 days later in the contralateral footpad with PPD together with either anti-rMIF or control (non-immune) antibody. As expected, RT-PCR analysis showed an increase in total MIF mRNA when inflamed footpads were compared to control, non-inflamed footpads. Furthermore, mice (n=6) treated with anti-MIF antibody showed a significantly reduced inflammatory reaction as assessed by swelling scores and histopathology compared to mice (n=6) treated with control antibody. Of note, *in situ* hybridization studies localized MIF mRNA to cell clusters which stained for macrophage-specific markers (Ram.11) rather than T-cell-specific markers (Thy-1). The important role of macrophage-derived MIF in DTH was confirmed *in vitro* by the observation that PPD itself induced the secretion of appreciable amounts of MIF in RAW 264.7 monocytes and peritoneal exudate macrophages.

These studies indicate that macrophage-derived MIF plays an important role in the classic DTH response and suggest that MIF may have a broader, pro-inflammatory function in promoting macrophage/T-cell interactions.

C2-107 DIMERS OF PEPTIDE/MHC MOLECULES $(\alpha\beta)_2$ ACTIVATE T CELLS IN SOLUTION, J. Jay Boniface*, Jen-Tsan Chi#, Daniel S. Lyons# and Mark M. Davis*#, Department of Microbiology and Immunology* and Howard Hughes Medical Institute#, Stanford University School of Medicine, Stanford, California.

Antigen recognition by T lymphocytes (T cells) is dependent on the binding peptide/MHC molecules to specific T cell receptors (TCR). Crystallographic studies by Brown et al. have shown that class II MHC molecules can exist as dimers of MHC heterodimers $(\alpha\beta)_2$, and the authors speculated that dimerization may be a key event in the activation of T cells through the TCR. To test this hypothesis we prepared peptide/MHC dimers by chemical crosslinking. Carbodiimide chemistry was chosen to form covalent amide bonds from adjacent $-NH_3^+$ and COO^- groups abundantly located at the dimer interface in the crystal structure. Peptide/MHC dimers prepared by this strategy bind a variety of monoclonal antibodies and activate antigen specific T cells when coated on microtiter plates. Normally the affinity of TCR molecules for peptide/MHC complexes is low and characterized by a rapid off-rate. In contrast, BIAcore analysis of the binding of dimeric peptide/MHC molecules to TCR indicates that the off-rate is 10-40 fold slower. These data indicate that both TCR binding sites on the dimer are accessible and functional. Most interestingly, peptide/MHC dimers but not monomers $(\alpha\beta)$ are able to activate T cells from TCR transgenic mice when incubated in solution with cells and without other stimulants. Studies are underway to determine whether the avidity effect alone (due to two binding sites/dimer) is sufficient for T cell activation or if the orientation of these dimers is unique and essential for their functional properties.

C2-108 DIFFERENTIAL EXPRESSION OF ALTERNATE mB7-2 TRANSCRIPTS, Frank Borriello[1], Jennifer Oliveros[1], Andy Chen[1], Gordon J. Freeman[2], Lee M. Nadler[2], Arlene H. Sharpe[1], [1]Departments of Pathology, Brigham and Women's Hospital; [2]Department of Medicine, Dana Farber Cancer Institute; Boston, MA 02115

The murine B7-2 (mB7-2) costimulatory molecule is expressed on antigen presenting cells early during the course of an immune response, suggesting that it may play a pivotal role in the decision between T cell activation and anergy. Murine B7-2 mRNA displays a restricted pattern of expression: It is inducible in B cells, T cells, NK cells, and dendritic cells, but constitutively expressed in unstimulated monocytes. The constitutive and inducible expression of mB7-2 on distinct cell types indicates that mB7-2 is regulated differentially. To further characterize mB7-2 transcripts, we employed 5' RACE and RT-PCR to examine transcripts expressed in a variety of types of APCs and examined their genomic organization. We report here that the mB7-2 locus consists of 12 exons and demonstrate that exons 1 through 5 can be used in alternative fashions to produce 5 distinct transcripts, differing in their 5' untranslated and signal regions. The expression of these transcripts differs in distinct types of APCs and is modulated by stimuli that activate B cells. These results demonstrate that mB7-2 transcripts are differentially regulated in a tissue-specific fashion and in response to activation stimuli.

C2-109 **INTRACELLULAR SIGNALING VIA THE L-SELECTIN RECEPTOR**

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During an inflammation circulating leukocytes emigrate from blood into the surrounding tissues, a process including marginalisation, rolling, firm adhesion of leukocytes to endothelial cells and finally extravasation. Rolling as the initial step in the adhesive interaction between activated leukocytes and the endothelium is mediated by selectins. It is unknown, however, whether the L-selectin-receptor functions as an active signaling molecule or as a passive "anchor". In the present study we demonstrate a stimulation of the src-tyrosine kinase p56lck and a tyrosine phosphorylation of the L-selectin receptor after stimulation of CEM-cells via their L-selectin receptor using the L-selectin antibody DREG56 (2µg/ml). Upon tyrosine-phosphorylation of the receptor molecule the GDP/GTP-exchange protein Sos, but not Vav, associates with L-selectin mediating a 4-fold activation of the small G-protein p21ras. Ras triggers a 5-fold activation of its downstream targets MAP-kinase and Rac2, a small G-protein involved in membrane ruffling and H_2O_2 release. Transient transfection of CEM-cells with transdominant inhibitory N-17Ras abolishes the activation of Rac2 after cellular selectin stimulation. The physiological significance of the identified signaling cascade Lck, Sos, Ras, MAP-Kinase and Rac2 after selectin stimulation is demonstrated by an actin filament polymerisation, measured as increased TRITC-Phalloidin binding in FACS-analysis upon cellular L-selectin triggering. The actin filament polymerisation is blocked by transient expression of transdominant inhibitory Ras in CEM cells. In summary, our data demonstrate that the L-selectin-receptor mediates rolling via activation of src-kinases, the Ras-pathway and reorganisation of the cytoskeleton.

Supported by DFG.

C2-110 **MIF IS A GLUCOCORTICOID-INDUCED CYTOKINE WHICH COUNTER-REGULATES STEROID INHIBITION OF MACROPHAGE CYTOKINE PRODUCTION,** Thierry Calandra, Jürgen Bernhagen, Lori Spiegel, Christine Metz, Anthony Cerami and Richard Bucala, The Picower Institute for Medical Research, Manhasset, NY 11030

Glucocorticoids exert profound effects on the immune system. In contrast to other hormones, however, no physiological mediator has been identified that counter-regulates their anti-inflammatory and immunosuppressive effects. Recent investigations of the MIF protein, described originally as a product of activated T lymphocytes, have established it be both a pituitary hormone and a monocyte/macrophage cytokine secreted in response to inflammatory stimuli [*Nature* 1993;365:765; *J Exp Med* 1994;179:1895]. MIF exhibits important pro-inflammatory activities *in vivo* and plays a critical role in septic shock.

Since glucocorticoids are potent inhibitors of macrophage cytokine production, we examined their effect on MIF secretion. In contrast to expectations, steroids induced rather than inhibited MIF secretion by macrophages. MIF secretion was induced by extremely low concentrations of glucocorticoids (10^{-10}M-10^{-14}M of dexamethasone or hydrocortisone) which are well within physiological range. Macrophage MIF secretion was detected within 2 to 3 hr of dexamethasone (10^{-10}M) stimulation. *In vivo*, serum MIF peaked 3 to 6 hr after the intravenous injection of dexamethasone (1-10 mg/kg) into rats. Recombinant human MIF (0.01 to 100 ng/ml) was found to override in a dose-dependent fashion dexamethasone (10^{-9}M) inhibition of TNFα, IL-1β, IL-6 and IL-8 secretion by LPS-stimulated human mononuclear cells. Furthermore, small amount of exogenously administered rMIF completely overcame the protective effect of dexamethasone against lethal endotoxemia in mice.

Glucocorticoids were found to induce the secretion of both central (pituitary) and peripheral (macrophage and T lymphocyte) MIF, which then acts to modulate the immunosuppressive effects of steroids (*see also accompanying abstract*). Taken together these data suggest that MIF is a critical cytokine of the immune and neuroendocrine systems, which acts in concert with glucocorticoids to regulate inflammatory and immune responses.

C2-111 ASSOCIATION BETWEEN CD22 AND PROTEIN TYROSINE PHOSPHATASE 1C. Mary-Ann Campbell and Norman Klinman, Dept of Immunology, The Scripps Research Institute, 10666, N. Torrey Pines Rd, La Jolla CA 92037.

The induction of novel protein tyrosine phosphorylation is one of the earliest biochemical events stimulated by crosslinking of the B cell antigen receptor, membrane immunoglobulin (mIg), and is essential for subsequent B cell activation. CD22 is a B lymphocyte specific cell surface glycoprotein that is a major substrate for protein tyrosine kinases following stimulation through the antigen receptor. Previous studies have suggested that CD22 might be a component of a membrane Ig-coupled signal transduction pathway. To determine if tyrosine phosphorylation of CD22 promoted its interaction with downstream elements of a signal transduction pathway in B cells, we looked for molecules associating with CD22 following anti-Ig stimulation of B cells. A 60kD molecule was found to associate with CD22 following B cell stimulation with either anti-Ig, anti-µ or anti-δ antibodies. The interaction between CD22 and p60 is dependent on tyrosine phosphorylation of CD22 as treatment of the p60-CD22 complex with potato acid phosphatase to remove phosphotyrosine from CD22 was found to disrupt the interaction between CD22 and p60. However, p60 itself does not appear to be significantly phosphorylated on tyrosine within the complex. The association between CD22 and p60 is stable even in the presence of denaturing detergent such as SDS. This is in contrast to the previously reported association between CD22 and both membrane Ig and a tyrosine kinase. Our data suggested that the interaction between p60 and CD22 might be mediated via the binding of one or more src-homology 2 (SH-2) domains in p60 to phosphotyrosine in CD22. Western blotting of anti-CD22 immunoprecipitates with antisera directed against the 65kDa, SH-2 domain-containing protein tyrosine phosphatase, PTP1C, revealed that p60 is PTP1C. We are presently attempting to determine the functional significance of the PTP1C-CD22 interaction.

C2-112 THE ROLE OF CD72 IN B CELL MATURATION: B CELL RECEPTOR (BCR)-INDUCED APOPTOSIS IN IMMATURE B CELLS IS BLOCKED BY CD72 SIGNALING. Ju-Fay Chang and Jane R. Parnes. Dept. of Medicine, Div. of Rheumat. and Immunol., Stanford University School of Medicine, Stanford, CA 94305.

WEHI-231 is an IgM expressing B cell lymphoma line that can be induced to undergo apoptosis upon anti-IgM stimulation and has been accepted as a model system to study the mechanisms of tolerance in immature B cells. Recently, WEHI-231 has also been used to demonstrated that a signal mediated by the CD40:CD40L interaction can rescue WEHI-231 cells from BCR-mediated apoptosis. We have examined the role of the B cell surface molecule, CD72 (Lyb-2) in BCR-mediated apoptosis using the WEHI-231 model system. CD72 (Lyb-2) is a 45-KD glycoprotein expressed on pre-B, immature, and mature B cells but not on terminally differentiated plasma cells. CD72 has also been shown to interact with the pan T cell protein CD5, and anti-CD72 mAb can induce B cell proliferation. Our studies show that crosslinking of both CD72 and IgM by specific mAbs rescued WEHI-231 cells from anti-IgM-induced cell death as measured by ^{3}H-thymidine incorporation. Addition of anti-CD72 to anti-IgM treated cultures markedly reduced anti-IgM induced DNA fragmentation by WEHI-231 cells, indicating that signals from CD72 are able to block BCR-induced apoptosis in these cells. Since CD5 is a ligand for CD72, we examined the ability of CD5:CD72 interaction to induce "anti-apoptotic" signals in WEHI-231 cells upon anti-IgM stimulation. Our results show that addition of soluble CD5 purified from EL4 cells to anti-IgM treated cultures also rescued WEHI-231 cells from anti-IgM-induced cell death. These findings suggest that CD72 can send a signal for continued maturation/activation of immature B cells.

C2-113 SELECTIVE INHIBITION OF B CELL ANTIGEN RECEPTOR COMPLEX MEDIATED SIGNAL TRANSDUCTION. Marcus R. Clark. Departments of Medicine and Pathology. University of Chicago, Chicago IL 60637.

The B cell antigen receptor complex (BCR) contains two transmembrane proteins, Ig-α and Ig-β which are invested with most, if not all, of the signalling capacity of the intact receptor complex. When the cytoplasmic domains of each of these proteins is expressed independently in B cell lines Ig-α, but not Ig-β, efficiently induces tyrosine kinase activation and calcium mobilization. This function of Ig-α may be dependent upon its ability to interact with, via unique amino acids within its cytoplasmic domain, members of the Src-family of tyrosine kinases including Fyn and Lyn. We postulated that disruption of this interaction would attenuate BCR mediated tyrosine kinase activation. Therefore we established clones of the B cell lymphoma M12 expressing either the tyrosine kinase binding domain of Ig-α (Ig-αb) alone, Ig-αb targeted to the plasma membrane with the Src myristylation sequence (Ig-αbm) or a similar construct containing, instead of Ig-αb, the corresponding sequences of Ig-β (Ig-βbm). We then assayed the ability of the native BCR to activate proximal signal transduction pathways. In cells expressing Ig-αbm, inductive tyrosine phosphorylation of cellular substrates was inhibited by up to 90%. Analysis of multiple clones revealed that the degree of inhibition was proportional to the amount of inhibitory peptide expressed. Less inhibition was seen in those clones expressing Ig-αb and essentially no inhibition was seen in clones expressing Ig-βbm or in those expressing the Src myristylation sequence alone. In contrast both Ig-αbm and Ig-βbm inhibited BCR mediated calcium mobilization. These results suggest that inhibitory peptides can be used to uncouple the antigen receptors from pathways of cellular activation.

C2-114 SORTING SIGNALS IN THE MHC CLASS II INVARIANT CHAIN CYTOPLASMIC TAIL AND TRANSMEMBRANE REGION DETERMINE TRAFFICKING TO AN ENDOCYTIC PROCESSING COMPARTMENT, James F. Collawn, C. Greg Odorizzi, Ian S. Trowbridge, Luzheng Xue, Colin R. Hopkins, Cynthia D. Davis, Department of Cell Biology, University of Alabama at Birmingham, Birmingham, AL 35294

Targeting of MHC class II molecules to the endocytic compartment where they encounter processed antigen is determined by the invariant chain (Ii). By analysis of Ii-transferrin receptor (TR) chimera trafficking, we have identified sorting signals in the Ii cytoplasmic tail and transmembrane region that mediate this process. Two non-tyrosine-based sorting signals in the Ii cytoplasmic tail were identified that mediate localization to plasma membrane clathrin-coated pits and promote rapid endocytosis. Leu^{7} and Ile^{8} were required for the activity of the signal most distal to the cell membrane whereas Pro^{15,} Met^{16,} Leu^{17} were important for the membrane-proximal signal. The same or overlapping non-tyrosine-based sorting signals are essential for delivery of Ii-TR chimeras, either by an intracellular route or via the plasma membrane, to an endocytic compartment where they are rapidly degraded. The Ii transmembrane region is also required for efficient delivery to this endocytic processing compartment and contains a signal distinct from the Ii cytoplasmic tail. More than 80% of the Ii-TR chimera containing the Ii cytoplasmic tail and transmembrane region is delivered directly to the endocytic pathway by an intracellular route, implying that the Ii sorting signals are efficiently recognized by sorting machinery located in the *trans*-Golgi.

C2-115 **DISTINCT T CELL RECEPTOR SIGNALLING PATHWAYS CONTROL LYMPHOKINE INDUCTION AND PROGRAMMED CELL DEATH IN MATURE T LYMPHOCYTES.** Behazine Combadiere, Mathew Freedman, Michael J. Lenardo, Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, Bethesda, MD, 20892.

Mature T lymphocytes that are progressing through the cell cycle are high susceptible to T cell receptor (TCR) induced programmed cell-death (PCD). Previous work has established the importance of phosphotyrosine containing motifs in the CD3-zeta and CD3-epsilon chains in activation and lymphokine induction processes. We have evaluated the roles of these activation motifs in the CD3-ζ and CD3-ε chains in PCD. We have used a large number of different truncations and point mutations in the CD3-ζ and CD3-ε chains as chimeric molecule containing the extracellular and transmembrane domain of TAC antigen. We find that specific alterations of these chains have distinct effects on lymphokine induction and PCD. We have also found that specific mutants have effects on the induction of Fas and Fas-ligand after ligation of chimeric receptors.Taken together, our results suggest that distinct signaling pathways govern activation process as opposed to the induction of programmed cell death in mature T lymphocytes.

C2-116 REGULATION OF EXPRESSION OF THE IL-2 RECEPTOR α CHAIN (CD25) IN HUMAN B LYMPHOCYTES

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CD25 is the 55kDa inducible component of the IL-2 receptor. IL-4 appears to be the sole cytokine which positively modulates CD25 expression in human B cells, but not in T cells where a distinct group of cytokines influences CD25 induction. Stimulation of B cells with anti-Ig and anti-CD40 antibodies also promotes CD25 induction, albeit to differing extentS. Induction of maximal CD25 expression, like that of CD23, requires stimulation of the IP_3 / Ca^{2+} and cAMP generating pathways, although elevation of intracellular cAMP alone leads to enhanced cell surface CD25 levels. Chronic phorbol ester treatment abolishes the ability of IL-4 to induce CD25 expression. IL-4 also influences the activities of number of transcription factors which interact with the CD25 promoter. Thus, while the cytokine has no striking effects upon Sp1 levels, IL-4 enhances the activity of a binding factor which recognises a motif in the CD25 promoter which has homology to known IL-4 response elements located upstream of the Cε and CD23 genes. Conversely, treatment of human tonsillar B cells or B cell lines with IL-4 appears to attenuate AP2 transcription factor activity.

C2-117 MOLECULAR BASIS of "NEGATIVE" SIGNALING by FcγRIIB1: INVOLVEMENT of the PHOSPHOTYROSINE PHOSPHATASE PTP-1C. Daniele D'Ambrosio,* Keli H. Hippen,* Stacey Minskoff,# Ira Mellmann,# Kathy A. Siminovitch@ and John C. Cambier*.

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Co-ligation of FcγRIIB1 with the B cell antigen receptor (BCR) leads to abortive BCR signaling. A 13 amino acid motif contained in the cytoplasmic tail of FcγRIIB1 has been shown to mediate this function. Further BCR-FcγRIIB1 co-ligation dependent phosphorylation of a tyrosine in this "inhibitor" motif is necessary for FcγRIIB1 function. Using a synthetic tyrosine phosphorylated "inhibitor" motif peptide with the sequence EAENTIT(p)YSLLKH to probe B cell lysates, we identified the phosphotyrosine phosphatase PTP-1C as one of three phosphopeptide associating proteins. Co-ligation of BCR with FcγRIIB1 resulted in detectable FcγRIIB1 tyrosine phosphorylation dependent association of PTP-1C with the FcγRIIB1. Mutational analysis of FcγRIIB1 identified the tyrosine of the "inhibitor" motif as necessary for negative signaling as well as PTP-1C association. PTP-1C was found to associate with the tyrosine phosphorylated FcγRIIB1 via a direct SH2-phosphotyrosine interaction. Finally FcγRIIB1 function was investigated in the Motheaten (PTP-1C deficient) mouse. In this mouse BCR and FcγRIIB1 mediated functions are severely impaired suggesting that PTP-1C may act at multiple levels to regulate B cell activation. These results identify PTP-1C as an important effector of BCR-FcγRIIB1 negative signal cooperativity. Recruitment of PTP-1C in proximity of the activated BCR complex by association with tyrosine phosphorylated FcγRIIB1 may allow dephosphorylation of critical signaling molecules leading to inhibition of B cell activation.

C2-118 SLAM: A NOVEL CO-STIMULATORY MOLECULE FOR HUMAN T CELLS, Jan E. de Vries, Gregorio Aversa, Chia-Chun J. Chang, José M. Carballido, Hans Yssel and Benjamin G. Cocks, Human Immunology Department, DNAX Research Institute of Molecular and Cellular Biology, 901 California Avenue, Palo Alto, CA 94304-1104

Optimal T-cell activation and T-cell expansion requires T-cell receptor (TcR) triggering and co-stimulatory signals provided by accessory cells. We cloned a gene belonging to the Ig-gene superfamily encoding a glycosylated 70 Kd protein with a core of 40 Kd designated SLAM (signaling lymphocytic activation molecule), which acts as a co-stimulatory molecule for human T-cell activation. SLAM is exclusively expressed on lymphoid cells and can be induced rapidly with mRNA transcripts and surface expression detectable 1-2 hr following activation. Antibodies against SLAM act agonistically to enhance optimal antigen-specific proliferation and cytokine production by $CD4^+$ T cells. Particularly, the production of IFN-γ was strongly upregulated, even in T-helper type 2 (Th2) $CD4^+$ T-cell clones. Thus, SLAM is a novel T-cell co-stimulatory molecule, which, when engaged, induces a Th0/Th1 cytokine production profile.

C2-119 T CELL REGULATION BY CELL SURFACE MONO-ADP RIBOSYLATION, Gunther Dennert, Jin Wang, Eiji Nemoto, Alexander Kots and Harvey Kaslow, Department of Microbiology, University of Southern California School of Medicine, Los Angeles CA 90033

Incubation of cytotoxic T cells with NAD causes almost complete suppression of ability to proliferate in response to antigen as well as significant inhibition of target lysis. Effects are seen after 3h of incubation with concentrations as low as 1μM NAD and are sustained for hours after NAD removal. Suppression was found to be due to inhibition of cell activation and weak conjugate formation between CTL and targets. NAD metabolites, nicotinamide, ADP-ribose and cyclic ADP-ribose fail to mimic the NAD effect, suggesting that NAD-glycohydrolase or ADP-ribose cyclase are not involved. Incubation of intact CTL with [^{32}P]NAD leads to incorporation of label, demonstrated to be mono-ADP-ribose attached to arginines. The enzyme responsible is demonstrated to be a cell surface ADP-ribosyltransferase, anchored in the outer leaflet of the cell membrane by a glycosyl-phosphatidylinositol (GPI) residue. Treatment of CTL with bacterial GPI specific phospholipase C (PIPLC) causes quantitative release of the enzyme and failure of [^{32}P]NAD to ADP-ribosylate cell surface proteins. Support for the notion that GPI-anchored ADP-ribosyltransferase is responsible for the inhibitory action of NAD is provided by the finding that CTL treated with PIPLC, lacking ADP-ribosyltransferase are insensitive to the suppressive effects of NAD. Analysis of the precise effects of ecto-NAD on antigen stimulated transmembrane signalling revealed that anti-CD3 mediated signal transduction assayed by Ca^{2+} mobilization or generation of inositolphosphates is not affected. However signalling through CD8 is completely inhibited. It is shown that the expression of CD8 is not decreased by NAD nor is CD8 ADP-ribosylated. Experiments will be presented showing that a CD8 associated regulatory protein is responsible for the NAD mediated effect and is ADP-ribosylated by the transferase. It is concluded that CTL can be regulated by a novel, so far undescribed mechanism that utilizes extracellular NAD to posttranslationally modify proteins that play a pivotal role in CD8 mediated transmembrane signalling. (see also Wang, J. et al. J. Immunol. 153: Nov. 1994)

C2-120 A 69kd PROTEIN ASSOCIATED WITH LIPOPOLYSACCHARIDE-INDUCED SIGNAL TRANSDUCTION,
Bruce H. Devens, Satoshi Fukuse, Toshiro Maeda, and David R. Webb, Institute of Immunology, Syntex Discovery Research, Palo Alto, CA 94303.

Lipopolysaccharide (LPS) triggers various cellular responses in mammalian cells. Several proteins have been reported to possess LPS binding capacity but the signal transduction molecule(s) through which the LPS-induced signal is transmitted have not been identified. A rabbit antiserum (5299) diluted 1:10,000, as well as purified IgG from this serum, was found to activate the human monocytic cell line THP-1 to produce tumor necrosis factor (TNF), while control sera or IgG failed to induce TNF production. Endotoxin contamination was demonstrated to be not responsible for this effect. THP-1 cells desensitized by incubation for 24h with LPS respond normally to induction with phorbol ester but not LPS or antiserum 5299 suggesting a common signalling pathway. Western blotting with antiserum 5299 demonstrated a 69kd membrane protein. This protein rapidly appeared in a Triton-X 100 insoluble fraction after stimulation with LPS or 5299 IgG fraction, suggesting association of the 69 kd protein with the cytoskeleton. Two mutant cell lines from γ-irradiated THP-1 lack responsiveness to LPS as well as antiserum 5299. These cell lines lack the 69kd protein in the Triton-X insoluble fraction after stimulation with LPS. It is postulated that p69 functions in the transduction of LPS-induced signals.

C2-121 IDENTIFICATION OF AMINO ACIDS IN THE I-DOMAIN OF CD11a IMPORTANT FOR LFA-1 BINDING TO ICAM-1.
C.P. Edwards, M. Champe, T.N. Gonzalez, L.G. Presta, S.C. Bodary, and P.W. Berman. *Department of Immunology.* Genentech, Inc.

Leukocyte Function Antigen-1 (LFA-1) is a heterodimer composed of an alpha chain (CD11a) and a beta chain (CD18) which has been shown to bind to 3 distinct ligands, Intercellular Adhesion Molecules-1, -2, or -3 (ICAM-1, -2, -3). LFA-1/ICAM-1 interactions are the best understood and it is generally accepted that the I-domain of CD11a is critical for this interaction since numerous anti-LFA-1 antibodies that block LFA-1 binding to ICAM-1 map to the I-domain; moreover, CD11a I-domain-IgG chimeras bind ICAM-1. No one, however, has shown that the I-domain in the context of the entire LFA-1 molecule is critical for this interaction or has defined amino acids within this region important for ICAM-1 binding. In these studies we took two approaches to define I-domain regions involved in ICAM-1 binding. First, we exploited the observation that murine LFA-1 does not bind to human ICAM-1 and generated human/murine I-domain chimeras. Secondly, we examined the potential contribution of key amino acids conserved across species and family members.

Initially, we swapped the murine CD11a I-domain into the human CD11a chain (mu3) and demonstrated that unlike wild-type human CD11a, this chimera when expressed with the human CD18 chain in 293 cells failed to bind human ICAM-1. The epitopes of six anti-human CD11a blocking antibodies have been mapped to the I-domain using human/murine chimeras. Given that the mu3 construct failed to bind to human ICAM-1, we assayed whether these other human/murine I-domain chimeras affected LFA-1 adhesion to ICAM-1. Surprisingly, these chimeras bound full length human ICAM-1 with approximately the same efficiency as wild-type LFA-1, demonstrating that residues that map to the epitope of these blocking antibodies are not critical for the LFA-1/ICAM-1 interactions.

There is approximately 55% homology among the I-domains of the ß2 integrin family members and between murine and human CD11a. Two of these family members, Mac-1 and LFA-1, bind ICAM-1. Human LFA-1 can bind to murine ICAM-1. These data suggest that amino acids conserved across the I-domain are important for binding to ICAM-1. In these studies, we also mutated conserved amino acids in the human CD11a I-domain. Several of these mutations abolished LFA-1 binding to ICAM-1 without significantly affecting the gross conformation of the CD11a as assayed by antibody binding.

C2-122 BOTH IG-α AND IG-β ARE REQUIRED TO INDUCE APOPTOSIS IN WEHI-231 CELLS.
Bartholomew J Eisfelder, Jeannie Tseng, Young Jae Lee, Shara Kabak, and Marcus R Clark. Departments of Medicine and Pathology. University of Chicago, Chicago,IL 60637.

Stimulation of the B-cell antigen receptor complex in immature B-cells induces apoptosis. Although a great deal has been learned about the mediators of this process, little is known concerning which specific component(s) of the antigen receptor complex initiate the apoptotic signal. To address this question we developed a system in which we could examine the ability of Ig-α and Ig-β, either expressed individually or together, to induce apoptosis. Receptor chimeras were made by fusing the extracellular and transmembrane domains of the platelet derived growth factor receptor (PDGFR) alpha or beta chains to the cytoplasmic tail of the Ig-α or Ig-β chain (PDGFRβ/Ig-α, PDGFRβ/Ig-β, PDGFRα/Ig-β). We stably expressed these constructs individually and in combination in the immature B-cell line WEHI-231. To stimulate the chimeras we first added PDGFBB ligand which formed homodimers in single-chain transfected cells and homo- and heterodimers in the double-chain transfected cells. We then added antibodies specific for each receptor to achieve higher orders of aggregation. While cross-linking of Ig-α or Ig-β homodimers induced tyrosine phosphorylation of multiple cellular substrates, neither chain individually induced apoptosis. In contrast, aggregation of chimeric receptor heterodimers, which contain both Ig-α and Ig-β, induce an apoptotic signal indistinguishable from that induced via the endogenous antigen receptor. Thus, we have shown that both Ig-α and Ig-β are necessary and sufficient to induce apoptosis in WEHI-231 cells. It is not clear from this data if Ig-α and Ig-β generate independent signals or if the two chains combine to activate a single apoptotic pathway. However, it is clear that Ig-α and Ig-β contribute non-redundant signalling capacities to the B-cell antigen receptor complex.

C2-123 EFFECT OF ANTI-CD45 mAb ON LYMPHOCYTE FUNCTION,
Chester Elias*, J. Spellberg*, E.C.Butcher# and D.P.Andrew*. Amgen Inc, Thousand Oaks, California* and Dept of Pathology, Stanford University#.

To identify cell signalling/adhesion molecules on T cells we raised mAbs to a CD8 T cell lymphoma, TK1 and searched for mAbs which induced homotypic aggregation of this cell line. Further characterization of one of these aggregation inducing mAbs showed it to recognize CD45. TK1 cell aggregation by anti-CD45 mAb was prevented by low temperature and cytochalasin B but not by inhibition of protein kinase C, G-proteins, adenylate cyclase, tyrosine kinases and tyrosine phosphatases. However, a study of the expression of CD45 by cell lines and their ability to aggregate in response to anti-CD45 mAb showed that there was no correlation between CD45 expression by these cell lines and their aggregation on addition of anti-CD45 mAb. Therefore, the homotypic aggregation observed is not due to cross-linking of CD45 on different cells by mAb. Anti-CD45 mAb induced TK1 aggregation apparently involves a novel adhesion route as blocking anti-LFA-1, anti-alpha4 and anti-CD44 mAbs did not inhibit aggregation of TK1 cells by anti-CD45 mAb.

We also examined the ability of anti-CD45 to modulate the immune response in vitro. Signalling via CD45 strongly inhibits anti-CD3 induced T cell proliferation as well as antigen specific T cell proliferation of T cell clone D10.G4.1 to ovalbumin. Anti-CD45 mAb shows inhibitory effects on B cells, reducing LPS induced B cell proliferation. We also observed that at low doses of anti-CD45 there was an augmentation of allospecific T cell proliferation in a Mixed Lymphocyte Reaction.

C2-124 DIFFERENTIAL KINASE ACTIVATION IN SUBSETS OF CD4 T CELLS, Donna L. Farber, Kim Bottomly, and Oreste Acuto, Section of Immunobiology and the Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, CT 06510, and Molecular Immunology, Department of Immunology, Pasteur Institute, 25, rue du Docteur Roux, 75724 Paris Cedex 15, France

Separation of mouse CD4+ T cells on the basis of CD45RB isoform expression results in two major subsets, designated CD45RBlo and CD45RBhi, which functionally represent memory and naive T cells, respectively. These subsets differ in the type of cytokines they produce, their activation requirements through the T cell receptor complex (TCR/CD3), and their sensitivity to negative signalling through CD4. To determine the molecular basis for these functional disparities, we analyzed the tyrosine phosphorylated substrates and specific kinase activities following activation through TCR/CD3 and CD4 in the two subsets. Striking differences were found in the complement of tyrosine phosphorylated proteins produced following TCR/CD3 stimulation in CD45RBlo versus CD45RBhi cells. Fewer tyrosine phosphorylated substrates appeared in activated CD45RBlo cells when compared to CD45RBhi cells or whole CD4 T cells; most noteworthy was the absence of strongly phosphorylated bands between 70-80 kdal in TCR/CD3-stimulated CD45RBlo cells. Because the two kinases ZAP-70 (70 kdal), and syk (72-74 kdal) are known to be phosphorylated and activated following TCR/CD3 stimulation, we examined the activation state of these two kinases in the memory and naive CD4+ subsets. Following TCR/CD3 stimulation, ZAP-70 was heavily phosphorylated in both naive CD45RBhi cells and whole CD4+ T cells, but not in memory (CD45RBlo) cells. By contrast, significant phosphorylation of syk was observed following TCR/CD3 stimulation of CD45RBlo cells but not of CD45RBhi and whole CD4+ cells. Differences in tyrosine phosphorylated substrates were also observed following crosslinking of CD4 in the two subsets, specifically in the extent of phosphorylation of a novel 36 kdal band that appears only after CD4 crosslinking. We also investigated p56lck kinase activity and found no differences in CD4- associated or whole lck kinase activity in CD45RB subsets. These results suggest that the different activation requirements and cytokine profiles of CD45RB subsets may be controlled by the differential activation of kinases associated with the TCR/CD3 complex, namely ZAP-70 and syk. Whether this differential kinase activation directly leads to distinct downstream pathways is currently being investigated.

C2-125 REGULATION OF *IL2 GENE EXPRESSION* IN CD8+ T CELLS, Rosalynde J. Finch and Philip D. Greenberg, Dept of Immunology, University of Washington, Seattle, WA 98195

The focus of our studies is to examine the molecular basis for the failure of the majority of mature CD8+ T cells to produce IL2 as an autocrine growth factor. CD8+ T cells may initially produce IL2 *in vivo*, but eventually lose this ability through an unknown mechanism. Instead this obligate proliferative signal is delivered in a paracrine manner from CD4+ T cells that release IL-2 locally in response to antigen recognition. However, there are conditions under which CD8+ T cells are capable of producing IL2. CD8+ T cell clones derived from TCR transgenic mice (provided by Frank Fitch) are capable of IL2 expression if stimulated by target cells expressing B7, but irreversibly lose the ability to produce IL2 if target cells are encountered that lack the ligand for CD28. The isolation of these T cell clones provides the opportunity to study IL2 regulation in primary CD8+ T cells and to evaluate the molecular basis for the loss of IL2 expression in mature CD8+ T cells. *In vitro* studies using transformed CD4+ T cells have been very informative in defining the regulation of the IL2 gene in IL2 producing cells, but there is little known regarding the regulation of IL2 in the CD8+ T cell subset. To determine if loss of IL2 expression in CD8+ T cells reflects the presence of an inhibitor, *in vivo* footprinting of non-transformed T cell clones is currently in progress to look for the presence of novel transcription factor binding sites in the IL2 promoter that could potentially bind an inhibitor of IL2 transcription in CD8+ T cells. Alternatively, CD8+ T cells may lack one or more of the transcription factors necessary for IL2 expression. Transactivation of the transcription factor binding sites required for IL2 production is being evaluated by stable transfection into non-transformed T cell clones of Lac-Z reporter gene constructs containing the cis-acting IL2 promoter elements. These constructs include the β-actin promoter as a positive control, the 300 bp IL2 promoter, and tandem repeats of individual enhancer elements that bind the following transcription factors: Oct, AP1, NFκB, NFAT, and CD28RC. In a non-transformed CD4+ T cell clone used as a positive control, transactivation of the reporter gene constructs increased 5X to 25X following stimulation through the TCR. We are currently using this system to evaluate transactivation of cis-elements of the IL2 promoter in CD8+ T cell clones capable of IL2 production, and in CD8+ T cell clones that have lost the ability to produce IL2. The CD8+ T cell clones are currently being expanded for analysis, and the results of these studies will be described.

C2-126 THE ROLE OF THE RAF AND MEKK PATHWAYS, AND OF PHOSPHATASES PP1 AND PP2A, IN REGULATION OF THE NF-κB TRANSCRIPTION FACTOR FAMILY, Timothy S. Finco, Jeanette L. Cheshire, and Albert S. Baldwin, Jr., Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC 27514

The NF-κB transcription factor family regulates a number of genes whose products are important in immune responses, including T and B lymphocyte activation. Induction of NF-κB involves release from its cytoplasmic inhibitor IκB, followed by translocation of the active complex into the nucleus where it modulates target gene expression. The identification of signal transducing molecules which participate in the activation of NF-κB has been the focus of recent research. Previously, we demonstrated that expression of activated forms of Ras and Raf resulted in κB-dependent gene expression. We have now extended these studies by showing that MEKK and the downstream kinase MEK also activate gene expression through κB sites and that this activation is inhibited by co-expression of IκBα. Furthermore, we are studying the ability of dominant negative forms of MEK and MAP kinase to block induction of NF-κB by a variety of agents to determine the necessity of this pathway for activation. It is critical to elucidate the exact mechanism(s) by which these signal transducers mediate their effect on NF-κB activity. Our current research is also directed toward answering this important question. In addition, we are studying the role phosphatases play in the control of NF-κB. We have found that a variety of phosphatase inhibitors which target PP1 and PP2A activate NF-κB by a pathway seemingly identical to that utilized by more physiological inducers such as TNFα. Similarities include activation through a redox-sensitive pathway involving the phosphorylation and subsequent degradation of IκBα. Furthermore, it appears that PP2A, but not PP1, is the phosphatase which regulates NF-κB within the cell.

C2-127 HUMAN PP76: A TYROSINE PHOSPHOPROTEIN WHICH ASSOCIATES WITH GRB2 AND OTHER SIGNALLING MOLECULES IN T CELLS, Paul R. Findell, Janet K. Jackman, Qiming Sun, Christoph W. Turck, David G. Motto, Gary A. Koretsky, and Gary A. Peltz. Department of Leukocyte Biology, Syntex Discovery Research, Palo Alto, CA 94304

Grb2 and associated tyrosine phosphoproteins form a multicomponent protein complex in antigen receptor-activated T cells. To molecularly characterize cytoplasmic proteins mediating the membrane-proximal signalling events in human T cells, the cDNA encoding the human 76 kDa tyrosine phosphoprotein (pp76) which associates with Grb2 was cloned and analyzed. This cDNA encodes a novel 533 amino acid protein with a single SH2 domain at its carboxy terminus. Although no tyrosine, serine/threonine, or lipid kinase domains were present in the predicted amino acid sequence, it contains several potential motifs recognized by SH2 and SH3 domains. RNA blot analysis demonstrated that pp76 mRNA is expressed solely in peripheral blood leukocytes, thymus and spleen; and in human T cell and monocytic cell lines. A GST-fusion protein containing the predicted SH2 domain of pp76 co-precipitated two tyrosine phosphoproteins of 60 and 120 kDa. Co-precipitation studies performed using GST-fusion proteins and specific antisera indicated that other effector molecules, including phosphoinositide 3-Kinase and phospholipase C gamma-1 interact with pp76. In addition, another GRB2 -associated tyrosine phosphoprotein, pp36, was also shown to interact with these two enzymes and pp76.

C2-128 ENDOCYTOSIS OF T-BAM (CD40-L) DETACHES T-BAM FROM CD40 TO REGULATE THE DURATION OF HELPER INTERACTIONS. Sarah M. Fortune, Christopher J. Gamper, Aileen M. Cleary, Michael J. Yellin and Seth Lederman. Columbia University, New York, NY 10032

T-BAM is an essential surface-effector molecule for T cell helper function that is expressed after TcR stimulation, mediates B cell signalling by direct cell-cell contact and is rapidly down-regulated by endocytosis after interaction with cells expressing its high affinity B cell surface receptor CD40. To study the functional significance of T-BAM endocytosis we generated stable rT-BAM$^+$ transfectants of both kidney 293 cells or the T cell Jurkat B2.7 and analyzed down-modulation of rT-BAM induced by CD40$^+$ cells by two-color FACS. In contrast to activated T cells or T-BAM$^+$ Jurkat D1.1 cells, both the 293/rT-BAM and B2.7/rT-BAM transfectants are relatively inefficient at down-regulating rT-BAM by cytochalasin-B inhibitable endocytosis. However, co-culture of the rT-BAM$^+$ transfectants with CD40$^+$ cells, followed by disrupting cell pairs, results in modest down-modulation that is cytochalasin-B insensitive and is associated with the appearance of T-BAM on the surface of the B cells. The appearance of T-BAM on the B cell surface is not inhibited by cycloheximide and appears to be the result of transfer of rT-BAM from the transfectants to the CD40$^+$ cells. In contrast, T-BAM$^+$ T cells that efficiently endocytose T-BAM (e.g. activated normal CD4$^+$ T cells or Jurkat D1.1) do not transfer T-BAM measurably to CD40$^+$ cells. Similar to the T-BAM:CD40 interaction that results in endocytosis, the rT-BAM:CD40 interaction that results in transfer requires cell-cell contact and is inhibited by pre-treatment with either anti-T-BAM or anti-CD40 mAbs. Further, SDS-PAGE revealed that transferred T-BAM migrates equivalently to normal T-BAM and thus is unlikely to result from proteolytic cleavage in the extracellular domain. Together these observations suggest that on cells that are inefficient at endocytosing T-BAM, patching of T-BAM:CD40 pairs may occur that results in transfer (of patches) when cell pairs are disrupted. The fact that cells which efficiently endocytose T-BAM do not maintain these T-BAM:CD40 structures suggests that the endocytosis of T-BAM normally plays an important role in detaching T-BAM from CD40 molecules. Therefore, endocytosis appears to be a biological solution to the problem of disengaging the high-affinity, multivalent T-BAM:CD40 pairing by an irreversible "unzippering" process that also renders T helper cells incapable of performing subsequent promiscuous B cell activations.

C2-129 LIGATION OF SURFACE IMMUNOGLOBULIN D INDUCES APOPTOSIS IN MATURE MURINE B CELLS, Elizabeth Furrie, Gilles Jolly and Lee Leserman, Centre d'Immunologie INSERM-CNRS de Marseille-Luminy, 13288 Marseille CEDEX 9, France

IgM and IgD, the two surface immunoglobulin receptors on mature B cells, have the same antigen specificity. Surface IgM is expressed on developing B cells and has been strongly implicated in selection of the B cell repertoire. In contrast, surface IgD it is only expressed on mature B cells and is down regulated on B cell activation by mitogenic stimulators or anti-IgM antibodies. While the expression of sIgM and IgD appears to be differentially affected by contact with antigen, a distinct role for one or the other molecule in signaling or antigen presentation by mature B cells has not been described. We have shown that crosslinking of IgD by a polyclonal antibody can induce apoptosis of murine splenic B cells in a manner which is independent of the immunoglobulin Fc receptor. On FACS analysis of these cells there was a massive down regulation of surface IgD after 18 hours in culture with IgD-specific antibodies (70% reduction of expression). Treatment with anti-IgM antibodies induced 40% reduction in the expression of sIgM. On examination of the cells 8 hours after removal of the anti-IgD or anti-IgM antibodies we observed full recovery of IgM but only 40% recovery of sIgD. However, a small population of B cells expressing high levels of IgD were shown to be activated by thymidine incorporation, rather than undergoing apoptosis or down-modulating their surface IgD after treatment with the polyclonal antibody for 18 hours. We postulate that sIgD-mediated signals in mature splenic B cells may play a role in regulation of activation in the mature peripheral B cell population, notably in the case when antigen is contacted in the absence of T cell help.

C2-130 **Ca ENTRY VIA Na/Ca EXCHANGE FOLLOWING INTRACELLULAR STORE DEPLETION IN T LYMPHOCYTES, Jeffrey P. Gardner, M. Balasubramanyam, Christine Rohowsky-Kochan and John P. Reeves, Departments of Pediatrics, Neurosciences, Physiology and the Hypertension Research Center, UMD-New Jersey Medical School, Newark, NJ 07103**

Lymphocyte activation is accompanied by Ca mobilization from intracellular stores and Ca entry from the extracellular space. To examine Na/Ca exchange activity and the influence of store depletion on cytosolic calcium (Ca_i) regulation, ^{45}Ca entry and Ca_i were studied in human peripheral T lymphocytes in which cytosolic Na (Na_i) was increased by ouabain pretreatment and/or the Na gradient was decreased by reduction of extracellular Na concentration. SBFI-loaded cells treated with 0.1 mM ouabain for 30 minutes (37°C) increased Na_i from 21 mM to 43 mM. Ouabain-treated cells suspended in low-Na medium showed a 30-65% increase in ^{45}Ca uptake compared to cells in 140 mM Na-medium. Enhanced ^{45}Ca influx was entirely dependent on ouabain-pretreatment and reversal of the Na gradient. The effect of the Na gradient on Ca_i was studied in cells (+/- ouabain treatment) incubated with fura-2 AM in Ca-containing or Ca-free medium for 30 minutes and suspended in Na- or Na-free medium. Ouabain pretreatment and exposure to low Na-medium resulted in elevated Ca_i; this response was increased in cells loaded in Ca-free medium. As preincubation of cells in Ca-free medium enhanced the ouabain-dependent increase in Ca_i, effects of the microsomal Ca-ATPase inhibitor thapsigargin (TG) on Ca influx were studied. TG stimulated Ca entry following ouabain pretreatment and reversal of the Na gradient. Increases in Ca_i due to Na/Ca exchange mechanisms were retained in the presence of $LaCl_3$ inhibition of store dependent calcium influx pathways. These results show the Na/Ca exchanger is present in T lymphocytes and suggest this transporter modulates Ca influx following store depletion.

C2-131 OX40 RECEPTOR-LIGAND INTERACTION, Wayne Godfrey, Marwan Harara, David Buck and Edgar Engleman, Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305

The human 106/OX40 antigen (106Ag) is a tightly regulated T cell surface activation marker (CD4 > CD8) of the nerve growth factor receptor/tumor necrosis factor receptor (NGFR/TNFR) family, which is expressed during normal immune responses and in acute and chronic autoimmune diseases. We have recently demonstrated that crosslinking of the 106Ag on CD4+ T cells strongly costimulates phorbol ester and anti-CD3 induced proliferation. The magnitude of this effect is similar to that induced by cocrosslinking of anti-CD3 and anti-CD28, and anti-CD3 and anti-LFA-1. Using a soluble form of the receptor, the extracellular region fused with human immunoglobulin Fc (106-Ig), we have begun to characterize the expression pattern of the human OX40 ligand. No binding of the 106-Ig was detected on resting peripheral blood cells, and only trace staining was noted on activated B cells and T cells. Screening of 40 tumor lines revealed trace staining of a Burkitt lymphoma, Jiyoye, and an EBV transformed B lymphoblastoid cell line (LCL), MSAB. After activation with PMA/ionomycin for three days the MSAB cell line became markedly positive. We expression cloned the human OX40 ligand cDNA from a library derived from activated MSAB cells. The encoded protein was identified as gp34, a type II transmembrane antigen previously known to be expressed only by human T cell lymphotropic virus (HTLV-I) infected cells. We describe gp34 as a new member of the TNF family, and find that the recombinant ligand expressed in COS cells costimulates phorbol ester (PMA), phytohemagglutinin (PHA), and anti-CD3 induced CD4+ T cell proliferation. We have subsequently found the ligand to be expressed on in vitro activated B cells and on activated T cell lines. We speculate that the OX40 receptor-ligand interaction may be involved in T cell-B cell interactions as well as autocrine T cell stimulation.

C2-132 CD45 ISOFORM SPECIFIC REGULATION OF T CELL RECEPTOR SIGNALLING, Catriona F. Grant, Tom J. Novak and Kim Bottomly, Section of Immunobiology, Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, CT 06510.

The transmembrane tyrosine phosphatase CD45 is required for signalling through the T cell receptor. Expression of CD45 isoforms varies on populations of T cells. To determine how expression of particular CD45 isoforms may affect T cell receptor signalling pathways, T cell transfectants have been made expressing different isoforms of CD45. Additionally these cell lines have been transfected with cDNA encoding the well characterized conalbumin specific T cell receptor D10. Tyrosine phosphorylation of substrates after stimulation with antibodies directed against CD3, and alpha/beta chains of the T cell receptor and CD4 have been analyzed. Isoform specific outcomes of early biochemical signalling events such as tyrosine phosphorylation and specific kinase activation are being correlated with cytokine production. We have previously shown CD45 isoform specific regulation of cytokine production is apparent following T cell receptor stimulation. In order to test the hypothesis that T cell receptor ligands of lower affinity are more dependent on CD45, a panel of clonotypic antibodies having varying affinities to the T cell receptor will be used.

C2-133 LFA-1/ICAM-3 MEDIATED EVENTS CAN DECREASE THE PROLIFERATIVE RESPONSE OF ACTIVATED T CELLS. Jonathan M. Green and Craig B. Thompson, Department of Medicine and The Gwen Knapp Center for Lupus and Immunology Research, The University of Chicago, Chicago, IL 60637

T lymphocytes can be activated and induced to proliferate in the absence of accessory cells by a combination of phorbol ester and CD28 ligation. This treatment also leads to homotypic adhesion mediated by LFA-1/ICAM interactions. We have found that prevention of the homotypic interactions between T cells activated by PMA + α-CD28 results in a 2-5 fold increase in the proliferative response. This occurred whether the homotypic interactions were prevented by anti-CD11a or anti-CD18 antibodies, by the use of plate immobilized antibodies against other cell surface molecules, or by culture in flat bottom wells, and was dependent upon signaling through CD28. The increased proliferation was most likely due to interference with a negative signal delivered to the T cell as a result of ICAM-3 mediated events. When an agonistic anti-ICAM-3 antibody was added to T cell cultures activated with PMA + α-CD28 in the presence of anti-LFA-1 antibodies, proliferation was decreased back to baseline levels and homotypic aggregation was re-induced. If this reaggregation was prevented, addition of anti-ICAM-3 had no effect, suggesting that the decrease in proliferation was not mediated directly through ICAM-3, but via an as yet undefined receptor/ligand pair. The secondary aggregation induced by ICAM-3 ligation was not inhibited by blocking antibodies against CD11a, CD18, ICAM-1, β-1 integrin, CD45, class I MHC, class II MHC, CD2, LFA-3 or VLA-4. These data demonstrate LFA-1/ICAM-3 interactions between T cells regulate an LFA-1 independent pathway that results in homotypic adhesion and a downregulation of the proliferative response of activated T cells.

C2-134 **Activation of the Ras-signaling pathway by the CD40-Receptor via tyrosine phosphorylation and diglyceride release,** E.Gulbins*, B.Brenner", C.Langlet#, O.Linderkamp", K.M.Coggeshall+, F.Lang*, *I.Institute of Physiology, University of Tuebingen, 72076 Tuebingen, Germany, "Dep. of Pediatrics, University of Heidelberg, 69210 Heidelberg, Germany, +Department of Microbiology, Ohio State University, Columbus, Oh 43210, USA, # CNRS/INSERM, Centre d'Immunologie de Marseille-Luminy, 13288 Marseille, France

The CD40 receptor has been shown to function as an important molecule regulating B-cell proliferation, maturation, antibody class switching and prevention of apoptosis. In the present study we identified signal transduction events triggered by crosslinking of the CD40 receptor. Stimulation of Daudi cells with anti-CD40 resulted in activation of p21ras, an important switchpoint in the regulation of cell growth and differentiation, and the downstream target of Ras, MAP-kinase (MAP-K). Herbimycin A or calphostin pretreatment partially blocked Ras activation, combination of the two inhibitors abolished Ras activation upon CD40 triggering completely. These results indicate a function of tyrosine kinases and lipids in Ras activation after CD40 stimulation: The kinase activity of p56lyn and p58blk increased 5-8-fold after CD40 crosslinking whereas two other members of the src-kinase family, p59fyn and p56lck, showed no significant change of kinase activity. Additionally we detected a lecithine degradation and an increase of diacylgycerol synthesis after anti-CD40. Stimulation of Ras can be mediated by the guanine nucleotide exchange factors SOS/Grb2 or - in hematopoietic cells - Vav, which is regulated by tyrosine phosphorylation and diglyceride binding. Vav displayed a 5-fold catalytic GDP/GTP-exchange activity on Ras in vitro after anti-CD40 stimulation, which was partially inhibted by Herbimycin or calphostin and completely inhibited by both inhibtors. A functional relation of Vav and Ras in vivo is further indicated by coimmunoprecipitation experiments revealing a CD40 activation dependent association of Vav and Ras almost entirely in the membranous fraction. Increased tyrosine phosphorylation of Vav after anti-CD40 correlated with increased association of Vav and Ras. These results demonstrate the activation of a signaling cascade from src-kinases and phospholipases to Vav, Ras and MAP-K, which might be important in the mediation of biological effects after CD40 receptor engagement. Supported by DFG.

C2-135 MUTATIONAL ANALYSIS AND AN ALTERNATIVELY SPLICED PRODUCTS OF B7 DEFINES ITS CD28/CTLA4 BINDING SITE ON IgC-LIKE DOMAIN, Yong Guo, Yan Wu, Min Zhao, Xian-Peng Kon and Yang Liu. Michael Heidelberger Division of Immunology, Department of Pathology and Kaplan Comprehensive Cancer Center, New York University Medical Center, New York, N.Y. 10016

Costimulatory molecules B7 and B7-2 interact with T cell surface receptors CD28/CTLA4 and deliver a costimulatory signal essential for T cell growth. However, the structure basis of this interaction is not known. B7 and B7-2 are members of immunoglobulin super family and the extracellular portion consists of an IgV-like domain and an IgC-like domain. Here we report that a alternatively spliced form of B7 mRNA maps the CD28/CTLA4 binding site to IgC-like domain encoded by B7 exon 3. Consistent with this notion, a mutation of Cys165 to Gly which destroys the disulfate chain in the IgC-like domain, eliminates B7 binding to CD28Ig and reduces its binding to CTLA4Ig by 10-fold. Mutational analysis reveals that amino acid residues clustered in two regions in the IgC-like domain of B7, located in or near the connecting loops between strands BC, and DE. These two regions are critical for B7 binding to CD28/CTLA4 and also clustered to form a localized binding domain centered at Tyr201, which is conserved among human B7, B7-2 and murine B7, and in murine B7-2 has been conservatively replaced by Phe. In addition, a comparison of the effects of mutations on the binding of CD28 and CTLA4 reveals that CD28 and CTLA4 binds to the same site on the B7 molecule. These results have important implication on the functional role of CTLA4 and CD28 in T cell costimulation. The structure of CD28/CTLA4 binding site also provides valuable information for immune intervention targeted at the B7/B7-2:CD28/CTLA4 interactions.

C2-136 MECHANISMS OF CYTOKINE ACTION IN LEYDIG CELLS. D. Buck Hales, Xiangquan Li. Dept. Physiology and Biophysics, Univ of Illinois at Chicago, Chicago, IL 60612-7342

We have demonstrated that the interleukin-1 (IL-1) and tumor necrosis factor-α (TNF) inhibit Leydig cell testosterone production primarily by inhibiting the expression of cytochrome P450c17. We now examine the mechanisms through which cytokines regulate P450c17 gene expression in Leydig cells. The 5'-flanking region of the *Cyp17* gene (the gene that encodes P450c17) was isolated from a mouse genomic library and cloned into the pGSVOCATt vector. The *Cyp17*-CAT constructs were transiently transfected into MA-10 tumor Leydig cells and co-transfected with SV2β-Gal. Cells were treated with 500μM 8-Br-cAMP plus hIL-1α, mTNFα or phorbol 12-myristate 13-acetate (PMA, 10 nM), and CAT and Gal activities were determined. The greatest cAMP-induced increase in CAT activity was seen in cells transfected with the -1021/+25-CAT construct, 7.7± 2.1 fold compared to untreated control. IL-1 (5 ng/ml) caused a 43.4±11.8% inhibition of cAMP-stimulated activity. Increasing concentrations of IL-1 up to 100 ng/ml caused no further decrease. In contrast, TNF caused a dose-dependent decrease in cAMP-stimulated CAT activity. Treatment with 10 ng/ml TNF resulted in a 83.8±5.1% inhibition of cAMP-stimulated activity. PMA treatment resulted in a 71.5 ±8.6% inhibition of cAMP-stimulated activity. The PKC inhibitor, calphostin C, completely reversed both the TNF- and PMA-mediated inhibition. The translocation of PKCα was analyzed by fractionation of cells into nuclear and cytosolic fractions and quantitated by Western blot. PKCα was distributed 55/46%, 60/39%, and 29/71% cytosol/nucleus in control, cAMP and TNF treated cells, respectively. Cells were examined by indirect immunofluoresence for PKCα translocation. PMA and TNF caused a marked translocation of fluoresence to the nucleus and plasma membrane compared to controls. These data suggest that activation and translocation of PKC are involved in the TNF-mediated inhibition of cAMP-stimulated *Cyp17* gene expression. (Supported by HD 27571)

C2-137 EXPRESSION CLONING OF THE HUMAN CD97 ANTIGEN

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In the Activation Section of the Fifth International Workshop on Leucocyte Differentiation Antigens CD97 has been defined by four distinct mAbs. All mAbs recognize a monomeric glycoprotein of 75-85 kDa. Granulocytes and monocytes constitutively express the CD97 antigen. On resting lymphocytes CD97 is expressed only at low density, but cellular activation rapidly upregulates surface expression of the molecule (Eichler et al. 1994: *Scand. J. Immunol.* 39, 111-115). Enhanced expression of CD97 upon activation was found to be dependent on *de novo* RNA and protein synthesis.

We have isolated a cDNA encoding human CD97 by expression cloning in COS cells and immunomagnetic selection. The size of the complete cDNA is in accordance with a 3-kb transcript that was revealed by RNA blot analysis. The clone contains a single open reading frame of 1617 bp. A signal peptide is followed by a polypeptide chain with a predicted molecular mass of 56 kDa. CD97 has an extended extracellular region with several potential sites for N-glycosylation. Preliminary analysis for sequence homologies indicates the existence of EGF-like domains.

C2-138 PHOSPHATIDYLCHOLINE-SPECIFIC PHOSPHOLIPASES IN LYMPHOCYTE MATURATION AND PROLIFERATION, Margaret M. Harnett, Jonathan Gilbert, Pamela Reid and Sandra Gardner, Division of Biochemistry and Molecular Biology, University of Glasgow, Glasgow G12 8QQ, UK.

B and T cells respond to antigen via clonotypic antigen receptors. The earliest signalling events following B or T cell activation include the activation of protein tyrosine kinases (PTKs) and the phospholipase C (PLC)-mediated hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) to generate the intracellular second messengers, IP_3 and diacylglycerol. Although receptor-mediated hydrolysis of PIP_2 has been implicated in the generation of proliferative responses in many cell types, it is evident that other phospholipid classes, notably phosphatidylcholine (PC) are also hydrolysed by receptor-coupled phospholipases and may give rise to DAG (PLC) and other biologically active lipids, such as phosphatidic acid (PA, PLD) and arachidonic acid (PLA_2), in response to a wide range of growth factors and mitogens. We now present evidence that the antigen receptors on B (sIg) and T (TcR/CD3) cells are differentially coupled to one, or more, of the these PC-signalling pathways in a maturation state-dependent manner. For example, whilst the antigen receptors on mature B cells are not coupled to either PC-PLC or PC-PLD activities, ligation of the TCR induces PC-PLD activation which is maximal at optimally stimulatory concentrations of anti-CD3 antibodies. In addition, the antigen receptors on mature B and T cells are not coupled to PC-specific $cPLA_2$ activition. Interestingly, $cPLA_2$ is not expressed by either splenic or tonsillar B and T cells or indeed by a wide range of B and T cell lines. However, $cPLA_2$ is expressed in thymocytes and lymphocytes obtained from immature spleens, perhaps suggesting a role for $cPLA_2$ in lymphocyte maturation.

C2-139 DIVERGING SIGNALS CONTROL PROLIFERATION AND DIFFERENTIATION OF SELF REACTIVE B LYMPHOCYTES, Jim I. Healy, Martin McMahon*, Micheal P. Cooke, David Mack, Mark M. Davis, Christopher C. Goodnow, *DNAX Research Institute, Palo Alto, CA, Howard Hughes Medical Institute, Stanford University School of Medicine, Stanford, CA.

Antigen receptor signalling exerts negative and positive controls on B lymphocyte survival, multiplication and differentiation, but the signals involved in negative versus positive control have not been defined. Self reactive B lymphocytes that have been rendered anergic by chronic exposure to self antigen have a proximal block in the antigen receptor signalling cascade manifest as a decreased ability to phosphorylate their receptor or flux calcium. Functionally, these cells lack a cyclosporin-sensitive mitogenic response to receptor engagement but retain cyclosporin-resistant signaling which prevents B cell differentiation into plasma cells. Data will be presented showing that the latter signaling correlates with elevated MAPK activity, hyperphosphorylated RSK and increased nuclear EGR-1. Activation of the MAPK/RSK pathway with phorbol esters inhibits BLIMP expression, syndecan expression, CD72 down modulation, J chain transcription and IgM secretion. Selective activation of the MAPK/RSK pathway in self-reactive B cells may negatively regulate B cell differentiation and thus prevent autoantibody production.

C2-140 Structure/function studies of p56*lck* SH2 domain
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Deletion studies have previously shown that the SH2 domain of p56*lck* has a very important role in T cell signalling. The domain binds tyrosine phosphorylated proteins and may act to bring certain components of the signalling complex into close proximity with the kinase domain. Tyrosine phosphorylated peptides that bind the SH2 domain *in vitro* include the C-terminal autoregulatory peptide of p56*lck* and a peptide from the hamster polyoma virus middle T antigen. We will present evidence that the SH2 domain also interacts directly with phospho-peptides derived from the T-cell activation motif of CD3 zeta chain. To further explore the peptide specificity of the SH2 domain a number of mutations have been introduced by site-directed mutagenesis into the pY and pY+3 binding pockets. The effects of these substitutions have been studied by *in vitro* peptide binding assays, BIAcore biosensor studies and GST-fishing from T cell lysates. The results of these experiments will be presented including evidence that substitutions at one site in the pY+3 pocket selectively effects peptide binding of the SH2 domain. This site has been shown to be phosphorylated *in vivo* and therefore may act as a cellular switch in controlling p56*lck* function.

C2-141 **AN ACTIVATION PROTEIN (1A11) POSSIBLY INVOLVED IN T CELL STIMULATION**,
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T cell activation is characterized by proliferation, lymphokine secretion, and upregulation of different membrane proteins such as CD2 or CD25. Some of these activation molecules have also costimulatory functions in T cell activation.

In order to find T cell activation molecules we immunized BALB/c mice with PHA-blasts and obtained a hybridoma which produces a monoclonal antibody against an activation structure on T cells and monocytes called 1A11. This molecule has an apparent molecular weight of 130/40 kD by immunoprecipitation under reducing as well as non-reducing conditions. 1A11 is upregulated by PHA stimulation from 5% to 15% over a stimulation period of 8 days. More importantly, the expression of 1A11 is upregulated on in vivo activated T cells such as synovial T cells from patients with rheumatoid arthritis (25-50%). Double staining revealed that 1A11 is primarily expressed on CD8 positive T cells with only very low expression on CD4 positive cells. Finally, preliminary results indicate that 1A11 might be involved in T cell receptor independent T cell activation in combination with the phorbol ester PdBu. Taken together, we identified an activation protein which is mainly expressed on CD8 positive cells and which, in addition, appears to have costimulatory functions.

C2-142 EXPRESSION OF A SOLUBLE ISOFORM OF MURINE FAS PROTECTS A SUBSET OF INTRAHEPATIC T CELLS FROM APOPTOSIS,
Dennis P. M. Hughes and I. Nicholas Crispe, Section of Immunobiology, Yale University School of Medicine, New Haven, CT 06510

Fas is a cell-surface receptor capable of inducing apoptosis. Its ability to signal is known to be controlled both by the regulation of mRNA expression and by modulation of downstream signaling events. We have identified a form of murine Fas which arises from alternate splicing to a previously undescribed exon within the *fas* gene. The function of this isoform of Fas, which we term Fas B, was analysed in transfection experiments. Overexpression of the cDNA for Fas B protected cell lines from death induced by the Fas Ligand (FasL), while the parental cell lines remained susceptible. Fas B may also act to protect certain cells from Fas-mediated apoptosis *in vivo*. We have recently reported identification of two subsets of TCR αβ+ cells in the liver of normal mice. (Huang *et al.*, *Int. Immunology* 6:533, 1994) One subset consists of activated CD4+ and CD8+ T cells, while the other is made up of cells expressing neither CD4 nor CD8 (DN T cells) which are undergoing apoptosis. While both cell populations expressed a high level of the transmembrane form of Fas, the apoptotic DN T cells expressed very little Fas B, while the other population expressed very high levels of Fas B message. This suggests that alternative splicing of Fas message helps regulate susceptibility to Fas-mediated apoptosis *in vivo*.

C2-143 PARTIAL PURIFICATION AND CHARACTERIZATION OF A DNA-BINDING PROTEIN SPECIFIC FOR AN IL-4 ACTIVATION RESPONSIVE ELEMENT,
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An activation responsive element (ARE, P1, CS1) within the IL-4 promoter is located between -88 to -60 relative to the site of transcriptional initiation and contributes to the activation-dependent transcription of IL-4 in T-cells. This element participates in inducible as well as constitutive DNA-protein interactions and shares significant sequence identity and protein-binding characteristics with an IL-2 regulatory site that is a target for the inducible binding of NF-AT. DNA-affinity chromatography was used to purify ARE binding proteins from crude nuclear extracts of stimulated T-cells. The partially purified protein(s) form a single specific DNA-binding complex of unique mobility when compared to those that form with crude extracts. Subsequences critical for binding of the purified protein(s) are identical to those required for complex formation from crude extracts. UV crosslinking of the partially purified material with the ARE results in the formation of a single DNA-protein complex that migrates with an apparent molecular weight of ~43kDa on SDS-PAGE. Despite the partial sequence identity between the ARE and NF-AT elements, the protein(s) has at least a 10-fold higher affinity for ARE than for the NF-AT binding site. These data suggest that the protein(s) purified in this study is unique to the regulation of IL-4 and may be a site of Th2 specific activation signals.

C2-144 EVIDENCE FOR AN ACTIVE "OFF-SIGNAL" FOR CD8 MEDIATED CTL BINDING TO CLASS I PROTEIN, Paige L. Jensen & Matthew F. Mescher, Dept. of Lab. Med. and Pathology, University of Minnesota, Minneapolis, MN 55455

The CD8 receptor of cytotoxic T lymphocytes (CTL) has been shown to serve both adhesion and cosignalling roles in CTL activation. Previous work in the laboratory has suggested the presence of active on and off signals for CD8-mediated binding. Treatment of CTL with fluid phase anti-TCR mAb activates CD8-dependent binding to non-antigen class I protein, and inhibitors of tyrosine phosphorylation block this "on" signal. Recently, work in the laboratory has suggested that protein kinase C (PKC) may be responsible for delivering an "off-signal" to CD8. In binding assays measuring TCR-activated CD8-mediated adhesion to class I, the PKC activator PMA significantly reduced triggered CD8-mediated binding. In contrast, the PKC inhibitor bisindolylmaleimide,HCl significantly increased binding. Experiments are in progress to examine rates of de-adhesion for the CD8-mediated triggered binding with and without the presence of PKC inhibitors and activators. Additionally, experiments are in progress to examine changes in the phosphorylation of the CD8α chain in response to agents which activate or inactivate CD8-mediated binding. Other groups have shown that PKC can phosphorylate CD8α and this could be involved in the mechanism for regulating avidity of CD8 binding. In conclusion, this work aims to better define what constitutes the necessary signals for deactivation of CD8-mediated binding.

C2-145 IDENTIFICATION OF A NOVEL GROWTH-RELATED 38-KD INTRACELLULAR PHOSPHOPROTEIN IN T CELLS. Malek Kamoun, Jianping Qui and Ha Won Kim. Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia PA 19096.

The CD2 cytoplasmic domain between amino acid residues 258 and 280 appears to be important in T cell signal transduction. This region contains two repeats of the sequence motif PPPGHR, thought to form a structure involved in signal transduction. To study the structural nature of this region, we produced a series of mAbs that were raised against a synthetic peptide (ATSQHPPPPPGHRSQ) derived from the human CD2 molecule. Many of these strongly reacted with phosphorylated intracellular proteins. Immunoprecipitates obtained with one of these mAbs (3E10), using ^{32}P labeled cell lysates of unstimulated human T and B cell lines and thymocytes, revealed a prominent 38-kD phosphoprotein in these cells. Other less prominent phosphorylated protein bands coprecipitated with the 38-kD protein were also observed. A similar finding was observed with a CD2-negative Jurkat mutant, indicating that the expression and phosphorylation of the 38-kD protein is not CD2-dependent. Anti-phosphotyrosine western blots did not reveal the presence of phosphotyrosine residues. A time course analysis of phosphorylation in resting and IL2-stimulated peripheral blood T cells revealed that this phosphorylation is inducible (over a 10-fold increase) by IL-2 and is cell cycle dependent. Similar findings were also observed with the IL-2 dependent murine CTLL-2 cells, suggesting that the phosphorylation of this 38-kD protein is cell cycle regulated. Immunoprecipitation obtained from ^{35}S-methionine labeled cell lysates revealed, in addition to CD2, two intracellular proteins which migrated as two doublets with an apparent mw of a 37- to 38-kD and 27- to 28-kD.

200 pmoles of the 38-kD protein were affinity-purified from a large scale culture of Jurkat cells and were then separated by SDS-PAGE and electrotransferred onto a high retention PVDF membrane. Internal peptides were obtained by in situ digestion and an amino acid sequence obtained from one of these peptides revealed a novel protein sequence.

The CD2 PPPGHR sequence motif or a similar consensus motif could represent a new region of homology present in a number of proteins which may have diverse cellular function. The 38-kD protein and proteins that coprecipitated using mAb 3E10 may represent a novel functional complex which is regulated by a kinase cascade implicated in cell growth and differentiation.

C2-146 LYMPHOTACTIN TRANSGENIC MICE: *IN VIVO* ANALYSIS OF A NEW CLASS OF CHEMOTACTIC CYTOKINE, Gregory S. Kelner, Andrew G. D. Bean, Kevin B. Bacon, Thomas J. Schall, and Albert Zlotnik. DNAX Research Institute of Cellular and Molecular Biology. Palo Alto, CA. 94304.

We have recently reported the isolation and characterization of Lymphotactin, a new class of chemotactic cytokine (Science 1994, in press). This cytokine elicits a chemotactic response from CD8+ T cells, αβTCR+CD4-CD8- T-cells, fetal liver cells, and bone marrow cells. Lymphotactin is produced by a subpopulation of immature thymocytes known as Pro T-cells, CD8+ T-cells, and αβTCR+CD4-CD8- T-cells. We have constructed a Lymphotactin transgenic mouse with expression of the protein directed by the lck promoter. Our goal is to further investigate the *in vivo* role of this cytokine in the immune response.

C2-147 A NEW ASPECT OF IL-2 R SIGNALING PATHWAY IN MURINE T LYMPHOCYTES. Young Ho Kim*, Meredith A. Buchholz and Albert A. Nordin. *Department of Microbiology, College of Natural Sciences, Kyungpook National University, Taegu 702-701, Korea. Clinical Immunology Section, Gerontology Research Center, NIA, NIH, 4940 Eastern Avenue, Baltimore, MD 21224, USA

T lymphocytes play a central role in the immune response by regulating not only the function of other lymphocytes, but also of monocytes and hematopoietic cells. Upon perturbation of TCR by the specific agonist, Resting (G_0) T cells express an array of genes required to reenter and progress through the cell cycle. IL-2 and IL-2R are among the more critical genes that must be expressed to estabilish the growth factor-dependent proliferation of the activated T cells. The addition of rIL-2 to anti-CD3 activated murine T cells resulted in an increased level of tyrosine phophorylation of a single 97 kDa protein. The degree of tyrosine phosphorylation palleled the amount of rIL-2 added and correlated with the extent of DNA synthesis. The treatment of IL-2 resulted in a transient increase in $p56^{lck}$ kinase activity. When G_0 T cells were activated by phorbol dibutyrate (PBu_2) in the absence of IL-2, the high affinity IL-2R expressed failed to induce a proliferative signal by the addition of physiological level of rIL-2, and neither the tyrosisne phosphorylation of the 97 kDa protein nor the transient increase in $p56^{lck}$ kinase activity was detected. Northern blot analysis of the total RNA extracted from these cells showed the accumulatin of IL-2Rα specific mRNA but none of the mRNA specific for c-myc, cyclin D2, cyclin D3, and cdc2 was expressed. The addition of 100 nM rIL-2 to T cells activated by PBu_2 was able to induce a proliferative response, and under these conditions tyrosine phosphorylation of 97 kDa protein, the transient increase in $p56^{lck}$ kinase activity, and specific mRNA for IL-2Rα, c-myc, cyclin D2, cyclin D3, cdk2, and cdc2 were detected.

These results demonstrate that the IL-2-induced upregulation of IL-2Rα is independent of $p56^{lck}$ kinase activity and the tyrosine phosphorylation of 97 kDa protein, both of which are apparently required for the expression of c-myc, cyclin D2, cyclin D3, cdk2, and cdc2, suggesting at least two different signaling pathways in IL-2R in murine T cells.

C2-148 CO-LIGATION OF MOUSE COMPLEMENT RECEPTORS 2 AND 1 (CR2 AND CR1) WITH sIgM RESCUES SPLENIC B CELLS AND WEHI-231 CELLS FROM ANTI-sIgM INDUCED APOPTOSIS, Yuko Kozono, Richard C. Duke, Mary S. Schleicher and V. Michael Holers, Department of Medicine, Divisions of Rheumatology and Medical Oncology and Department of Immunology, University of Colorado Health Science Center, Denver, CO 80262

Recent studies have shown that complement receptors play important roles in both T-dependent and T-independent B lymphocyte responses to low doses of Ags in vivo. Complement is activated by Ag-Ab complexes and microorganisms, resulting in covalent binding of many C3 molecules in forms that ligate CR1 and CR2. We hypothesized that complement-bound Ags might crosslink CR2 and/or CR1 with sIgM, alter the signal transduced, and potentially rescue B cells from apoptosis that could be caused by binding of Ag alone. To test this hypothesis, we have utilized mouse splenic resting B cells (ρ=1.079) and WEHI-231 cells in which apoptosis can be induced by sIgM ligation. We have found that co-crosslinking of mouse CR2 and CR1 with sIgM using anti-CR2/CR1 mAb 7E9 rescues resting B cells and WEHI-231 cells from apoptosis to a comparable level as found using soluble mouse CD40L. Both splenic B cells and WEHI-231 cells require co-crosslinking of CR2/CR1 with sIgM to be rescued from apoptosis. The CR2/CR1 effect is additive with CD40L. These results support the hypothesis that co-clustering of sIgM, CR1, CR2 and likely the CD19 complex substantially alters the nature of the sIgM signal transduced and promotes the B lymphocyte response to non-self Ags.

C2-149 SUBUNITS OF THE INTERLEUKIN 15 RECEPTOR, Satoru Kumaki, Judith G.Giri, Minoo Ahdieh, Kenneth Grabstein, Kurt Shanebeck, Hans D.Ochs*, David Cosman and Dirk Anderson, Immunex Research and Development Corp., Seattle, WA 98101 and *Department of Pediatrics, University of Washington, Seattle, WA 98195

IL-15 is a novel cytokine identified as a T cell growth factor in culture supernatants of the monkey kidney epithelial cell line, CV-1/EBNA. The biological activities of IL-15 are similar to those of IL-2 in assays measuring T cell proliferation and generation of cytolytic effector cells in vitro. In the present study, we have investigated the nature of the IL-15 receptor and possible utilization of the three subunits of the IL-2 receptor α, β and γ. First, we ruled out the involvement of IL-2 receptor α chain by the inability of α chain specific antibodies to block IL-15 activity. Next, we demonstrated that monoclonal antibodies against the β chain block IL-15 binding and IL-15-induced activities. To confirm the involvement of the β chain, we reconstituted the human β chain into a mouse IL-3 dependent pro-B cell line, BAF/B03, and showed that the transfected cells acquired the abilities to proliferate and to induce c-myc, c-fos and c-jun oncogenes in response to IL-15. Finally, involvement of the IL-2 receptor γ chain was examined. The combination of the IL-2 receptor β and γ chains, but neither subunit alone, bound IL-15 on transfected COS cells. We also demonstrated that the γ chain is required for IL-15-induced c-myc and c-fos induction in the mouse fibroblast cell line, L929, utilizing cells stably transfected with the human IL-2 receptor β chain with or without the γ chain. These data show that the β and γ chains of the IL-2 receptor are components of the functional IL-15 receptor complex. It was interesting that a mouse IL-15 responsive T cell line showed a much higher number of IL-15 binding sites than could be accounted for by the number of β and γ sites, providing evidence for a molecule capable of binding IL-15 independently of the β and γ chains. Additionally, some mouse cell lines could not bind or respond to IL-15 although they were able to bind IL-2 with high-affinity and respond to IL-2. As we showed that the mouse $\beta\gamma$ heterodimer can not bind IL-15 or IL-2, these cell lines may lack an IL-15 specific receptor component. In human cells, we demonstrated that IL-15 can bind to EBV-transformed B cell lines established from XSCID patients that lack surface expression of the γ chain. Collectively, these data indicate that there is an IL-15 specific receptor subunit in both mouse and human cells. Progress towards the molecular characterization of this novel receptor component will be presented.

C2-150 SYK RECRUITMENT TO IG-α AND ITS PHOSPHORYLATION ARE ESSENTIAL FOR B CELL ANTIGEN RECEPTOR SIGNALING, Tomohiro Kurosaki[1], Sara A. Johnson[2], Kiyonao Sada[4], Hirohei Yamamura[4], and John C. Cambier[2,3]
[1]Department of Cardiovascular Molecular Biology, Lederle Laboratories, Pearl River, N.Y. 10965 and Section of Immunobiology, Yale University School of Medicine, New Haven, CT 06510-8023 [2]Department of Pediatrics, Division of Basic Science, National Jewish Center for Immunology and Respiratory Medicine, 1400 Jackson Street, Denver, CO 80206 [3]Department of Immunology, University of Colorado Health Science Center, Denver, CO 80220 [4]Department of Biochemistry, Fukui Medical School, Matuoka, Fukui, 910-11, Japan

The B cell antigen receptor (BCR) is a multimeric protein complex consisting of membrane immunoglobulin and the Ig-α/β heterodimer that mediates intracellular signaling by coupling the receptor to protein tyrosine kinases (PTKs). We showed previously that Syk is essential for coupling the BCR to phosphatidylinositol (PtdIns) pathway. To explore the mechanism(s) by which Syk participates in BCR signaling, we have studied Syk mutants that interfere with signal transduction. B cells expressing both N- and C-terminal SH2 mutants of Syk exhibited similar BCR-induced tyrosine phosphorylation pattern to Syk-deficient cells. Phosphorylation of the Igα antigen receptor homology 1 (ARH1) motif increased binding of Syk. This increased binding was dependent of both of the Syk SH2 domains. Furthermore, a mutant in which Syk putative autophsophorylation site is changed to phe, did not induce the tyrosine phosphorylation of phospholipase C (PLC)-γ2 upon BCR stimulation. These findings demonstrate that both recruitment of Syk to tyrosine phosphorylated Igα and its phosphorylation are required for coupling the BCR to activation of PLC-γ2.

C2-151 DISSECTING BIOCHEMICAL AND FUNCTIONAL RESPONSES TO TCR OR CD28 ACTIVATION USING HUMAN CD4+ T CELL CLONES. Riitta Lahesmaa, Andrea Allsup, Janet Jackman, Carol Soderberg, Paul Findell, and Gary Peltz. Department of Leukocyte Biology, Syntex Research, Palo Alto, CA 94304.

A panel of human CD4+ T cell clones was utilized to dissect and analyze the biochemical and functional consequences of activation of CD3 and CD28. In contrast to transformed T cell lines which require only one (TCR-mediated) activating signal; human T cell clones require two distinct activating signals (TCR-mediated plus a costimulatory signal) to trigger proliferation or cytokine production. CD28-mediated costimulatory signals induced Th1- and Th2-like T cell clones to proliferate. However, CD28 ligation induced IFN-γ production by Th1 cells; but not IL-4 production by Th2 cells, even though other activating stimuli triggered IL-4 production by Th2 cells. To molecularly characterize receptor-activated proximal signaling events occurring in T cell clones, tyrosine phophorylated proteins co-precipitating with a GST/Grb2 fusion protein after receptor activation were analyzed. Grb-2 interacts with several proteins which become tyrosine phosphorylated upon T cell activation to form a multi-protein complex which regulates intracellular signal transduction. Ligation of CD28, but not other costimulatory signals studied induced the tyrosine phosphorylation of two Grb2 binding proteins. A third Grb2 binding protein became tyrosine phophorylated in response to combined CD3 and CD28 activation. Interestingly, pretreatment with exogenous cytokines modulated the tyrosine phosphorylation of these Grb2-associated proteins.

C2-152 **T CELL SIGNALLING THROUGH THY-1 IS MEDIATED BY THE ASSOCIATION OF p100 WITH THE KINASE FYN.** A. Lehuen, L. Beaudoin, J.F. Kearney*, J.-F. Bach and R. C. Monteiro. INSERM U25, Hôpital Necker, 75743 Paris, France;* Div. of Develop. & Clin. Immunol., UAB, AL 35294. Thy-1 molecules which lack a transmembrane domain, can nonetheless induce T cell activation; it has thus been suggested that a separate transmembrane molecule associated with Thy-1 is required for signal transduction. We have previously characterized a transmembrane protein with an Mr of 100,000 (p100), which is non-covalently bound to two glycosyl-phosphatidylinositol (GPI)-linked molecules, Thy-1 and ThB. The differential expression of p100 on CD4 cells allow us to investigate the role of p100 in signal transduction through Thy-1 molecules. We found that only $p100^+$ CD4 cells proliferate and release cytokines in response to cross linkage of Thy-1 whereas both populations, $p100^+$ and $p100^-$, express high levels of Thy-1 molecules on their surfaces. Control stimulation by anti-CD3 antibodies or concanavalin A induces identical thymidine uptake of both populations of CD4 cells. However, these two populations of CD4 cells have different cytokine profiles after activation through CD3; only $p100^+$CD4 cells release IL-2 and IFN-γ, whereas both populations release IL-4. In vitro kinase assay reveals that p100 is associated to kinase activity, $p59^{fyn}$, and that phosphorylated proteins of 90, 59, 56 and 33 kDa are coprecipitated with Thy-1 only in $p100^+$ CD4 cells. Altogether, these data strongly suggest that p100 is the signal-transducing molecule which mediates activation through Thy-1. Moreover, the expression of p100 in activated CD4 cells in vivo could be relevant to the proposed function of Thy-1 in cell activation as an accessory signalling molecule.

C2-153 MOLECULAR BASIS FOR THE CO-ASSOCIATION BETWEEN CD4 AND SPECIFIC ISOFORMS OF THE CD45 TYROSINE PHOSPHATASE. David Leitenberg, Thomas Novak, Catriona F. Grant, B.R. Smith, and Kim Bottomly. Departments of Immunobiology and Laboratory Medicine, Yale University School of Medicine, New Haven, CT 06510.

The CD45 tyrosine phosphatase is thought to play an important role in regulating T lymphocyte activation, but the function of the different isoforms of CD45 is not known. A variety of T cell transfectants have been prepared which express individual CD45 isoforms. We have previously shown that T cells expressing the null isoform of CD45 are preferentially stimulated to secrete IL-2 following antigen-specific stimulation, compared to cells that express the CD45ABC isoform. One hypothesis for the preferential activation of cells which express the null isoform of CD45 is that it specifically interacts with other molecules important in T cell receptor (TcR) signaling, altering their phosphorylation status, and affecting the character of the signal transduction pathway. In this report we demonstrate that the null isoform of CD45 preferentially co-associates with CD4 compared to the ABC isoform. The CD45 ABC transfectants are not refractory to co-capping however, since CD3 and CD4 co-associate normally. These data are consistent with the hypothesis that CD45 isoform specific regulation of T cell function is based on intermolecular associations which regulate substrate access to the tyrosine phosphatase domains. The basis for this interaction and the requirements for CD45 tyrosine phosphatase activity and CD4-associated tyrosine kinase activity for preferential IL-2 production will be examined using T cell transfectants expressing distinct isoforms of CD45 (including B only, C only, and exon-1 isoforms), glycosyl phosphatidyl inositol-linked forms of CD45 (lacking tyrosine phosphatase domains), and mutant forms of CD4 which vary in their extracellular and cytoplasmic domains.

C2-154 THE EXPRESSION OF A DOMINANT NEGATIVE cJUN ALTERS INDUCTION OF AP-1 COMPONENTS IN T CELL LINES, Joi M. Lenczowski*, Charles M. Zacharchuk, Michael Birrer, and Jonathan D. Ashwell, LICB, NCI, NIH, Bethesda, MD, 20814, *HHMI-NIH Research Scholars Program. Antigenic stimulation of T lymphocytes induces transcription of a large number of genes, including members of the proto-oncogene fos and jun families. Fos and Jun family members are subunits of the AP-1 and NF-AT transcriptional complexes, which are important in the induction of IL-2 production. While PMA stimulation also induces AP-1 in T cells, induction of the NF-AT complex requires additional signals. TAM-67, a dominant negative form of cJun, has been shown to block trans-activation of several constitutive and inducible AP-1 reporters. This dominant negative molecule retains both the dimerization and the DNA-binding domains, but lacks the transcriptional activation domain. Stable expression of TAM-67 in the Jurkat cell line inhibits activation-induced IL-2 production and response to PMA stimulation, as measured by transient transfection with an AP-1 responsive reporter. Evaluation of the stably transfected cell lines has also revealed reduced induction of RNA and protein expression of the Jun and Fos family members, indicating alterations in the regulation of steady state message levels. This effect is consistent with evidence for AP-1 sites present in the c-jun promoter acting to positively autoregulate c-jun transcription or may be due to post-transcriptional destabilization of message. Additionally, gel shift assays have indicated a novel AP-1 binding complex in the TAM-67 expressing cells. The dysregulation of endogenous Jun and Fos family members due to expression of TAM-67 may explain some of the functional effects of this dominant negative construct and should allow further investigation of the function of Jun and Fos family members in T cells.

C2-155 ANCHORING OF PROTEIN KINASE A IS REQUIRED FOR MEDIATING THE INHIBITORY EFFECTS OF 3',5'-CYCLIC ADENOSINE MONOPHOSPHATE ON IL-2 TRANSCRIPTION IN HUMAN T CELLS, R. Owen Lockerbie, Adam Kashishian, Lynn Courtney, John D. Scott and W. Michael Gallatin. Cell Adhesion Group, ICOS Corporation, Bothell WA 98021 and Vollum Institute, Oregon Health Sciences University, Portland, OR 97201

The signalling pathways controlling cytokine gene expression and proliferation in T lymphocytes are not fully understood at present. However, intracellular elevation of cAMP with subsequent activation of cAMP-dependent protein kinase A (PKA) mediates an inhibitory effect on TCR-CD3-induced T cell replication. Recent studies have indicated that diverse biochemical effects of different agonists that stimulate cAMP production may occur through activation of compartmentalized pools of PKA. Of the several PKA subtypes, there is evidence that compartmentalization of type II PKA is maintained through protein-protein interactions between the regulatory (R) subunit and specific anchoring proteins (AKAPs). All RII AKAPs so far studied have been found to bind to the same or overlapping sites on RII through a conserved amphipathic helical secondary structure binding motif. To investigate the role of AKAPs in PKA mediated regulation of T cell activation, we have used Human T cells that have been stably transfected with a reporter gene construct containing the minimal IL-2 promoter fused to the lacZ gene encoding β-galactosidase (β-gal). Quantitation of IL-2 transcription is achieved through fluorescence-activated cell sorter analysis of β-gal activity following activation with phorbol ester and ionomycin. Forskolin and IBMX reduces PMA/ionomycin induced β-gal activity by approx. 50%. This blockade is completely reversed by both a myristilated PKA inhibitor peptide and a myristilated amphipathic AKAP peptide that blocks anchoring protein interaction with the RII regulatory subunit of PKA. The effects of the drug/peptide treatments are mirrored by Northern blot analysis of IL-2 message. These results strongly suggest the involvement of PKA and its anchoring through an AKAP(s) in regulating IL-2 gene expression.

C2-156 A CONFORMATIONAL ASSAY FOR T CELL RECEPTOR AND MHC CLASS II ENGAGEMENT, Daniel S. Lyons[1], John J. Boniface[2], and Mark M. Davis[1,2], [1]Program in Immunology, [2]Department of Microbiology and Immunology, and the Howard Hughes Medical Institute, Stanford University School of Medicine, Stanford, CA 94305

Interactions between the T cell receptor (TCR) and the class II Major Histocompatibility Complex (MHC) peptide complex have proven difficult to study due to their low affinity. Additionally, little is known about the nature of the changes that occur in these molecules upon binding. In these experiments the interaction between the TCR and MHC class II peptide complex is studied using Circular Dichroism (CD) in the near UV. This technique allows the measurement of changes in the environment of aromatic residues such as Tyr, Trp, and Phe upon interactions between the two proteins. Difference spectra between a mixture of TCR and MHC class II versus the spectra of the proteins alone gives a measure of the relative amount of TCR-MHC class II complex formed at equilibrium. For this study a soluble form of the 2B4 TCR, specific for Moth Cytochrome c (MCC) residues 88-103, and a soluble form of mouse class II MHC (I-E^k), which binds MCC specifically, were used. These experiments were performed over a series of concentrations of the TCR and MHC class II mixture to determine an affinity (K_d) for this interaction. Use of this technique has allowed the determination of K_d values both for a non-peptide loaded 'empty' MHC class II molecule and for several specific MHC class II MCC peptide complexes (IE^k-MCC). In addition this technique is compared to results obtained using surface plasmon resonance to monitor binding of the TCR and MHC class II peptide complexes. These experiments provide direct evidence for a conformational change being important in TCR-ligand interactions, and will allow a more detailed understanding of the physical forces involved in these interactions.

C2-157 INTERLEUKIN 12 UPREGULATES THE T CELL IMMUNE RESPONSE, Maguire H.C. Jr., Department of Medicine, Thomas Jefferson University School of Medicine, Philadelphia, PA 19107

Interleukin 12, a heterodimeric molecule made up of disulfide linked 35 kD and 40 kD chains, has multiple effects on the immune response including directing stimulated T helper cells to cells of a Th-1 cytokine profile, with suppression of Th-2 cells. We hypothesized that in vivo this would result in an immunoadjuvant effect in the area of delayed type hypersensitivity (DTH). We have investigated this issue using, as experimental model, allergic contact dermatitis (ACD) in mice treated with murine recombinant IL-12. We found that 1. Systemic IL-12 given at sensitization significantly increased the induced sensitivity, 2. IL-12 at the time of ACD challenge failed to up-regulate the DTH reaction, 3. Effective doses of IL-12 ranged from 0.3-2 .0 micrograms and were non-toxic, and 4. Cyclophosphamide and IL-12 synergize as immunoadjuvants. It is likely that IL-12 will be a practical immunoadjuvant in man.

C2-158 TCR AND CD8-DEPENDENT TYROSINE PHOSPHORYLATION IN CTL: ACTIVATION OF $p56^{lck}$ BY CD8 BINDING TO CLASS I, Matthew F. Mescher[1], Alberto Anel[2], Dayue Shen[1] and Alan Kleinfeld[2], [1]Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN 55455 and [2]Medical Biology Institute, LaJolla CA 92031.

Activation of CTL effector function (degranulation) can be divided into two steps. Engaging the TCR with fluid phase anti-TCR mAb activates CD8 to mediate CTL adhesion to class I MHC protein. 'Activated' CD8 then generates a costimulatory signal upon binding to class I. When CD8 binds class I, PI hydrolysis, a rise in $[Ca^{2+}]_{int}$ and degranulation are activated. In this two step process, TCR engagement with fluid phase anti-TCR mAb results in increased tyrosine phosphorylation of a discrete subset of cellular substrates, i.e. in the absence of a costimulatory signal from CD8 only some substrates undergo increased phosphorylation. Increased phosphorylation of additional substrates occurs when CD8 then binds to class I (non-antigen), resulting in a phosphorylation pattern comparable to that obtained when cells are stimulated with class I alloantigen (a ligand for both TCR and CD8). Tyrosine phosphorylation of $p56^{lck}$ increases upon stimulation with anti-TCR mAb and subsequently decreases to below basal level when CD8 binds class I, accompanied by a substantial increase in $p56^{lck}$ kinase activity. These results are consistent with a two step model for TCR activation of CD8-class I interactions, and directly demonstrate that CD8 binding to class I leads to upregulation of $p56^{lck}$ activity.

C2-159 **NEGATIVE SIGNALLING VIA CD4 IN $CD45RB^{lo}$ CD4+ T CELLS**, Daniela P. Metz, Donna Farber and Kim Bottomly, Department of Immunobiology, Yale University, School of Medicine, 310 Cedar Street, New Haven, CT 06510.

Naive and memory T cells distinguished by CD45RB expression have been shown to differ in their activation requirements. One set of studies revealed that in contrast to $CD45RB^{hi}$ CD4 T cells (naive cells), $CD45RB^{lo}$ (memory) $CD4^+$ T cells did not proliferate to anti-CD3 (29B) in the presence of Class II^+ syngeneic APCs (splenocytes). These data stood in contrast to vigorous proliferation of $CD45RB^{lo}$ cells in the presence of plate-bound anti-CD3. The failure to respond was controlled by the presence of MHC Class II in that $CD45RB^{lo}$ $CD4^+$ cells responded to anti-CD3 presented by MHC Class II deficient cells. Since CD4 is the only natural ligand known to specifically bind to MHC Class II, the engagement of CD4 by MHC Class II in the presence of anti-CD3 was thought to deliver a negative signal to $CD45RB^{lo}$ but not to $CD45RB^{hi}$ $CD4^+$ T cells. To test this hypothesis further, we employed CD4-deficient mice, kindly provided by Dan Littman. These mice have a high frequency of $CD4^-$ and $CD8^-$ (DN) T cells in the periphery. In contrast to $CD4^+$ $CD45RB^{lo}$ cells, the DN $CD45RB^{lo}$ T cells proliferated to anti-CD3 in the presence of splenic APC (Class II^+). Thus, both CD4 and MHC Class II are required for negative signalling seen in $CD45RB^{lo}$ $CD4^+$ T cells. Furthermore, the CD4:MHC Class II interaction leads to negative signalling in memory cells but not naive cells. We are presently investigating the mechanism of negative signalling.

C2-160 PHOSPHORYLATION OF THE T CELL RECEPTOR CD3-ε SUBUNIT AND THE IDENTIFICATION OF ASSOCIATED PROTEINS, Markus Metzger, Craig H. Hall and Cox Terhorst, Department of Medicine, Division of Immunology, Harvard Medical School, Beth Israel Hospital, Boston, MA 02215

Recent findings support an important role of signaling through the TCR/CD3-ε subunit in early T cell differentiation (Wang et al., 1994, Levelt et al., 1994). Phosphorylation of the CD3 subunits upon T cell antigen receptor (TCR) activation involves the interaction with nonreceptor kinases (PTKs). Early events in signaling were examined in transfected COS cells. When coexpressed with the src kinase p59*fyn*, ζ becomes tyrosine phosphorylated, wheras ε, γ or δ did not. Strikingly, mutants of CD3-ε, which in contrast to the wt were shown to be expressed on the cell surface (Mallabiabarreno et al., 1992), could be phosphorylated by the src kinases *fyn*T and *lck*. Moreover, coexpression of either p56*lck* or p59*fyn* with a cell surface expressed CD8-ε fusion protein led to tyrosine phosphorylation of ε in COS cells. Thus, cell surface expression of CD3-ε was a prerequisite for ε phosphorylation. Additionally, coexpression of neither CD45, which is known to upregulate src-kinase activity, nor ZAP-70 or SYK had any effect on the phosphorylation state of ε. Furthermore, phosphorylation of and binding to the *fyn* kinase in vitro and in vivo upon CD8 engagement was investigated by constructing mutated CD8-ε fusion proteins, which lack either of the two tyrosines present in the CD3-ε "Reth"-motif. The evaluation of further ε associated proteins is in progress.

C2-161 Bcl-x_S CAN ANTAGONIZE THE ANTI-APOPTOTIC PROPERTIES OF Bcl-x_L, Andy J. Minn, Lawrence H. Boise, and Craig B. Thompson, Gwen Knapp Center for Lupus and Immunology Research, University of Chicago, Chicago, IL 60637

Our laboratory has recently cloned a gene *bcl-x*, whose product shares extensive homology to Bcl-2 and is dramatically induced upon T cell activation. Alternative splicing of *bcl-x* yields two mRNA products, *bcl-*x_L and *bcl-*x_S. Initial studies demonstrated that Bcl-x_L, similarly to Bcl-2, protects cells from programmed cell death induced by growth factor withdrawal. *bcl-*x_S uses an alternative 5' donor splice site to remove 63 of the most conserved amino acids shared between Bcl-x_L and other Bcl-2 family members. In contrast to Bcl-x_L, Bcl-x_S was initially shown to inhibit the anti-apoptotic effects of Bcl-2. Studies from other laboratories have demonstrated that *bax* is another gene whose product can antagonize the properties of Bcl-2. Bax can either homodimerize or heterodimerize with Bcl-2. We and others have demonstrated that Bax can also form heterodimers with Bcl-x_L, but overexpression does not seem to antagonize the function of Bcl-x_L. In order to see if Bcl-x_S can antagonize Bcl-x_L and/or form a physical association, we coexpressed the two genes in an IL-3 dependent prolymphocytic cell line FL5.12. By analyzing various clones that express different levels of Bcl-x_S relative to Bcl-x_L and inducing apoptosis by removal of IL-3, we show that Bcl-x_S can indeed inhibit the protective effects of Bcl-x_L. Preliminary metabolic labelling and immunoprecipitation studies suggest that Bcl-x_S does not associate with Bcl-x_L or Bax. Additional interaction studies with Bcl-x will be presented.

C2-162 IL-2 SIGNALING: FUNCTIONAL ACTIVATION OF JAK1 AND JAK3 KINASES THROUGH SELECTIVE ASSOCIATION WITH THE IL-2Rβ AND γCHAINS, Tadaaki Miyazaki*^, Atsuo Kawahara*, Hodaka Fujii*, Yoko Nakagawa*, Yasuhiro Minami*, Zhao-Jun Liu*, Isao Oishi*, Edward L. Barsoumian^, Olli Silvennoinen+, Bruce A. Witthuhn+, James N. Ihle+ and Tadatsugu Taniguchi*, *Institute for Molecular and Cellular Biology, Osaka Univ., Osaka 565, Japan, ^Dept. Molecular and Cellular Biology, Nippon Boehringer Ingelheim Co.,Ltd., Hyogo 666-01, Japan, +Dept. Biochemistry, St. Jude Children's Research Hospital, Memphis, TN 38101, U.S.A.

The interleukin-2 receptor (IL-2R) consists of IL-2Rα, β and γ chains. The IL-2Rγ chain is commonly used in the receptors for IL-4, IL-7 and IL-9. Recently, IL-2 stimulation was shown to induce the tyrosine phosphorylation and activation of the *Janus* kinases, Jak1 and Jak3.

We present data on the selective association of Jak1 and Jak3 with the cytoplasmic domains of IL-2Rβ and γ chains, respectively. Association of Jak1 with the IL-2Rβ required the "serine-rich" region of IL-2Rβ, whereas association of Jak3 with the IL-2Rγ required the COOH-terminal region of IL-2Rγ, a critical region commonly deleted in many XSCID patients. Both receptor regions are necessary for IL-2 signaling. Ectopic expression of Jak1 and Jak3 in IL-2 responsive, hematopoietic cells augmented activation of the c-*fos* promoter by IL-2. Antisense oligonucleotides for Jak1 and Jak3 selectively inhibited IL-2-induced thymidine uptake. Furthermore, we examined the functional role of Jak3 by the expression study in Jak3-negative fibroblasts which express reconstituted IL-2R.

The experimental findings demonstrate the essential role of Jak1 and Jak3 for IL-2 signaling through the selective association with the IL-2R β and γ chains.

C2-163 DIFFERENCES IN ANTIGEN RECEPTOR SIGNALLING IN IMMATURE AND MATURE STAGE B CELLS, John G. Monroe, Amanda Norvell and Robert J. Wechsler, Dept. of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA 19104

During their development, B cells pass through a maturational stage in which encounter with antigen leads to tolerance rather than activation. At least three mechanisms for achieving B cell tolerance have been reported; deletion, anergy and receptor editing. We have utilized an in vitro culture system to determine whether cross-linking sIgM on tolerance-susceptible sIgM+IgD-B cells results in deletion by apoptosis. In contrast to the effect of sIgM crosslinking on mature splenic B cells, treatment of immature, bone marrow derived B cells results in significant levels of apoptotic death. Antigen receptor mediated apoptosis is detectable by 14 hours after sIgM engagement. Moreover, cycloheximide, which has previously been shown to prevent B cell tolerance induction, specifically block the sIgM induced apoptosis observed in the immature B cells. Similarly, immature B cells from the neonatal spleen are also susceptible to apoptosis following sIgM crosslinking, although they manifest somewhat higher levels of unstimulated apoptosis as compared to bone marrow-derived B cells. Thus immature stage B cells respond differently to antigen receptor induced signals than do mature stage which are activated by these signals. We have also been studying the biochemical basis for these developmentally related differences in sIgM signalling. Recently, we have studied the antigen receptor associated tyrosine kinases expressed and activated by sIgM crosslinking in immature and mature stage B cells. Consistent with previous reports, we demonstrate that mature B cells express *syk, lyn, blk, hck,* and *fyn*; in addition, we find that they express significant amounts of the src-family kinase p55*fgr*. Immature B cells from day 3 mouse spleen show similar expression and activity of *syk, lyn, blk* and *hck*. However, these cells have marked deficiencies in *fyn* and *fgr* expression. High levels of *fyn* and *fgr* are detectable in splenic B cells beginning at 4 weeks of age, coincident with the appearance of cells that proliferate in response to receptor crosslinking. These observations indicate that expression of the src-family kinases *fyn* and *fgr* is developmentally regulated in murine B lymphocytes. Moreover, they suggest that these kinases may be important for mature B cell responses to antigen receptor stimulation, and that their absence may contribute to the unresponsiveness and tolerance susceptibility of immature B cells.

C2-164 CHARACTERIZATION OF INVARIANT CHAIN GENE REGULATION, Bethany B. Moore and Patricia P. Jones, Department of Biological Sciences, Stanford University, Stanford, CA 94305-5020

Class II MHC molecules are expressed on a variety of antigen presenting cells(APCs) and are critical for initiating immune responses by CD4+ T cells. Proper functioning of these heterodimers depends on their non-covalent association with a nonpolymorphic glycoprotein called invariant chain (Ii). Consistent with their cooperative roles, class II and Ii are co-regulated. Expression of these genes shows the same tissue specificity and cytokine inducibility. Accordingly, the Ii gene shares the conserved MHC class II promoter sequence elements; H, X, and Y, in the Ii upstream enhancer. However, the other Ii regulatory elements appear to be distinct and unrelated to class II genes. For example, Ii has a unique tissue specific promoter as well as an intronic enhancer. This intronic enhancer was initially mapped to a 2.4kb genomic fragment. By analyzing a series of nested deletions, we have identified an approximately 255bp region which significantly enhances transcription in conjunction with the Ii minimal promoter. This enhancer is constituitively active in both B and monocyte cell lines. Preliminary data indicates the presence of a number of specific nuclear proteins present in APCs which can bind to this region of DNA. Characterization of these interactions is currently underway.

C2-165 SIGNALING BY SH-PTP1 IN NORMAL AND MOTHEATEN B CELLS, Monica J.S. Nadler, J. Simon Anderson, Benjamin G. Neel and Henry H. Wortis, Department of Molecular Medicine, Beth Israel Hospital, Boston, MA 02215, and Department of Pathology, Tufts University, Boston, MA 02111

Protein tyrosine phosphorylation, which is regulated by the opposing actions of protein tyrosine kinases and protein tyrosine phosphatases (PTPs), is a key regulatory mechanism for cell growth and differentiation. However, little is known of how PTPs participate in signaling pathways. Mutations in the SH2-containing PTP, SH-PTP1 (also known as PTP1C, HCP and SHP), that result in the loss of cellular protein (*me/me*) or a PTP with severely reduced activity (me^{v}/me^{v}) cause the *motheaten* mouse. *Motheaten* mice have multiple defects throughout the hematopoietic cell lineage in T cells, NK cells, erythroid precursor cells, macrophages and B cells. In particular, *motheaten* B cells appear to be in a state of chronic stimulation. There is a depletion of B cell precursors, a corresponding accumulation of atypical plasma cells, hypergammaglobulinemia and increased serum levels of IgG and IgM, and multiple autoantibodies. In addition, the majority of *motheaten* B cells have a B1 phenotype which is normally restricted to a small population of B cells. Together, these observations suggest that SH-PTP1 may act as a negative regulator of B cell signaling pathways. In order to generate a model system to study how SH-PTP1 participates in B cell signaling, we have immortalized mature splenic sIgM+ B cells from *motheaten* and normal littermate mice with an oncogene-containing retroviral construct. Preliminary characterization of normal *vs. me/me* cell lines suggests that SH-PTP1 may participate downstream of sIgM crosslinking.

C2-166 MODULATION OF NF-κB NUCLEAR LOCALIZATION BY THE IMMUNOSUPPRESSANT 15-DEOXYSPERGUALIN, Steven G. Nadler and Jeffrey S. Cleaveland. Dept of Autoimmunity, Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, WA 98121.

We have been studying the mechanism of action of the immunosuppressive agent 15-deoxyspergualin (DSG) which is currently in phase I/II clinical trials for prevention of transplant rejection, HAMA response and treatment for autoimmune disease. Since it was recently shown that that DSG inhibits kappa Ig light chain production in pre-B cells we studied the effects of DSG on the transcription factor NF-κB which is important for kappa light chain expression, as well as, a number of other immunologically relevant proteins such as TNF-α, IL-2Rα and IL-6. DSG, in a dose dependent manner, inhibited the nuclear localization of NF-κB in 70Z/3 mouse pre-B cells, mouse L1210 T-cells and human THP-1 monocytes, in response to various activators. In addition to effects on NF-κB, there were slight effects on other transcription factors such as octamer binding protein, however, the most dramatic effects were on NF-κB. Current studies are aimed at trying to identify at the molecular level the exact mechanism of inhibition of NF-κB nuclear localization by DSG. One possibility is that DSG may be inhibiting the "chaperoning" of transcription factors from the cytosol to the nucleus. Previous studies have shown that DSG specifically interacts with Hsc70 a member of the Hsp70 family of heat shock proteins which have been shown to play a role in nuclear transport. These possibilities are being investigated and will be presented.

C2-167 DECREASED IgG1 PRODUCTION IN RESPONSE TO T CELL-DEPENDENT ANTIGENS IN TRANSGENIC MICE THAT EXPRESS ELEVATED CD72 CONSTITUTIVELY ON B CELLS, Henry E. Neuman de Vegvar[1], Yu Fang[2], Dale T. Umetsu[2], and Jane R. Parnes[1], Departments of [1]Medicine and [2]Pediatrics, Stanford University, Stanford, CA 94305

The cell surface glycoprotein CD72 is expressed on all mouse and human B lymphocytes from their early precursors to mature B cells but not after they differentiate into plasma cells. CD72 is a ligand for CD5, a cell surface molecule of T cells and a subset of B cells. Previous studies with antibodies against CD72 *in vitro* suggested that it has an important role in the proliferation and differentiation of B cells in T cell-dependent responses. Anti-CD72 antibodies also reduced the production of IgG1 by mouse B cells presumably by inhibiting IL-4-induced class-switching. To assess the function of CD72 *in vivo*, we have generated transgenic mice in which the $CD72^{a}$ allele is expressed in $CD72^{b}$ mice under the control of the immunoglobulin kappa light chain promoter and enhancers. The total amount of CD72 (endogenous and transgenic) on B cells in the transgenic mice is about twice as high as that in non-transgenic mice, regardless of whether the cells express kappa or lambda chains. Fourteen days after immunization with the T cell-dependent antigen trinitrophenyl-keyhole limpet hemocyanin (TNP-KLH), TNP-specific IgG1 was reduced by about 65% in transgenic mice as compared to their non-transgenic litter mates. Both groups made indistinguishable amounts of TNP-specific IgM, IgG2a, IgG2b, and IgG3. In response to the type 2 T cell-independent antigen TNP-Ficoll, both groups also had similar levels of TNP-specific IgM and IgG. These data confirm a role for CD72 in regulating IgG1 made in responses to T cell-dependent antigens.

C2-168 EFFECTS OF THE ENGAGEMENT OF SURFACE MHC II ON Th CELL PROLIFERATION. Quoc V. Nguyen and Rebecca L. King. Department of Pediatrics, SUNY Health Science Center, Syracuse NY 13210.

Class II major histocompatibility complex (MHC II) plays key role in recognition of self, signal transduction and antigen presentation in antigen presenting cells. Activated human T cells, but not murine T cells, express surface MHC II. Its role in the regulation of T cell function is still not well characterized. We have used Th clones restricted to DR5 and tetanus toxoid to address the effect of the ligand-MHC binding on Th functions. The binding of whole and Fab of 9.3F10 anti-class II monoclonal antibody (mAb) to Th cells inhibited Th proliferation in a dose-dependent fashion. A down regulation of phosphotyrosine kinase activity was found in such Th cells. Both bivalent and monovalent engagements were not associated with apoptosis as evidenced by the absence of DNA fragmentation on gel electrophoresis. A common inhibitory mechanism generated by crosslinking binding (whole mAb) and by monovalent binding (Fab) may not be likely because Th clones expressed different bcl-2 species and levels for each type of binding. Other ligands of MHC II, including peptides known to bind to the antigen presenting groove or outside of the groove, are being used in further studies. Results may implicate a different role (other than the classic help to B cells) of MHC II bearing Th cells in the regulation of human immune response.

C2-169 MOLECULES INVOLVED IN ALTERNATIVE COSTIMULATION BY T-LYMPHOMAS. John D. Nieland, Mariëlle C. Haks, Tobias F. Rinke de Wit*, Rienk Offringa**, and Ada M. Kruisbeek, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, * Dept Immunology, Erasmus University, Rotterdam, **Dept. of Immunohaematology and Bloodbank, University Hospital Leiden, The Netherlands.

Activation of antigen specific T-cells requires more than signalling through the TCR/CD3 complex. A second or costimulatory signal is also required. The best known receptor-ligand interaction through which second signals can be transduced is formed between CD28 on T-cells and B7 on APC's. Nevertheless, some B7-negative cell lines appear capable of providing second signals under certain conditions. For instance, the peptide transporter defective T-lymphoma RMA-S does not express B7, but can effectively induce peptide specific CTL responses in vitro, once loaded with an exogenous source of antigenic peptide. Such responses cannot be blocked by anti-B7 reagents (i.e. anti B7 or CTLA4-Ig), suggesting that B7 independent costimulatory pathways may exist.
This notion was further investigated in various models. First, RMA-S can support clonal expansion of purified CD4 or CD8 T-cells from unprimed mice activated with ConA or immobilised anti-CD3. In fact, also the parental RMA cell line, as well as at least one other T-cell lymphoma (i.e., EL4), can aid in such anti-CD3-induced clonal expansion in a dose dependent fashion. This expansion is accompanied with IL2 and IFNγ production. Secondly, also costimulation of antigen-specific T-cell proliferation of both class I and class II restricted T-cell clones can be provided by B7-negative T-lymphoma cells. To identify the molecules mediating costimulation by T-lymphomas, we prepared a cDNA library of RMA-S in pcDNA-1, and set up a system to identify costimulatory molecules by functional screening. We screened about 25000 cDNA molecules and isolated 12 cDNA molecules which upon COS transfection enabled the COS cells to costimulate a ConA activated T-cell response. The lenghts of these molecules were between 400 bp and 2 Kb, and we have partially sequenced 11 molecules. Those we consider potentially most interesting include a molecule with homology to a GTP binding protein of the RAS gene superfamily; a molecule with weak homology with G-CSF; and a molecule with homology with a mrp protein. Protein expression analysis is currently performed, as is a more extensive biological function analysis, with stable transfectants, of the nature of costimulatory effects induced.

C2-170 **EXPRESSION OF COSTIMULATORY MOLECULES BY CNS-RESIDENT ANTIGEN PRESENTING CELLS.** Kelly M. Nikcevich†, Stephen D. Hurst†, Terrence A. Barrett†§, and Stephen D. Miller†. Department of Microbiology/Immunology† and Department of Medicine§, Northwestern University Medical School, Chicago, IL 60611.

Astrocytes, microglia, and oligodendrocytes are resident cells of the CNS that may serve as effectual APCs during the Th cell-mediated immune response to myelin components during multiple sclerosis (MS) and experimental autoimmune encephalomyelitis (EAE). Although these cell types do not constitutively express MHC class II molecules, expression may be induced during active EAE or by *in vitro* incubation in the presence of IFN-γ. Each cell type has been shown to stimulate antigen-specific T cells *in vitro* after induction of class II expression suggesting that each is able to deliver co-stimulatory signals to T cells, however, the expression of these molecules has not yet been confirmed. We have determined that primary murine astrocytes constitutively express both B7-1 and B7-2 molecules as determined by FACS and PCR analyses. While surface expression of these molecules increases steadily upon incubation with IFN-γ or TNF-α the mRNA levels appear to be unchanged. Unlike primary rat astrocytes, ICAM-1 expression is down-regulated in the presence of both of these cytokines. In *in vitro* proliferation assays, primary murine astrocytes, both IFN-γ-induced and non-induced, were able to stimulate proliferation of an ovalbumin-specific memory T cell line. In contrast, only the IFN-γ-induced astrocytes were able to stimulate proliferation of naive transgenic ovalbumin-specific T cells. These data suggest that differential expression of B7-1 and B7-2 co-stimulatory molecules may affect the ability of a "non-professional" APC to stimulate memory but not naive antigen-specific T cells. These data also suggest a model by which astrocytes or other CNS-resident APCs present myelin antigens to stimulate myelin-reactive T cells in the CNS during EAE or MS that may not be involved in the initial priming of the auto-reactive T cells (*i.e.*, epitope spreading).

C2-171 THE EFFECT OF CD28 COSTIMULATION ON T CELL SURVIVAL DURING ACTIVATION. Patricia J. Noel, Jonathan M. Green, Lawrence H. Boise and Craig B.Thompson. The Gwen Knapp Center, Howard Hughes Medical Institute, The University of Chicago, 924 East 57th Street, Chicago, IL 60637

Maximal activation of T cells requires stimulation of the T cell receptor (TCR) via Ag/MHC complexes as well as a second, costimulatory signal. We have recently shown that mice that are deficient for the T cell surface receptor CD28 have significantly reduced T cell proliferative responses when stimulated with α-CD3 in the presence of antigen presenting cells (APCs) when compared to wild-type cells. Additionally, provision of exogenous IL-2 cannot completely restore proliferation to wild-type levels. When CD28/B7 interactions are blocked in wild-type cells, proliferation is equal to that seen in CD28-deficient cells underscoring the importance of costimulation via CD28 during a proliferative response. One possible explanation for this phenomenon is provided by the observation that CD28-deficient T cells that are activated in the presence of APCs have reduced viability as compared to wild-type cells under the same conditions. When activated with α-CD3 alone, wild-type T cells survive significantly better than CD28-deficient cells at 48 and 72 hours. This effect is potentiated by providing costimulation with α-CD28. Previous data from our lab has shown that CD28 costimulation enhances the viability of activated T cells through the induction of extrinsic and intrinsic survival factors. Specifically, the survival gene *bcl-x$_L$* protects against a variety of agents which induce apoptosis via an IL-2 independent mechanism. Western analysis has shown that Bcl-x$_L$ is induced in wild-type cells after induction with α-CD3 and that the induction is greatly enhanced by activation with α-CD3 + α-CD28. In contrast, in CD28-deficient cells Bcl-x$_L$ levels are severely reduced in T cells activated with α-CD3 and, as expected, costimulation with α-CD28 does not enhance the induction. Thus, it appears likely that the reduced Bcl-x$_L$ levels contribute to the increased cell death seen in CD28-deficient cells. Data will be presented on the effect of both extrinsic survival factors such as IL-2 and intrinsic factors such as Bcl-x$_L$ and Bcl-2 on activation-induced cell death as well as the potential role of a Fas-Fas ligand mediated cell death mechanism in CD28-deficient as compared to wild-type cells.

C2-172 CELL SURFACE EXPRESSION OF THE β SUBUNIT OF MITOCHONDRIAL ATP SYNTHASE: A NOVEL EARLY MARKER OF T-LYMPHOCYTE ACTIVATION, Allen J. Norin, Adam M. Borochov, Rimma Mushnitsky, Minoo Sadeghian, Nuria M. Lawson, Ballabh Das, Departments of Medicine, Surgery and Anatomy & Cell Biology, SUNY Health Science Center, Brooklyn, New York 11203.

The β- subunit of H^+ transporting ATP synthase (β- H^+ATPase) was thought to function exclusively in mitochondria as the catalytic site for ATP synthesis. We have recently demonstrated β- H^+ATPase on the surface of human tumor cell lines and its role as a ligand in the effector phase of lymphocyte mediated cytotoxicity (J Exp Med. 180:273, 1994). In the current study we investigated the expression of β- H^+ATPase during concannavalin A (Con A) and MLC induced lymphocyte activation. Most freshly isolated human T-lymphocytes do not express β- H^+ATPase on the exterior of the plasma membrane as determined by flow cytometry and Western Blot analysis. Within 6 hrs of stimulation with Con A approximately 40% of the T lymphocytes expressed cell surface β- H^+ATPase. (84% of the total cells are CD 3^+). With further culture (24 to 72hrs) the small T lymphocyte population had fewer cells that expressed high levels of β- H^+ATPase. However, the large T cell blasts that arose during days 1 to 3 of culture expressed high levels of surface β- H^+ATPase . The activated T cells also co-expressed CD25. Similarly T cell blasts arising from allo-MHC stimulation expressed high levels of β- H^+ATPase. T cell blasts that arose in "unstimulated" cultures also expressed high levels of surface β- H^+ATPase. Most of the CD3 $^+$ cells in the nylon column purified preparations used in these studies (16% of the total cells) expressed elevated levels of surface β- H^+ATPase. Approximately 30% of the $CD3^+$ cells were $CD16^+$ (presumably NK cells) whereas the majority were $CD4^+$, 8^+ ,16^+,$45^{+/-}$. These results suggest that acquisition of cell surface β- H^+ATPase is an early event in T lymphocyte activation. Further studies are required to determine whether surface β- H^+ATPase plays a physiologic role in maintenance of the activated state. Supported by NCI grant CA47548.

C2-173 **THE FK-506- AND CSA-SENSITIVE PHOSPHATASE, CALCINEURIN, ENHANCES ACTIVATION OF NF-κB IN SEVERAL CELL TYPES.** Edward A. O'Neill*, Gary Bren†, Nora Steffan†, Carlos V. Paya†, Michael J. Tocci*, Stephen J. O'Keefe* and Betsy Frantz*. *Department of Molecular Immunology, Merck Research Laboratories, Rahway, NJ 07065. †Department of Immunology, Mayo Clinic, Rochester, MN 55905

The transcription factor NF-κB plays a prominent role in a variety of immune and inflammatory responses. Through a combination of molecular and pharmacological techniques we demonstrate that the Ca^{2+}-regulated protein phosphatase calcineurin stimulates the activation of NF-κB in several different cell types by enhancing the degradation of IκB, the inhibitor of NF-κB. By transient expression of a constitutively active mutant calcineurin we demonstrate that this phosphatase acts in synergy with PMA to stimulate induction of an NF-κB promoter element in the human T cell line Jurkat and the human promonocytic cell line U937. With an electrophoretic mobility shift DNA binding assay we demonstrate that calcineurin increases the amount of nuclear NF-κB DNA binding activity in these cells as well as in the rat kidney fibroblast cell line NRK-52E. Finally, using IκB/MAD3 specific antiserum in Western immunoblots we demonstrate that calcineurin acts in synergy with PMA to accelerate the rates of phosphorylation and degradation of either transiently expressed IκB/MAD3 or endogenous IκB in both Jurkat and U937 cell lines. It remains to be determined precisely how calcineurin stimulates the phosphorylation of IκB. Since calcineurin is an FK-506- and cyclosporin A-sensitive enzyme these observations provide one explanation for how these compounds achieve broad immunosuppression. Further, these observations provide a possible explanation for mechanism-based toxicities associated with FK-506 and CsA by demonstrating that the drugs can inhibit the calcineurin-dependent activation of a ubiquitous transcription factor.

C2-174 MURINE FC γ RECEPTOR INTERACTIONS WITH CD38, Alyce M. Oliver and John F. Kearney, Department of Microbiology, The University of Alabama at Birmingham, Birmingham, AL 35294

Stages of B cell differentiation can be defined by cellular and molecular events that result in gene activation and expression of certain surface molecules. One such cell surface antigen is CD38 which is expressed on B cells and a subpopulation of T cells. Although the function of CD38 is unknown, murine CD38 has homology to ADP ribose cyclase as well as the recently identified BP-3 and BST-1 molecules. In addition to the enzyme activity of converting NAD^+ into ADPR, antibody crosslinking of CD38 can induce proliferation of resting B cells as detected by ^{3}H-Thymidine uptake. These results suggest that CD38 may function in B cell activation. In contrast to possible B cell activation signaling pathways, we have shown that this CD38 induced proliferative response can be attenuated by adding anti-Fc γ receptor antibodies in culture with anti-CD38 antibodies. FcγR appears to interact with CD38 by co-capping on the surface of B cells. Furthermore, upregulation of surface FcγR is observed when the CD38 cDNA is transfected into the BaF_3 cell line. These data indicate that CD38 responses can be modulated by the FcγR. Additional studies will investigate the functional interactions between CD38 and the FcγR. (Supported by USPHS grants AI30879, AI14782 and CA13148)

C2-175 CD45 EXPRESSION MODULATES THE ACTIVITY OF B CELL ANTIGEN RECEPTOR ASSOCIATED PROTEIN TYROSINE KINASES. Lily I. Pao[1], Michael Reth[2], and John C. Cambier[1], [1]Department of Pediatrics, Division of Basic Science, National Jewish Center for Immunology and Respiratory Medicine, 1400 Jackson St., Denver, CO 80206, and Department of Immunology, University of Colorado Health Science Center, Denver, CO 80220, [2]Max-Planck-Institüt für Immunobiologie, Stubeweg 51, D-79108, Freiburg, Germany.

CD45 expression is required for B cell antigen receptor (BCR) mediated signal transduction, including calcium mobilization and $p21^{ras}$ activation. To further elucidate the molecular basis of the CD45 requirement for signaling, we compared BCR mediated protein tyrosine kinase activation in $CD45^+$ and $CD45^-$ variants of J558Lμm3 cells. Anti-phosphotyrosine immunoblotting of whole cell lysates revealed receptor mediated protein tyrosine phosphorylation in both the presence and absence of CD45. Igα and Igβ were among the proteins inducibly tyrosine phosphorylated in both cells.

Given the likely role of Igα and Igβ phosphorylation in recruiting and activating tyrosine kinases, we assessed BCR association with Lyn and Syk, and the activity of these kinases following receptor stimulation. Lyn and Syk were associated with unligated receptors in both cells. Lyn recruitment to the receptor following stimulation was much greater in $CD45^+$ than $CD45^-$ cells, but Syk recruitment was comparable. Interestingly, studies using peptide substrates to measure Syk and Lyn activity in vitro indicated that BCR ligation leads to detectable activation of BCR associated kinases only in $CD45^+$ cells. Thus, the increased protein phosphorylation seen in $CD45^-$ cell lysates may reflect limited receptor mediated tyrosine kinase activation in the absence of counterbalancing protein dephoshorylation by CD45. Hence, in BCR signal transduction, CD45 expression may be required to properly recruit and activate receptor associated tyrosine kinases.

C2-176 **THE IMMUNOSUPPRESSIVE DRUG DEOXYSPERGUALIN INHIBITS FcGamma RECEPTOR OR LIPOPOLYSACCHARIDE INDUCED RELEASE OF TNF-alpha IN HUMAN MONOCYTES THROUGH INHIBITION OF THE TRANSCRIPTION FACTOR NF-kB.** Rankin, B.M., Kiener, P.A., Nadler, S.G. *From the Department of Autoimmunity and Transplantation, Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle WA 98121.*

Stimulation of the human monocytic cell line THP-1 by either crosslinking FcGamma receptor I or II induces the rapid phosphorylation of multiple intracellular substrates, Ca2+ mobilization, and the subsequent release of cytokines including TNF alpha. Lipopolysaccharide (LPS) stimulation of THP-1 cells also gives rise to protein phosphorylation and increases in the expression of TNF alpha even though the intracellular pathways involved in signaling through FcGamma receptors and LPS are thought to be different. Pretreatment of THP-1 cells for 72 hours with the immunosuppressive drug Deoxyspergualin (DSG), a synthetic analog derived from the antibiotic spergulin, leads to a dose dependent inhibition of TNF alpha release induced through either FcGamma receptor or LPS. DSG pretreatment was not able to significantly alter cell surface receptor expression or FcGamma receptor function in THP-1 cells as measured by phagocytosis of immune complexes and oxidative burst. In addition, DSG pretreatment did not inhibit FcGamma receptor induced protein phosphorylation or Ca2+ mobilization, indicating that DSG's inhibitory effects on TNF alpha release are down stream of the early signaling events. LPS stimulation of THP-1 cells induces nuclear expression of the DNA transcription factor NF-kB (p50/p65). Here we show that FcGamma triggering also results in significant NF-kB activation. In addition, we demonstrate that DSG pretreatment of THP-1 cells leads to a significant inhibition of NF-kB activation, which is normally induced through either FcGamma receptor crosslinking or LPS stimulation. This NF-kB inhibition may explain the immunosuppressant's mechanism of action in suppressing TNF alpha release.

C2-177 SIGNALING PATHWAYS IN MALIGNANT B-1 CLONES. Elizabeth Raveche, M. Balasubramanyam, J. P. Gardner, A. Dang. Dept. of Path. UMDNJ/NJMS Newark, NJ 07103

Alterations in surface receptors involved in signal transduction were studied in malignant B-1 clones which spontaneously develop in NZB mice. These clones serve as a model of human CLL and demonstrate altered expression of surface IgM and CD45, a tyrosine phosphatase. Malignant B-1 cells demonstrated decreased RNA levels of CD45 relative to IgM expression, while non-malignant B cells showed equal levels of IgM and CD45. CD45 exists in several isoforms and B cells predominantly express the highest molecular weight isoform (B220). RNA from both malignant and control B cells expressed this isoform. However, very little protein was detected in the malignant B-1 cells by Western analysis using anti-B220/6B2. Expression of this isoform depends in part upon post-translation glycosylation events which may be abnormal in malignant B-1 clone. F1 recipients of pre-malignant B-1 cells which had been sorted for IgM^{hi}, $B220/6B2^{negative}$ cells developed hyperdiploid malignant donor B-1 clones earlier than did recipients of NZB B-1 cells which were bright for B220/6B2. This indicated that abnormal expression of CD45 may be a pre-requisite for long term growth and malignant transformation. Thus alterations in CD45 may result in abnormal functioning of malignant B-1 cells which may further affect the proliferation of or signaling in these cells. Altered signaling was further analyzed by studying anti-IgM induced responses and cytosolic free calcium [Ca_i] signaling. Basal Ca_i monitored in fura-2 loaded cells was slightly lower in the malignant B-1 cells. However, marked differences were observed in the Ca_i profiles following treatment with anti-IgM. In the leukemic cells,Ca_i transiently increased followed by a decline to prestimulated levels. In contrast, B cells demonstrated a slower, smaller and sustained increase in Ca_i. Two minute pre-incubation with anti-CD45 reduced the anti-IgM stimulated Ca_i response in malignant B-1 cells; whereas nonleukemic cells were not affected. This work shows that alterations in CD45 and Ca signaling responses may play a role in malignant transformation. (NIH AI-29740)

C2-178 INDUCTION OF STABLE IgG 1 to IgA ISOTYPE SWITCH IN A MURINE MONOCLONAL ANTIBODY PRODUCING CELL LINE, Steven M. Rosen and Ellyn Fischberg -Bender, Roche Diagnostic Systems, Inc., Somerville, N.J. 08876.

The maturation and differentiation of B cells into immunoglobulin secreting cells of various isotypes has been reported in the literature to be modulated by several cytokines including IL-5, IL-4, IL-2, IFN-Beta , TGF-Beta and IFN -Gamma. These cytokines are thought to enable isotype switching by providing selective access of switch recombinases to heavy chain constant region genes. The ability to manipulate isotype expression of monoclonal antibody producing cell lines may be useful in certain situations. We have investigated the ability of IL-2 , IL-5 and TGF-Beta to induce the switch of RDS 21 11A6.1, a tetrahydrocannabinol (THC) specific, IgG1 producing hybridoma to the IgA isotype. The RDS 21 11A6.1 cell line is stable and does not undergo an isotype switch to IgA spontaneously after being carried in culture for several months. When 2×10^6 LPS stimulated RDS 21 11A6.1 cells were cultured in the presence of TGF-Beta and IL-5, we were unable to detect the presence of IgA secreting clones. However, when the same number of LPS stimulated cells were cultured for 10 days in the presence of TGF-Beta and IL-2, we were able to isolate 10 IgA positive clones. One of the isotype switched clones that had the highest binding activity in a THC specific solid phase enzyme immunoassay (EIA) was selected for subcloning by soft agar and limiting dilution. This subcloned IgA positive variant is stable in culture for at least 18 passages (6 weeks) and is capable of producing antibody in the absence of any further stimulation by LPS, IL-2 or TGF-Beta. The switched antibody producing clone has maintained it's specificity to THC as determined by a solid phase EIA.

C2-179 FAS/FAS LIGAND-DEPENDENT MURDER AND SUICIDE PATHWAYS IN THE REGULATION AND FUNCTION OF CD4+ T CELL RESPONSES, John H. Russell, Brian Rush and Ruduan Wang, Department of Molecular Biology and Pharmacology, Washington University School of Medicine, St. Louis, MO 63110

We have been interested in the role of TCR/CD3 stimulation coupled to fas ligand/fas activation as a potential mechanism of peripheral tolerance. Evidence is presented that the fas/fas ligand pathway serves both as a murder pathway for CD4+ lytic function and as a self-limiting, cell autonomous suicide pathway to limit CD4 cell expansion and inflammation. TCR/CD3-stimulated death of activated mature T cells is deficient in both *lpr* (fas-deficient) and *gld* (fas ligand-deficient) cells. Activated T cells express both fas and fas ligand and thus murder can take place between TCR/CD3-activated cells. However, "innocent" T cell bystanders are not killed during TCR/CD3-stimulated death as both the killer and the victim must be concomitantly stimulated through their TCR/CD3 complexes. This two signal requirement is most strikingly demonstrated with *gld* T cells. These cells require that both their TCR/CD3 complex and fas be stimulated simultaneously for optimal death. In other cells like the B cell lymphoma A20, anti-fas stimulates death in the absence of a second signal. Such cells can also be killed as "innocent" bystanders in a contact and density dependent interaction with fas ligand+ T cells undergoing TCR/CD3 stimulation. In contrast, TCR/CD3-stimulated death of T cells is density independent supporting a cell autonomous model of suicide.

Only a fraction of cells within a population of T cells is sensitive to TCR/CD3-stimulated death. Cells not dying in the first challenge are dramatically slowed in cell cycle progression, but do not die until they are removed from the stimulus and rechallenged. Therefore fas and fas ligand-dependent TCR/CD3-stimulated T cell death represents a self-limiting mechanism designed more to limit uncontrolled T cell expansion than an absolute mechanism of deletion analogous to that occurring in the thymus.

C2-180 DETERMINATION OF KEY RESIDUES ON ICAM-3 REQUIRED FOR BINDING TO LFA-1, Chanchal Sadhu, Brian Lipsky, Harold P. Erickson, Joel Hayflick, Ken O. Dick, W. Michael Gallatin and Donald E. Staunton, ICOS Corporation, Bothell, WA 98021 and Duke University Medical Center, Durham, NC 27110

The intercellular adhesion molecule -3 (ICAM-3) is a counter receptor for the leukointegrin LFA-1 which supports a number of cell-cell adhesion dependent functions. ICAM-3 is a transmembrane glycoprotein containing five immunoglobulin like domains and is a member of the immunoglobulin superfamily. In this report we describe the characterization of ICAM-3 in terms of its physical shape and the amino acid residues involved in binding to several anti-ICAM-3 monoclonal antibodies and LFA-1. Electron microscopic observations show that the ICAM-3 molecule is predominantly a straight rod of 15 nm in length which suggests a head to tail arrangement of the immunoglobulin-like domains. Of the nine ICAM-3 Mab tested six blocked the interaction with LFA-1 to varying degrees. Epitopes of all of the blocking Mabs were found to be domain 1 dependent. This observation and the characterization of LFA-1 binding to ICAM-3 mutants suggest that the domain 1 (the amino terminal domain) of ICAM-3 interacts with LFA-1. Amino acid substitution studies indicate that the residues E37, T38 and Q75 in ICAM-3 are required for LFA-1 binding. E37 and T38 form a conserved integrin binding site (IBS) which has also been shown to function as IBS in ICAM-1 and VCAM-1. The residue Q75, which is also required for LFA-1 dependent cell adhesion to ICAM-3, is conserved in ICAM-1 and ICAM-2 but not in VCAM-1 and hence may confer integrin binding specificity.

C2-181 **A ROLE FOR SLAM, A NOVEL T-CELL COSTIMULATORY MOLECULE, IN REGULATING HUMAN THYMOCYTE DEVELOPMENT.**

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SCID mice transplanted with pieces of fresh human fetal liver and fetal thymus develop a functional human thymus which sustains lymphopoiesis and in which negative and positive selection of T-cell precursors occurs. Recently a mAb (A12) was developed which recognized a T-cell costimulatory molecule, designated stimulatory lymphocytic activation molecule (SLAM). SLAM is expressed on 20-30% on the single positive (SP) $CD4^+$ and $CD8^+$ and 85%-90% on the double positive (DP) fetal or SCID-hu thymocytes. *In vivo* treatment with anti-SLAM mAb specifically downregulates SLAM expression on the SP $CD4^+$ and $CD8^+$ thymocytes, but not on the DP thymocytes. No increase in the T-cell activation antigens CD25, CD69, CD40L or CD71 are observed and the percentages of SP and DP cells do not change. However, anti-SLAM mAb administration results in a two-fold increase of $CD3^+$ T cells which are $CD45RA^+$, $CD45RO^-$ and $HLA\text{-}DR^-$. These cells represent the most mature thymic T cells which migrate to the periphery. In addition, thymocytes from anti-SLAM-treated SCID-hu mice show enhanced proliferative responses to mitogens *in vitro*. These data suggest that costimulation through SLAM enhances thymic T-cell maturation *in vivo*.

C2-182 DIFFERENTIAL UTILIZATION OF TCR-ASSOCIATED ACTIVATION PATHWAYS IN T CELL SUBSETS, Reinhard Schwinzer and Renate Siefken, Klinik für Abdominal- und Transplantationschirurgie, Medizinische Hochschule, Hannover, D-30625, Germany

Two peripheral T cell subsets can be defined in the human by the expression of the CD45RA and the CD45R0 isoform of the leukocyte common antigen. $CD45R0^+$ T cells are regarded to develop from antigen-stimulated $CD45RA^+$ T cells. This process is associated with various alterations in the response phenotype of the cells since $CD45R0^+$ cells produce a broader spectrum of lymphokines compared to $CD45RA^+$ cells. In the present study we asked whether differentiation of $CD45RA^+$ to $CD45R0^+$ cells is also associated with changes in signalling pathways. To this end the signals required for the induction of IL-2-synthesis were compared in isolated $CD4^+CD45RA^+$ and $CD4^+CD45R0^+$ T cells. Stimulation with crosslinked CD3/TcR antibodies triggered comparable early activation events (tyrosine phosphorylation, activation of $p56^{lck}$, Ca^{2+}-mobilisation) in $CD45RA^+$ and $CD45R0^+$ T cells but did not lead to the induction of IL-2-synthesis. In CD3/TcR-stimulated $CD45R0^+$ cells co-stimulation by accessory cells, anti-CD28 antibodies, and phorbolester was equally effective to induce IL-2-gene transcription and subsequent IL-2-production. In contrast, in $CD45RA^+$ cells only phorbolester was an effective co-stimulatory signal for the induction of CD3/TcR-initiated IL-2-synthesis. IL-2-induction (triggered by anti-CD3/TCR antibodies plus phorbolester) in $CD45R0^+$ cells was less susceptible to CsA-mediated inhibition compared to $CD45RA^+$ cells, suggesting the involvement of Ca^{2+}-independent control mechanisms in IL-2-gene transcription of $CD45R0^+$ cells. This assumption was supported by the observation that IL-2-production was significantly suppressed in $CD45RA^+$ cells by the Ca^{2+}-chelator EGTA whereas $CD45R0^+$ cells were less susceptible. These data suggest that CD3/TcR-associated signalling pathways differ between $CD45RA^+$ and $CD45R0^+$ T cells. Thus, alterations of signal transduction pathways may be one mechanism leading to distinct outcomes of similar stimuli in different T cell lineages and/or T cells belonging to different states of activation.

Supported by the DFG (Sonderforschungsbereich 265).

C2-183 DETECTION OF LIGAND(S) FOR A NOVEL MURINE HEMATOPOIETIC CELL SURFACE MOLECULE, 11F6, Tracy L. Sexton, R. Keith Humphries and Robert Kay, Department of Medical Genetics, University of British Columbia, British Columbia, Canada.

Many hematopoietic cell surface molecules are involved in signal transduction events leading to cellular activation and/or adhesion. In order to fully understand the functional role of these molecules, it is necessary to identify and characterize the ligands with which these molecules interact. Our research focuses on the identification of ligand(s) for 11F6, a cell surface molecule recently cloned in this lab. 11F6 is a transmembrane protein of 820 a.a's containing a somatomedin B domain and regions of homology to von Willebrand Factor and the selectin family. Flow cytometric analysis of normal murine bone marrow cells has revealed expression of 11F6 on a significant proportion of both myeloid and lymphoid cells.

At present, we are using two methods to detect the presence of cells expressing ligands for 11F6. The first method uses 11F6 retrovirally infected 3T3 cells as targets upon which fluorescently labelled test cells are added. Binding is monitored visually, then measured quantitatively using a fluorescence plate reader. Preliminary studies using ICAM-1 expressing cells as target controls and LFA-1 expressing test cells indicate that binding can be detected visually and measured quantitatively. In the second method, the extracellular domain of 11F6 is fused to the Fc region of hIgG1 to create a soluble fusion protein. This protein is fluorescently labelled and used as a probe to detect binding to various cell lines and primary cells. Quantitative measurements of binding are then determined using FACS. Using these two methods, we have screened various hematopoietic cell lines and primary cells; the results of which will be presented.

C2-184 **MICE DEFICIENT IN THE COSTIMULATORY MOLECULES, B7-1 AND B7-2** **Arlene H. Sharpe[1], Frank Borriello[1], Elizabeth A. Tivol[1], , Gordon J. Freeman[2] [1]Brigham and Women's Hospital, [2]Dana-Farber Cancer Institute, ,and [1,2]Harvard Medical School, Boston, MA, USA**

Signaling via the B7:CD28/CTLA4 costimulatory pathway can provide a potent costimulatory signal, preventing the induction of anergy. The discovery of a second costimulatory molecule, B7-2, as the major, early activating costimulator in this pathway indicates that signaling through this pathway is more complex than previously thought. It is unclear whether B7-1 (CD80) and B7-2 (CD86) mediate distinct or overlapping costimulatory functions. Although they demonstrate only modest amino acid conservation, B7-1 and B7-2 have been shown to equally costimulate T cell proliferation and IL-2 production through CD28 in vitro. The early expression of B7-2 has led to the hypothesis that B7-2 may participate in the initiation of the immune response, thereby playing a pivotal role in the decision between T cell activation and anergy, whereas mB7-1, being expressed later, may serve to amplify or regulate the immune response. To analyze the in vivo function of the costimulatory molecules B7-1 and B7-2, we have generated mice lacking or overexpressing B7-1 or B7-2. These mice provide a definitive means for determining whether B7-1 and B7-2 have complementary or overlapping functions in vivo. The importance of B7-1 for regulating in vivo T cell responses previously had been inferred from studies with CTLA4Ig fusion protein. The B7-1 deficient (B7 -/-) mouse strain provided the first in vivo evidence for the existence of functional alternative CTLA4 counter-receptors. Despite lacking B7-1 expression, activated B cells from B7-1 -/- mice still bound CTLA4, demonstrating that alternative CTLA4 ligand(s) exist. Initial evaluation of the B7-1 -/- mice revealed only a partial defect in the immune response due to the existence of additional CTLA4 counter-receptors. Unstimulated B7-1 -/- mice respond normally to mitogens. However, activated B7-1 -/- B cells have a 70% reduction in costimulation of the response to alloantigen, with a corresponding reduction in IL-2 production. The finding of functional alternative CTLA4 counter receptors in the B7-1 -/- mouse strain provided impetus to search for additional CTLA4 counter receptors and resulted in the cloning of murine mB7-2. Our initial characterization of the B7-2 deficient mouse strain is in progress.

C2-185 THE EFFECT OF BLOCKING CO-STIMULATION ON ANTI-CD3 IMMUNOSUPPRESSION IN VIVO

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2C11, an anti-murineCD3ε mAb, has been shown to significantly prolong skin graft survival in mice. Its in vivo immunosuppressive mechanisms include TCR modulation, T cell depletion, and a long-lasting state of unresponsiveness. We hypothesized that the T cell unresponsiveness may be a consequence of TCR triggering in the absence of CD28 mediated co-stimulation. Thus, blocking CD28/B7 interactions could potentially enhance anergy. We investigated the effects of CTLA4Ig, a CD28 antagonist, on anti-CD3 induced immunosuppression. Two approaches were used: treatment of mice with exogenous human CTLA4Ig, and CTLA4Ig transgenic mice. In both models, CTLA4Ig alone had no effect on skin graft rejection. However, anti-CD3 treatment of transgenic animals resulted in a significant prolongation of graft survival over anti-CD3 in nontransgenics (50%survival for no anti-CD3, 11 days; tg neg.+2C11, 25 days; tg pos.+2C11, 34 days) We next examined the effect of CLTA4Ig on anti-CD3 induced T cell unresponsiveness. CTLA4Ig did not affect early activation of T cells, as defined by upregulation of CD25, and lymphokine production, modulation of the TCR, or late events such as T cell depletion. However, CTLA4Ig blocked or possibly reversed the development of T cell unresponsiveness. Therefore, two conclusions can be drawn: The enhanced immunosuppression with CTLA4Ig is most likely not due to augmented anergy induction by anti-CD3. CTLA4Ig's effect on unresponsiveness is not a result of FcR mediated blockade of T cell activation. Current studies are underway to examine the individual roles of B7-1 and B7-2.

C2-186 CD43 IS A MURINE T CELL COSTIMULATORY RECEPTOR THAT FUNCTIONS INDEPENDENTLY OF CD28,

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Costimulation through CD28 ligation has been shown to play an important role in the development of a vigorous T cell immune response. However, CD28-deficient mice can mount an effective immune response, and T cells from CD28-deficient mice can be induced to proliferate *in vitro*. These data suggest that other costimulatory molecules may play a role in T cell activation. In our search for other costimulatory receptors on T cells, we have characterized a monoclonal antibody that can costimulate T cells in the absence of accessory cells. This mAb, called R2/60, was found to synergize with T cell receptor (TCR) engagement in inducing proliferation. This mAb does not react with CD28 and costimulates in a CD28 independent fashion since the mAb costimulates T cells from the CD28-deficient mice as well as normal. In addition, independent ligation of CD3 and the ligand recognized by R2/60 also results in T cell proliferation, suggesting that the two molecules do not have to co-localize in order to activate the R2/60 costimulatory pathway. Western analysis of whole cell lysates and immunoprecipitation of cell surface labeled proteins demonstrated that R2/60 reacted with two monomeric proteins with apparent molecular weights of 105 kD and 115 kD. Expression cloning of the R2/60 antigen identified the ligand as murine CD43. Further analysis of the role of CD43 in T cell activation demonstrated that anti-CD43 (R2/60) enhanced allogenic stimulation of T cells. The increased T cell proliferation observed in the presence of anti-CD43 was due to a direct effect on T cells and not mediated by anti-CD43 binding to APC since the R2/60 mAb also enhances a xenogenic MLR. Together, the data demonstrate that CD43 can serve as a receptor on T cells that can provide CD28-independent costimulation.

C2-187 ROLE OF THE CD40-CD40L INTERACTION IN T CELL CONTACT-DEPENDENT ACTIVATION OF MONOCYTE IL-1 SYNTHESIS.

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Most studies of the induction of cytokine synthesis in monocytes have employed an exogenous triggering agent such as LPS. However, in nonseptic inflammatory responses monocyte activation occurs as a result of T cell generated signals. In previous reports, we and others have demonstrated that contact-dependent T cell generated signals are capable of contributing to macrophage activation. We have shown that plasma membranes from anti-CD3 activated purified peripheral $CD4^+$ T cells (Tm^A) but not from resting $CD4^+$ T cells (Tm^R) induce monocytes to synthesize IL-1 in the absence of costimulatory cytokines. Studies to determine the expression kinetics of the molecule(s) unique to activated $CD4^+$ T cells which interact with monocytes to induce IL-1 revealed that optimal expression occurred at 6h post activation. This matched the previously reported kinetics of expression of CD40L on activated peripheral T cells, implicating the CD40-CD40L interaction as a candidate for the initiator of the IL-1 signaling event. The ability of Tm^A to induce IL-1 synthesis in resting monocytes could be markedly reduced by addition of a monoclonal anti-CD40L antibody. In addition, a monoclonal anti-CD40 IgM proved dramatic in its ability to induce resting monocytes to synthesize IL-1. These results demonstrate that the CD40-CD40L interaction provides a critical component of $CD4^+$ T cell contact-dependent activation of monocyte IL-1 synthesis.

C2-188 CHARACTERIZATION OF THE -73 TO -48 PROXIMAL REGULATORY ELEMENT IN THE INTERFERON-γ PROMOTER IN T CELLS, Marianne T. Sweetser, Laurie A. Penix*, William M. Weaver and Christopher B. Wilson, Departments of Pediatrics and Immunology, University of Washington, Seattle, WA 98195, *Department of Pediatrics, Yale University School of Medicine, New Haven, CT 06520

Interferon-γ (IFN-γ) is expressed following activation by primed or memory T cells and by the Th1 subset of CD4+ T cells, but not by naive T cells. This suggests that IFN-γ may be differentially regulated compared to the IL-2 gene. By transient transfection of β-galactosidase reporter constructs containing various amounts of 5' flank of the human IFN-γ gene into Jurkat T cells, we previously demonstrated that constructs containing the region between -108 and -40 conferred activation specific expression in T cells. Within this region are two essential regulatory elements, the -96 to -80 distal element and the -73 to -48 proximal element, which are conserved across species and deletion of which reduces expression by 70%. To determine whether the proximal element could confer T cell activation specific expression, a dimer of this element was inserted into the β-galactosidase reporter construct and tested by transfection into Jurkat T cells. The dimer of the proximal element gave expression comparable to the -108 to -40 construct, was dependent on induction with ionomycin and PMA and was blocked by cyclosporin A. The proximal element shares homology with the NF-IL2A element of the IL-2 promoter and in gel mobility shift assays binds multiple complexes. Unlike NF-IL2A which binds Oct-1, Oct-2 and fos/jun, the IFN-γ proximal element binds members of the ATF/CREB family as well as a member of the jun family. Mutations within the proximal element affect binding of the various ATF/CREB family members and reduce expression in transient transfection assays in Jurkat T cells. Differences in the proteins binding to the proximal element are also seen between Th1, Th2 and naive T cells which correlated with IFN-γ expression. These differences, in addition to methylation of a CpG dinucleotide within this element, may play a role in the differential expression of IFN-γ.

C2-189 REQUIREMENT OF PHOSPHOLIPASE C-γ2 ACTIVATION IN SURFACE IgM- INDUCED B CELL APOPTOSIS, Minoru Takata[1], Yoshimi Homma[2], and Tomohiro Kurosaki[1]. [1]Department of Cardiovascular Molecular Biology, Lederle Laboratories, Pearl River, NY 10965, and Section of Immunobiology, Yale University School of Medicine, New Haven, CT 06510-8023. [2]Department of Biosignal Research, Tokyo Metropolitan Institute of Gerontology, Itabashi-ku, Tokyo 173, Japan.

Immature B cells and some B cell lines undergo apoptotic cell death when activated through surface IgM, a phenomenon that is related to elimination of self-reactive B cells. Surface IgM stimu- lation induces the tyrosine phosphorylation of several cellular substrates, including phospholipase C (PLC)-γ2 which is involved in the activation of phosphatidylinositol pathway. Surface IgM-induced apoptosis was investigated with B cell lines deficient in Lyn, Syk, or PLC-γ2 which were generated by gene-targeting. In Syk- or PLC-γ2-deficient B cells, the induction of apoptosis was blocked, but was still observed in Lyn-deficient cells. Src homology 2 domains of PLC-γ2 were essential for both its activation and surface IgM-induced apoptosis. Since tyrosine phosphorylation of PLC-γ2 is mediated by Syk, these results indicate that activation of PLC-γ2 through Syk is required for surface IgM-induced apoptosis.

C2-190 **RAF-1, EITHER ALONE OR IN COMBINATION WITH CALCIUM IONOPHORE, ACTIVATES MULTIPLE PROMOTER ELEMENTS IMPORTANT IN T CELL ACTIVATION** David A. Taylor-Fishwick, Sharon A. Twitty and Jeffrey N. Siegel, Immune Cell Biology Program, Naval Medical Research Institute, Bethesda, MD.

Crosslinking of the T cell receptor for antigen (TCR) induces activation of tyrosine kinase activity leading to activation of Ras and a pathway causing increased intracellular Ca^{2+}. Several effectors of Ras have been identified including Raf-1, PI-3 kinase and MEK kinase. By transient co-tranfection of the human T-cell line Jurkat, we have explored the role of Raf-1 in regulating transcriptional events in TCR signal transduction. Using the CAT reporter gene, we have shown that a dominant negative form of Raf-1 (C4BR) completely inhibited PMA-and-ionomycin induced transcription by the IL-2 gene promoter as well as transcription mediated by several of its key promoter elements AP-1, NFAT and IL-2A. The Raf-1 dominant negative also inhibited TCR-mediated activation of the NFAT element. To further investigate the role of Raf-1, we transfected Jurkat cells with a constitutively active form of Raf-1 (BXB) and left cells untreated or stimulated them with PMA and/or ionomycin. We found several patterns of Raf-1 effects. On the AP-1 promoter element, constitutively active Raf-1 alone was sufficient to induce transcription of the reporter gene. Similarly, constitutively active Raf-1 was sufficient to induce transcription from the Elf-1 binding site in the GM-CSF promoter. In contrast, Raf-1 had no effect on the NFAT promoter element by itself but synergized with ionomycin to activate transcription in a dose-dependent manner. Finally, the effects of Raf-1 were studied on the expression of the surface marker CD69. PMA-induced expression of CD69 was inhibited by the dominant-negative Raf-1 construct while constitutively active Raf-1 alone induced CD69 expression. We conclude that Raf-1 activation is sufficient, by itself or in combination with a Ca^{2+}-dependent pathway, to activate multiple promoter elements involved in T cell activation.

C2-191 SIGNAL TRANSDUCTION PROPERTIES OF THE TRANSMEMBRANE AND CYTOPLASMIC DOMAINS OF THE TCR ß CHAIN, Georg Tiefenthaler and Ed Palmer, Basel Institute for Immunology, CH-4005 Basel, Switzerland.

The antigen specific T cell receptor (TCR) is composed of the α/β or γ/δ TCR heterodimers which are non covalently associated with a group of invariant polypeptides collectively referred to as CD3. While the antigen specificity of the TCR is determined by the variable domains of the α/β or γ/δ chains, the components of the CD3 complex are necessary for the assembly of, and signal transduction through, the TCR complex.
We were interested in the role of the constant domain of the TCR β chain in connecting antigen recognition with triggering T cell functions. To this end we introduced random single point mutations into the transmembrane and cytoplasmic domains of TCR β and introduced these mutant TCR β chains into a TCR β-negative T cell hybridoma.
First results show that some conserved amino acids in the transmembrane domain of the TCR β chain cannot be replaced without affecting proper assembly and/or expression of the TCR complex. On the other hand, other single amino acid substitutions allow TCR expression but modulate rather than abolish TCR signalling. An explanation for these unexpectedly subtle effects of single point mutations could be that signalling through TCR β is mediated by redundant features present either within the TCR β sequence alone or in TCR α as well. To test this possibility we are presently investigating the signalling properties of mutant TCRs containing multiple mutations in either TCR β or in TCR α and β.

C2-192 **Mapping and Discrimination of the Interaction of the Human B7 Receptors B7.1 and B7.2 with Human CD28.**

A. Truneh[1], M. Reddy[1], P. Ryan[1], D. Couez[1], R. Sekaly[2], D. Olive[3] and R. Sweet[1]: [1]SmithKline Beecham Pharmaceuticals, King of Prussia, Pennsylvania, USA, [2]IRCM, Montreal, Canada and [3]INSERM Unité 119, Marseille, France.

A model was developed to study the interaction of CD28 with the B7 counter-receptors, B7.1 (CD80) and B7.2. HuCD28 and HuB7 genes were transfected into murine T cell hybridomas and Lmtk- fibroblasts respectively. Uniformly high levels of cell surface CD28 and B7 expression were achieved by repetitive flow sorting and the expressed molecules retained all of the native mAb epitopes. The expressed HuCD28 molecule was fully functional as determined by its ability to mediate T cell activation, including Ca^{2+} mobilization, IL-2 promoter induction, IL-2 secretion, TNF-α production and apoptosis. The HuCD28 was also able to mediate cell attachment/adhesion to, and stimulation by, the B7 proteins on the Lmtk- cells. Although B7.1 and B7.2 share only 26% sequence identity in their extracellular domains, they were equivalent in their recognition of CD28 in these assays. Utilizing this system and site directed mutagenesis, we mapped residues on CD28 required for recognition of the B7 counter-receptors. The primary determinant of binding to both B7 proteins is the unique, and phylogenetically conserved, MYPPPY sequence located in the CDR3-like region. However, within this region certain conservative subtitutions discriminated between these recpetors, ablating binding to B7.2 without affecting the interaction with B7.1. A panel of mAbs which recognize distinct epitopes on HuCD28 or HuB7, and soluble Ig-fusion receptors including CTLA4-Ig, were used along with the CD28 mutants to further characterize the interactions between CD28 and B7. The epitopes recognized by these mAbs overlap with, or are in close proximity to, the recognition elements involved in CD28 and B7 interactions. We conclude that immunoreactivity of most of the anti-CD28 murine mAbs resides within the CDR-like regions of CD28, and that these regions, particularly CDR3, are involved in CD28-B7 receptor-counter-receptor recognition.

C2-193 **FUNCTIONAL CHARACTERIZATION AND EXPRESSION OF MURINE CTLA-4,** Theresa L. Walunas, Christina Y. Bakker, Deborah J. Lenschow, Craig B. Thompson and Jeffrey A. Bluestone. Committee on Immunology, the University of Chicago, Chicago, IL 60637

CD28 is a glycoprotein on T cells that binds to B7 family molecules, providing a co-stimulatory signal following TCR ligation, which is essential for IL-2 production and T cell proliferation. Less is known about the function and expression of CTLA-4, a CD28 homologue that also binds B7-1 and B7-2. We developed a panel of mAb against murine CTLA-4 and used these reagents to characterize CTLA-4 expression and function on resting and activated T cells. First, we have shown that CTLA-4 is not expressed on resting T cells but is maximally expressed 48 hours following activation with anti-CD3 mAb. Addition of the immunosuppressant, cyclosporin A, effectively blocks CTLA-4 induction following anti-CD3 signalling, even in the presence of IL-2. Furthermore, CD28 ligation by B7-2 is critical for maximal CTLA-4 expression. Anti-CD28 Fab fragments, CTLA4Ig and anti-B7-2, but not anti-B7-1 mAbs, block CTLA-4 expression when anti-CD3 is present. Similarly, T cells from CD28 deficient mice do not express similar levels of CTLA-4 following anti-CD3 activation. CD28 function in CTLA-4 expression could replaced only partially by the addition of IL-2. These results suggest that CTLA-4 is a highly regulated molecule whose expression is dependent on TCR ligation and CD28 co-stimulation.

Given the homology of CTLA-4 to CD28, it has been postulated that CTLA-4 may play a role in T cell co-stimulation. In fact, in allogeneic MLR, anti-CTLA-4 mAb augment T cell proliferation. However, monovalent Fab fragments, which cannot be crosslinked by FcR to transduce a signal, also augment proliferation, Moreover, intact anti-CTLA-4 mAb, but not Fab fragments, inhibited T cell proliferation to suboptimal doses of anti-CD3 in the presence of co-stimulation by anti-CD28 mAb. These results suggest that CTLA-4 may downregulate T cell activation via the interaction with a counter-receptor. Finally, studies are underway examining the role of CTLA-4 in *in vivo* immune responses.

C2-194 CTLA4Ig INHIBITS A T-CELL-DEPENDENT ANTIBODY RESPONSE IN CYNOMOLGUS MONKEYS, G. Warner[1], L. Johanson[1], D. Gonchoroff[1], R. Weiner[2], W. Shyu[2], N. Srinivas[2], P. Linsley[3], K. Cabrian[4], and T. Davidson[1]. Departments of Biologics Evaluation[1], Metabolism and Pharmacokinetics[2], Autoimmunity[3], and Bio-Process Research[4], Pharmaceutical Research Institute, Bristol-Myers Squibb Co., Syracuse, NY and Seattle, WA.

Studies were conducted to evaluate CTLA4Ig-mediated immunosuppression in non-human primates. Cynomolgus monkeys were administered 0, 0.67, 2.0 or 6.0 mg/kg CTLA4Ig intravenously, two-times per week for three weeks. On Day 1 (first day of dosing) and Day 102 animals were immunized with sheep red blood cells (SRBC: iv, 1.7 ml/kg of a 10% mixture). Serum and whole blood samples were obtained pre-dose and at least 4 weeks following primary and secondary immunization for assessment of anti-SRBC antibodies, serum gamma globulin levels, pharmacokinetics, clinical hematology and lymphocyte subpopulations ($CD2^+$, $CD4^+$, $CD8^+$, and $CD20^+$). A dose dependent decrease in anti-SRBC antibodies was observed following primary immunization, with maximal suppression of >95%. Monkeys treated with CTLA4Ig were able to respond to a secondary challenge with SRBCs (Day 102, no circulating levels of CTLA4Ig); the response in the high dose monkeys was similar to the primary response observed in control monkeys following a single immunization with SRBCs. No treatment-related changes were noted in the frequency of T-cells, or subpopulations of T-cells. Pharmacokinetic analysis suggests linearity between dose and serum levels of CTLA4Ig (C_{max} and AUC), with a T½ of between 106 and 161 hours. The results of this study indicate that CTLA4Ig can inhibit the antibody response directed against SRBCs in cynomolgus monkeys and that the cynomolgus monkey provides a useful model for determining the safety and pharmacology of CTLA4Ig in pre-clinical studies.

C2-195 TCR-CD4 and TCR-TCR Interactions in the TCR dependent signal transduction of T Cells

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Ligating the TCR or CD3 with monoclonal antibodies leads to proliferation of the mouse T cell line D10 and an increase in intracellular free calcium (Ca++). An exceptional D10 TCR antibody, 16A, was able to some extent stimulate proliferation of D10 but did not induce an increase in Ca++, even when extensively crosslinked with anti-immunoglobin. However when 16A was cross-linked to CD4 a rise in Ca++ was observed. Additional experiments were performed with a transfectant containing the D10 TCR and CD4, the results from these experiments were identical to those obtained from the original cell line. We also studied whether CD4 is necessary for the increases in Ca++ obtained with other anti-TCR antibodies. This was examined in a $CD4^-$ T cell transfected with cDNA:s encoding the D10 TCR. These cells could be induced to flux calcium by the same anti-TCR antibodies that gave this response in D10 cells, and again 16A failed to induce such a response. We have also studied the tyrosine phpsphorylation patterns resulting from CD4-TCR and the TCR-TCR interactions and the modulatory effect of the CD45 molecule on increases in Ca++ and tyrosine phosphorylation patterns resulting from these interactions. We found that these parameters were essentially similar in signal transduction resulting from CD4-TCR and the TCR-TCR interactions. Taken together these studies document that CD4-TCR interactions are an independent and in the case of 16A obligatory, component in the initiation of signal transduction by the TCR, but is not absolutely required for this. CD4 did not alter the emanating signal qualitatively. The results are consistent with a purely amplifying function for CD4 in the initiation of signal transduction. However this amplifying function can be essential for signalling and thus also have a qualitative dimension.

Antigen Presentation and Tolerance

C2-200 MHC CLASS II MOLECULES BIND UNFOLDED PROTEINS IN THE ENDOPLASMIC RETICULUM (ER) AND TRANSPORT THEM TO AN ENDOSOMAL COMPARTMENT. Gerald Aichinger, Lars Karlson, Per Peterson, RW Johnson PRI, The Scripps Research Insitute. 10666 North Torrey Pines Road. La Jolla, Ca 92037

The mechanism and site of loading of endogenous antigens, presented by MHC class II, has not been demonstrated. We coexpressed constructs of influenza hemagglutinin (HA) with MHC class II molecules, DR4Dw14 or DR1, in the absence of the invariant chain in HeLa cells and show that HA binds to class II molecules in the endoplasmic reticulum (ER). Subsequent folding of HA is inhibited in the presence of class II molecules as detected by conformation sensitive antibodies. Transport of HA out of the ER is dependent on correct folding (1, 2). In the presence of MHC class II molecules, however, unfolded HA is transported to the cell surface of HeLa cells and endocytosed into endosomal vesicles. As expected, coexpression of the invariant chain (p31/33) inhibits protein binding in the ER. The inhibition, however, is dependent on the expression level of the p31/33.

We conclude from our experiments, that low or absent expression of the invariant chain allows binding of unfolded or partially folded endogenous proteins to MHC class II molecules in the ER. Transport of the complexes to an endosomal compartment permits proteolysis and trimming of the bound protein and subsequent presentation by MHC class II molecules.

1. Copeland, C. S., et al. 1986. J. Cell Biol. 103:1179.
2. Gething, M.-J. et al. 1986. Cell 46: 939.

C2-201 STAINING OF ANTIGEN SPECIFIC T CELLS WITH MULTIMERIZED CLASS II MHC MOLECULES. John Altman, Michael McHeyzer-Williams, Yoshinori Fukui, Jay Boniface and Mark Davis, Department of Microbiology and Immunology and The Howard Hughes Medical Institute, Stanford University, Stanford, CA 94305-5428

Previously, we have shown that truncated soluble class II molecules produced as isolated subunits in *E. coli* can be folded *in vitro* and are indistinguishable from native proteins with respect to specific peptide binding and the abilitiy to stimulate antigen specific T cells. We have expanded the characterization of the *E. coli* derived proteins to include molecules designed to be multimeric in solution. One of the MHC multimers we have produced forms a complex with an αβ T cell receptor with a half life that is 10-20 fold longer than we observe for monomeric MHC molecules and is able to stain antigen specific T cells in flow cytometric applications. These data suggest that MHC multimers may be useful for tracking antigen specific T cells during the course of an immune response or the development of a specific T cell repertoire.

C2-202 IG-LINKED GENETIC SUSCEPTIBILITY TO AUTOIMMUNE DISEASE REFLECTS MOLDING OF THE T CELL REPERTOIRE BY IG DERIVED PEPTIDES *Anne C. Avery, #Alejandro Rodriguez, #C. Stephen Foster and *Harvey Cantor, *Department of Pathology, Harvard Medical School, Department of Ophthalmology, Harvard Medical School, Boston, Ma 14853

INTRODUCTION: Herpes stromal keratitis (HSK), is a virally induced immune-mediated disease which destroys host corneal tissues following infection with HSV-1. Studies of murine HSK have indicated that susceptibility is controlled by genes linked to the Igh allotype locus. Mice bearing the Igh^b allele (CB-17) are resistant to the development of stromal keratitis after ocular infection with HSV, whereas congenic mice bearing the Igh^d (CAL-20) or ^{c} are susceptible. Here we test the hypothesis that allotype-linked susceptibility to this disorder might reflect differential molding of the T cell repertoire by polymorphic variants of Ig-derived peptides.

RESULTS: Monoclonal $IgG2a^b$ antibody of irrelevant specificity, when given in a tolerogenic form, prevented the development of HSK. Similar administration of $IgG2a^c$ had no effect on the course of HSK. Conversely, T cells produced by immunization with $IgG2a^b$ in adjuvant could transfer disease to normally resistant nude mice whereas T cells produced by immunization $IgG2a^c$ could not. To define the peptides derived from $IgG2a^b$ that might be responsible for these effects, we synthesized peptides from regions of the $IgG2a^b$ molecule which differed from $IgG2a^c$, and which corresponded to T cell motifs. Tolerization with peptide 249-264 from $IgG2a^b$, but not peptides from other regions of$IgG2a^b$, succeeded in preventing HSK.

CONCLUSION: HSK may be mediated by a T cell clone which is deleted by mice bearing $IgG2a^b$. Thus, Ig-derived peptides may have a significant influence on the molding of the T cell repertoire and may explain other allotype-linked disease associations. Our results also raise the possibility that successful treatment of autoimmune disease with pooled human Ig may result from modulating T cells which are cross reactive with Ig-derived peptides.

C2-203 EPITOPE REPETITIVENESS AS A SELF/NON-SELF DISCRIMINATOR FOR B CELLS Martin F. Bachmann, Urs Hoffmann-Rohrer, Hans Hengartner, Rolf M. Zinkernagel, Institute for Experimental Immunology, University Hospital Zürich, 8091 Zürich, Switzerland

The glycoprotein (G) of vesicular stomatitis virus (VSV) exists in three different forms: a highly repetitive (organized) form present in the viral envelope, a poorly organized form associated with cell membranes and micells and a non-organized, soluble form lacking the transmembrane and cytoplasmic region. We found that the highly organized form of VSV-G behaves like a strong T helper cell independent type I antigen, whereas the poorly organized form was a week type II antigen only. Furthermore isotype switched memory B cells could be activated by the highly organized but not by the poorly organized form of VSV-G, indicating that B cell receptor crosslinking is a key event for B cell activation.

Transgenic mice expressing the membrane form of VSV-G exhibited an unresponsive (anergic) B cell repertoire. However, these unresponsive B cells could be activated by the highly organized from of VSV-G, breaking B cell tolerance. Conversely, VSV-G specific mature T helper cells were peripherally tolerized by the non-organized form of VSV-G expressed in a different transgenic mouse line, leading to phenomenological B cell unresponsiveness. Thus, the highly organized form of VSV-G on one side of the antigenic spectrum induced poorly controlled primary and secondary B cell responses, culminating in a break of B cell tolerance. The non-organized form of VSV-G, on the other side of the spectrum, tolerized mature peripheral T helper cells, leading to apparent B cell unresponsiveness. Since many if not most viruses and bacterial cell surfaces exhibit a high degree of antigenic organization comparably to VSV whereas vertebrate cell membranes are poorly- or non-organized, these results indicate that antigen organization is taken as a marker for foreigness by B cells. Thus, antigen orgnization is an important factor for self/non-self discrimination by the immune system.

C2-204 GENETICALLY ATTENUATED BACTERIAL SUPERANTIGEN VACCINES

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Bacterial superantigens are associated with several serious diseases. These exotoxins bind to class II molecules of the major histocompatibility complex (MHC) and the pathological effects are a consequence of a massive stimulation of T cells. In an effort to develop an effective vaccine, we have tested two kinds of staphylococcal enterotoxin A site-directed mutants for their efficacy as potential vaccines: Y92A, which has diminished MHC class II binding, and Y64A, which has attenuated T-cell receptor recognition. T-cell responses *in vitro* to the SEA mutants were greatly reduced, and their toxicity in mice was attenuated. Mice vaccinated with mutant toxins had high levels of antibody against SEA and were fully protected against a challenge with nonmutant toxin. In addition, responding T cell receptor profiles from mice immunized with SEA Y92A suggested this mutant was being presented by a conventional, non-superantigenic pathway.

C2-205 CD1 MOLECULES: A THIRD PATHWAY OF ANTIGEN PRESENTATION. Evan M. Beckman, Steven A. Porcelli, Stephen Furlong and Michael B. Brenner. Lymphocyte Biology Section, Dept. of Rheumatology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115.

MHC class I and class II molecules present peptide antigens to αβ T cells. An analogous antigen presenting function has been proposed for the non-MHC encoded CD1 molecules, expressed on most professional antigen presenting cells. We have previously demonstrated that CD1 molecules are recognized as ligands by certain autoreactive $CD4^-CD8^-$ T cells and isolated multiple examples of αβ T cells restricted by either CD1b or CD1c to foreign microbial antigens. Although associated with β2-microglobulin, the nonpolymorphic CD1 glycoproteins have little direct sequence homology with MHC class I or class II molecules and are predicted to have a different secondary structure in their α2 domains. This suggested that the antigens presented by CD1 might be quite different from peptide antigens bound by MHC molecules. To determine the chemical nature of the antigens presented by CD1 molecules, we purified the CD1b restricted antigen recognized by one representative CD4-CD8- αβ T cell line from *Mycobacteria tuberculosis* sonicates. The protease resistant and hydrophobic CD1b restricted antigen was purified by rpHPLC and shown to be mycolic acid, a family of long chain α-branched, β-hydroxy fatty acids unique to mycobacteria and several closely related species. The recognition in this system is not the result of a mitogenic response, as other mycobacteria specific T cells not restricted by CD1b do not respond to purified mycolic acid. Thus, at least one member of the CD1 family appears to have functionally diverged from classical MHC molecules to present lipid antigens. This non-MHC restricted recognition of nonprotein antigens enlarges the potential repertoire of antigens recognized by αβ T cells.

C2-206 **Trafficking of MHC class II molecules to the endocytic pathway.** *P. Benaroch*, M. Yilla G. Raposo and H.L. Ploegh.* *CNRS UPR415 Paris. France and M.I.T. E17-322. Cambridge .USA.*PB and MY contributed equally to this work*

We have examined trafficking of MHC class II molecules in human B cells exposed to concanamycin B, a highly specific inhibitor of the vacuolar H^+-ATPases required for acidification of the vacuolar system and for early to late endosomal transport. Neutralization of vacuolar compartments prevents breakdown of the invariant chain (Ii) and blocks conversion of MHC class II molecules to peptide-loaded, SDS stable αβ dimers. Ii remains associated with αβ and this complex accumulates internally, as ascertained biochemically and by morphological methods. In cells exposed to concanamycin B, but not to the protease inhibitor leupeptin, a slow increase (>20-fold) in surface expression of Ii, mostly complexed with αβ, is detected. This surface-disposed fraction of αβIi is nonetheless a minor population that reaches the cell surface directly, or is routed via early endosomes as intermediary stations. In concanamycin B-treated cells, the bulk of newly synthesized αβIi is no longer accessible to fluid phase endocytic markers. We concluded that the majority of αβIi is targeted directly from the TGN to the compartment for peptide loading, bypassing the cell surface and early endosomes en route to the endocytic pathway.

C2-207 ALLOREACTIVE T CELL RECOGNITION OF MHC-PEPTIDE COMPLEXES. Ned S. Braunstein, Miera Harris, Henry Rascoff, Yuan Zhang, Elizabeth Hexner, Catherine Grubin[†], Alexander Rudensky[†], Jose Eguia, and Govind Bhagat. Department of Medicine, College of P&S, Columbia University, New York, NY 10032 and [†]Howard Hughes Medical Institute and Department of Immunology, University of Washington School of Medicine, Seattle, WA 98195

To better understand the peptide dependency and specificity of alloreactive T cell responses *vis a vis* self-MHC restricted responses, we generated $CD4^+$ T hybridomas that are alloreactive for the complex $I\text{-}A^b$ + $E\alpha_{52\text{-}68}$. C3H mice were immunized with B10.A(5R) splenocytes. $CD4^+$ T cells recovered from the animals were re-stimulated *in vitro* with Eα peptide and $I\text{-}A^b$-positive, HLA-DM negative, T2 transfectants and fused. Of 44 hybridomas screened, 7 respond to T2-$I\text{-}A^b$ only in the presence of Eα peptide. These hybridomas also respond to B10.A(5R) splenocytes that constitutively express this MHC-peptide complex but do not respond to syngeneic C3H spleen cells. Surprisingly, the alloreactive T cells also respond to one or several murine $I\text{-}A^b$-positive APCs in the absence of the Eα peptide. At the very least, the results indicate that these alloreactive T cells respond to at least two different $I\text{-}A^b$-peptide complexes and may indicate that these T cells instead see a motif common to many $I\text{-}A^b$-peptide complexes. Experiments to address this are underway. Consistent with the hybridomas' common specificities, analysis of their TCR β chain sequences reveals that all of the alloreactive T cell hybridomas use Vβ6 and related Jβ segments.

To directly compare alloreactive and self-MHC restricted responses, we also generated, from B6 mice, a series of antigen-specific, self-MHC restricted T hybridomas that react with the same MHC-peptide complex. As expected, the self-MHC restricted T cells respond to a variety of $I\text{-}A^b$-positive APC only in the presence of the relevant antigen. Of interest, ~50% of these self-MHC restricted T cells, like the alloreactive T cells, use Vβ6 TCR. Experiments comparing the peptide fine specificity of the alloreactive and restricted T cells will be presented. These experiments should resolve whether alloreactive T cells discriminate amongst MHC peptide complexes with the same fine specificity as do self-MHC restricted T cells or if instead alloreactive T cells recognize motifs common to related MHC-peptide complexes.

C2-208 **STRUCTURAL COMPONENTS OF HUMAN TCRs WHICH RECOGNIZE INFLUENZA HEMAGGLUTININ 307-319 IN THE CONTEXT OF HLA-DR4 OR DR7**, James V. Brawley and Patrick Concannon, Virginia Mason Research Center, 1000 Seneca Street, Seattle, WA 98101 and Department of Immunology, University of Washington, Seattle, WA 98195

The human T cell clone 3BC6.6 reacts with the peptide antigen HA 307-319 only when presented by DR4 and utilizes a member of the Vα1 subfamily and Vβ3.1. A second T cell clone, JS515.11 which recognizes the HA 307-319 peptide only in the context of DR7, utilizes a different member of the Vα1 subfamily in conjunction with Vβ3.1. These receptors differ only by 3 positions within the Vα1 coding region and by a number of substitutions in the CDR3 regions of the α and β chain. In order to map sites on the TCR responsible for the DR restriction in this system, the TCR genes were cloned into expression vectors and introduced into a receptor negative Jurkat cell line. A restriction site was put into the TCRα genes allowing exchange of CDR3 regions between constructs. TCR constructs transfected into Jurkat cells demonstrated appropriate HLA-DR restricted antigen recognition. Hybrid receptors made by exchanging TCRα and β chains could be activated by αCD3 but did not recognize HA 307-319 presented by either DR4 or DR7. The response of receptors utilizing hybrid TCRα genes in which CDR1 and 2 from one T cell clone was grafted to CDR3 from the other mapped sequences important for HLA DR restriction to CDR3. While the original JS515.11 T cell clone was strictly monogamous, JS515.11 TCR transfectants could be activated by HA 307-319 presented by either DR4 or DR7 at high peptide concentrations. However, transfectants expressing the hybrid TCRα gene with CDRs 1 and 2 from 3BC6.6 and CDR3 from JS515.11 only responded in the context of DR7 regardless of peptide concentration. Together the data indicate that Jα determines class II restriction in this system, but show that Vα also affects the promiscuous recognition of DR.

C2-209 ROLE OF B-CELL TUMOR VS HOST BONE MARROW-DERIVED APC IN THE PRIMING OF TUMOR SPECIFIC CTL, Allen T. Bruce, Alex Y. C. Huang, Drew M. Pardoll and Hyam I. Levitsky, Department of Oncology, Johns Hopkins School of Medicine, Baltimore, MD 21205

It has been shown that activation of antigen-specific, MHC class I-restricted cytotoxic T lymphocytes (CTL) can lead to potent anti-tumor immunity. In order to generate improved cancer vaccines, it is important to understand how these cells are activated. Previous results have shown that in the case of an epithelially derived tumor line, priming of CTL against a MHC class I-specific antigen is mediated by host antigen presenting cells (APC). These observations raise the question as to whether tumors derived from cells that normally function as APC's can prime CTL directly. B cell lymphomas represent such a tumor, and have been shown to express co-stimulatory molecules necessary for T cell priming. We have developed a system with which we may assess the relative importance of tumor vs. host APC presentation of MHC class I antigen for CTL priming, using the B cell lymphoma, A20. This tumor has been shown to be an excellent APC in vitro, and expresses both B7-1 and B7-2. We have transduced A20 with influenza nucleoprotein (NP), a model antigen with well-characterized epitopes in K^d and D^b. Parent into F1 bone marrow chimeras enable the identification of the MHC restriction of CTL generated by priming with A20 NP or A20 NP co-transfected to express high levels of B7-1. Results of these experiments will be presented.

C2-210 **TCR-MEDIATED RECOGNITION OF ACTIVATED T LYMPHOCYTES BY HUMAN Vγ2Vδ2 T CELLS.** Jack F. Bukowski, Craig T. Morita, Hamid Band, and Michael B. Brenner. Department of Rheumatology/Immunology, Harvard Medical School, Boston, MA 02115.

T lymphocytes expressing the TCR variable (V) gene Vγ2 paired with Vδ2 (Vγ2Vδ2 T cells) are the predominant subpopulation of γδ T cells in human peripheral blood. In vitro, these Vγ2Vδ2 T cells undergo a polyclonal V gene-determined proliferation in response to a variety of microorganisms and tumor cells. In vivo, Vγ2Vδ2 T cells are expanded in the peripheral blood of patients with certain bacterial, parasitic, viral, and fungal infections, but their antigen specificity and function are unknown. This in vivo expansion is invariably accompanied by the presence of activated αβ T cells. Here we report that polyclonal Vγ2Vδ2 T cells purified (99.5% pure) from the peripheral blood mononuclear cells (PBMC) of normal donors proliferated in response to autologous or heterologous paraformaldehyde-fixed or mitomycin C-treated PHA blasts. Transfection into TCR^- recipient Jurkat cells of cDNA contructs containing TCR genes from a Vγ2Vδ2 but not a Vγ1Vδ1 T cell clone conferred upon the recipients the ability to recognize these PHA blasts as measured by IL-2 release. Resting T cells were not recognized. These results provide direct evidence for TCR-mediated recognition of a surface antigen on activated T lymphocytes by Vγ2Vδ2 T cells and suggest that these γδ T cells mediate an immunoregulatory function.

C2-211 DIFFERENTIAL RECOGNITION OF TCR CONTACT RESIDUES ON MHC CLASS II BOUND PEPTIDES: EFFECT ON T CELL SUBSETS AND SIGNAL TRANSDUCTION, Richard T. Carson and Dario A.A. Vignali, Dept of Immunology, St Jude Children's Research Hospital, 332 N. Lauderdale, Memphis, TN 38101.

The T cell antigen receptor (TCR) recognises peptides presented by major histocompatibility complex (MHC) molecules, but only a restricted number of these peptides residues interact with the TCR. Substitution of these TCR contact residues can produce peptides that are inhibitory or antagonistic without affecting binding of the peptide to the MHC molecule. The immunodominant epitope of hen egg lysozyme (HEL) 48-61 contains three known TCR contact residues; Tyr53, Leu56, and Glu57, which vary in their usage between different TCR. Peptides containing these residues in addition to the MHC anchor residues, 52 and 61, are sufficient for activation of T cell hybridomas that express CD4. However, some CD4 loss variants of these hybridomas are only activated by peptides containing the flanking residues, Trp62 and Trp63, suggesting that these may also be TCR contact residues. There is mounting evidence that CD4 facilitates recognition of peptides lacking Trp62 and Trp63 by an interaction with the TCR that does not appear to be solely dependent on $p56^{lck}$ or binding to MHC class II molecules.

A panel of analog peptides based on the HEL sequence 48-63 with double substitutions of residues 62 and 63 and/or alanine substitutions of residues 53, 56, and 57 have been produced. T cell hybridomas and TCR transgenic mice are being used to investigate the ability of these peptides to:

a) agonise, antagonise or inhibit T cell responses.
b) modulate the functional development of T cells towards subsets that secrete different cytokines.
c) alter signal transduction events.

The results of these studies will be presented and discussed.

C2-212 PEPTIDE BINDING AND PRESENTING PROPERTIES OF mCD1.

A. Raúl Castaño*, Shabnam Tagrin¶, Mitchell Kronenberg¶ and Per A. Peterson*.*The R. W. Johnson PRI at Scripps Research Institue and ¶ Dpt. Microbiology and Immunology, UCLA School of Medicine.

A peptide binding motif for mCD1 has been previously determined using a peptide display phage library and soluble mCD1/ß2m complexes expressed in *D. Melanogaster*. Synthetic peptides based on this motif have been used to analyze the structural requirements for peptide binding to CD1 in solution. Scatchard analysis of the binding of a ^{125}I labeled peptide showed a binding affinity for CD1 in the uM range, with a very fast association-dissociation kinetics ($t_{1/2}$ ~ 2 min). In opposition to classical class I molecules, the presence of Triton X-100 does not alter the kinetics, due to the higher stability of the CD1/ß2m complex, which does not dissociate at physiological temperatures.

N-terminus and C-terminus truncated variants of the reference peptide were analyzed in a competitive binding assay. The unlabeled reference peptide showed a dissociation constant of 10^{-7} M, similar to many class II-peptide interactions. Truncations at both N and C terminus outside the central core motif produced a steady reduction of the affinity of the binding. Truncations afecting the binding motif, completely abrogated peptide binding.

Alanine scanning mutagenesis verified the relative contribution of the putative anchor residues to the binding to CD1. Ala substitutions in positions 1, 4 or 7 decreased the binding affinity >250 fold, confirming the importance of these anchor positions. Substitutions of other residues slightly decreased or did not alter the binding capacity of the peptide. These data suggests that CD1 binds long peptides through a highly specific anchor region, with both N and C termini of the peptide protruding from the PBR and contributing to the overall affinity of the binding by multiple-low affinity interactions, in a similar way to class II-peptide interactions.

To study the physiological relevance of these data and the peptide presenting capabilities of mCD1, BALBc mice were immunized with CD1 expressing transfectants, loaded with a CD1-specific peptide. Spleen cells were isolated and CTL selected in vitro by multiple stimulations with peptide loaded CD1$^+$cells. Cytotoxic assays with appropriate target cells showed that T cells were able to recognize CD1-specific peptides in a CD1 dependent way. We are in the process of characterizing this response that would constitute, to our knowledge, the first example of CD1-restricted, peptide recognition by CTL.

C2-213 MHC CLASS II TRANSACTIVATOR (CIITA) REGULATES THE EXPRESSION OF MULTIPLE GENES IN VARIOUS CELL TYPES, Cheong-Hee Chang and Richard A. Flavell, Section of Immunobiology, HHMI, Yale School of Medicine, New Haven, CT 06510

CIITA has been shown to be required for the expression of MHC class II genes in both B cells and IFN-γ inducible cells. Here we demonstrate that CIITA not only activates MHC class II genes but also genes which are required for antigen presentation. Mutant HeLa cells, defective in the expression of classical MHC class II genes, Ii and the newly described HLA-DM genes, were used to study the role of CIITA in the regulation of these genes. Upon transfection with CIITA cDNA the mutant cells expressed all three genes suggesting that CIITA is a global regulator for the expression of genes involved in antigen presentation.

We also show that human T cells expressing MHC class II have CIITA transcripts while MHC class II negative human T cells and mouse T cells do not. The expression of MHC class II genes in mouse T cells can be reconstituted upon transfection with the human CIITA cDNA. These data indicate that the expression of CIITA explains the expression or lack of expression of MHC class II in human and mouse T cells respectively.

In order to study the role of CIITA in vivo, we generated CIITA deficient mice and the phenotype of mice will be discussed.

C2-214 BIASED ANTIGEN PRESENTATION BY F E T A L AND PLACENTAL MACROPHAGES, Ming-der Y. Chang, Houman Khalili, Sajata Kale Karihaloo, Department of Medicine, North Shore University Hospital-Cornell University College of Medicine, Manhasset, NY 11030

We have previously reported that placental and fetal macrophages of mid-gestational age have a decreased ability to present antigen to a panel of hybridomas. This reduced antigen presenting ability is not due to a decreased expression of MHC class II molecules, adhesion molecule ICAM-1 or co-stimulating factor B7. However, fetal and placental macrophages do not express detectable Ii chain mRNA which is known to have a major role in the class II-associated antigen processing pathway. Since fetal and placental macrophages can present neither antigenic peptides nor superantigen. the diminished Ii chain expression alone cannot account for the impaired antigen presenting function of fetal macrophages.

Although Ii chain negative fetal and placental macrophages cannot present antigen to some hybridomas, they can present antigens to T cell hybridomas whose recognition of antigen is independent of the presence of Ii chain in the antigen presenting cell. In addition, my data show that the ability of T cell hybridomas to recognize antigen presented by fetal macrophages is dependent on the presence one of the p41 form of Ii chain.

Furthermore, placental macrophages can activate a Th2 cell line, D10, at a level similar to that of adult macrophages, even though they are unable to activate a Th1 cell line, A.E7. Consistent with our result, previous studies have indicated that the immune response during pregnancy is biased towards humoral immunity (Th2 type immune response) and there is an increased synthesis of Th2 type cytokines at the maternal fetal interface. Therefore, the enhanced Th2 immune response during pregnancy may be, in part, directed by the antigen presenting cells at the uteroplacental site.

C2-215 NOVEL POLYMORPHISMS OF HUMAN TAP GENES IN HUMAN CANCERS. Hailei Chen and David P Carbone, Simmons CancerCenter, University of Texas Southwestern Medical Center, Dallas, Texas 75235

There are many potential mechanisms by which tumors might escape immune recognition, and one mechanism may involve deficits in the machinery of antigen presentation. Several genes are involved in the presentation of endogenous antigens, and we evaluated structural abnormalities in TAP genes (Transporters associated with the Antigen Processing). These gene products are presumed to mediate the delivery of peptides across the endoplasmic reticulum membrane for assembly to newly synthesized class I molecules. By utilizing single strand conformational polymorphism (SSCP) and DNA sequencing techniques, we are analyzing the structure of the two TAP genes in a hundred human cancer cell lines and freshly resected human tumors. In addition to the previously reported polymorphisms, we have found a novel allele of TAP 1 characterized by an ARG to GLN substitution at codon 659 in exon 10 in a human small cell lung cancer line, and another that changes codon 286 from SER to PHE in exon 4 from a breast cancer tumor. TAP 2 SSCP analysis also shows unique patterns in several exons where polymorphisms have not been reported. Thus, we have found novel TAP alleles in human tumors, not been reported in published data from non-tumor bearing individuals, suggesting that these alleles may be associated with cancer.

C2-216 PEPTIDE AND PROTEIN ANTIGENS REQUIRE DIFFERENT ANTIGEN PRESENTING CELL TYPES FOR THE PRIMING OF CD4+ T CELLS, Stephanie Constant, Theresa Pasqualini, Thomas Taylor, Ditza Levin, Richard Flavell and Kim Bottomly, Immunobiology Department, Yale University School of Medicine, New Haven, CT 06510

Priming of naive CD4+ T cells by protein antigen requires an antigen presenting cell (APC) that can take up the antigen, present peptide bound to MHC class II molecules, and also can stimulate T cell growth. We have used both in vivo and in vitro priming of CD4+ T cells to demonstrate that the APC used to prime naive CD4+ T cells with protein antigens depends on the form of that antigen. Using a series of MHC class II transgenic mice that differentially express I-E on their APC we show that, unlike priming to a peptide antigen, the presence of high numbers of I-E+ B cells but not dendritic cells (DC) is required for priming to an I-E restricted protein in vivo. A comparison of DC and B cells to stimulate naive TCR transgenic CD4+ T cells in vitro revealed that DC present peptide antigen extremely efficiently, even at low numbers, but present protein very poorly. In contrast B cells could prime equally well to both peptide and protein antigens. Furthermore, using antigen-specific B cells obtained from Ig receptor transgenic mice, we demonstrate that priming to a protein antigen using a population of B cells requires as few as 0.03% of the population to be specific for that antigen. We are currently using microbial-derived protein antigens to examine the importance of different APC types in microbial infections.

C2-217 UV INDUCED TOLERANCE AND INFLAMMATORY MACROPHAGIC ANTIGEN PRESENTING CELLS. K.D. Cooper, C. Hammerberg, N. Duraiswamy, Immunodermatology Unit, Department of Dermatology, University of Michigan, Ann Arbor, MI.

Contact sensitizers, tumor antigens, and microbial pathogens presented through UV-exposed skin result in T cell-mediated immune tolerance (inhibition of acquisition of responsiveness) to these normally potent immunogens. The antigen presenting cell (APC) in UV-exposed skin that delivers the signals inducing tolerance remains highly controversial and is the subject of this study. Application of the contact sensitizer, DNFB, to C3H/HeN mice immediately after a single dose of 72 mJ/cm^2 UVB (138 mJ/cm^2 total UVB) resulted in unresponsiveness to an initial DNFB ear challenge, but failed to block the development of responsiveness after a second sensitization on previously unexposed skin (no tolerance). A state of tolerance could only be achieved if a delay of 72 hours was allowed to elapse between the UV exposure and the initial sensitization. Epidermal cell suspensions (EC) were prepared from skin of normal controls (C-EC) and from skin exposed to the same UV dose 3 days prior (UV-EC). Three days after in vivo UV exposure, Langerhans cells (CD11b^{lo} Ia$^+$) were depleted and CD11b^{bright} Ia$^+$ macrophages had appeared in the epidermis along with GR-1$^+$ neutrophils. Intracutaneous injection of $DNBSO_3$-haptenated UV-EC, but not C-EC, resulted in the induction of locally-inducible antigen-specific tolerance to DNFB, indicating the presence and dominance of tolerogenic signal within in vivo-irradiated epidermis. Removal of CD11b$^+$ and class II MHC$^+$ cells within UV-EC showed that a CD11b$^+$ class II MHC$^+$ population was indeed critical for tolerance induction. In addition, tolerance induction by UV-EC was not due to surviving, UV-exposed Langerhans cells, because haptenated-3 day cultured EC from epidermis removed 5 hours after UV-exposure (before leukocytic infiltration) failed to induce a tolerogenic state. In vivo blockade of CD11b binding by peritoneal osmotic pump delivery of anti CD11b completely reversed UV-tolerance, restored immunologic responsiveness and reduced leukocytic infiltration. In conclusion, the ability of UV-exposed skin to induce peripheral adult tolerance to a normally potent immunogen is critically dependent on inflammatory class II MHC$^+$, CD11b^{bright} monocytic/macrophagic cells that infiltrate UV-irradiated skin at the same time the ability to tolerize is acquired.

C2-218 T CELL RECEPTOR REPERTOIRE OF H2-M3 SPECIFIC CYTOTOXIC T CELLS. Vikram Dabhi and Kirsten Fischer Lindahl. Howard Hughes Med. Inst., Depts. Microbiol. Biochem., Univ. Texas Southwestern Med. Ct., Dallas, TX 75235-9050.

M3 is a mouse major histocompatibility class Ib antigen with the unique ability to present formylated peptide to cytotoxic T cells. An endogenous formylated peptide, MTF, is derived from the N-terminus of the mitochondrial protein ND1. Cytotoxic T cells distinguish among M3 alleles of limited divergence and among four MTF peptides ($\alpha,\beta,\gamma,\delta$) differing by only a single amino acid at the sixth residue.

A panel of CD8+, peptide specific T cells has been cloned to determine the repertoire of T cell receptors (TCR) against M3. This repertoire is shaped by thymic maturation events that select for T cells which distinguish a self peptide differing from a non-self peptide by only a single methyl group. The TCR ß chain sequences from eight clones which recognize the γ peptide show no conservation of either Vß or Jß segment. TCR ß chain sequences from a total of 14 clones that recognize M3 complexed with other peptides also lack a conserved Vß or Jß segment. This ß chain variability is reflected in distinct patterns of reactivity by the clones to various M3 alleles and mutants.

An aspartic acid is conserved in the V-D-J junctional region from 11 of the 14 clones. This negatively charged residue may interact with a positively charged region identified near the peptide binding pocket of M3. TCR α chain analysis is in progress.

C2-219 CONTEXT-DEPENDENT PEPTIDE PRESENTATION Piergiuseppe De Berardinis*, Giuseppina Li Pira&, Maria N. Ombra°, Monica Autiero°, Daniela Fenoglio&, Fabrizio Manca& and John Guardiola°, *I.B.P.E., C.N.R., 80125 Naples, Italy, °I.I.G.B., C.N.R., Naples, & Dpt. Immunology, San Martino Hospital, Univ. Genoa

Helper T lymphocytes recognize antigenic peptides in association with class II MHC molecules on the surface of antigen presenting cells (APC). Recognition of an antigen thus requires its internalization and processing by the APC. Little is known about the process by which antigenic fragments are generated and delivered to class II molecules. Evidences from various antigen systems suggest that antigen processing can be one factor that determines the repertoire of immunogenic peptides. In the present study, we tested a possible positional effect on antigen processing and presentation by placing the antigenic sequence 231-246 of HIV gp120 (pep 24) into different positions of an unrelated carrier protein.

We purified two fusion proteins in which the pep 24 sequence is in a C-terminal position to the Glutathione-S-trasferase (GST-pep 24) or in a N-terminal position to GST (pep 24-GST). The two fusion proteins were separately used in a T cell proliferation assay with a human T-cell clone specific for pep 24. We observed proliferation when the autologous APC were pulsed with the synthetic peptide 231-246, the native protein gp120 and the fusion protein GST-pep 24. Proliferative response was not obtained with the protein pep 24-GST. Studies are in progress with another carrier protein and diverse antigenic peptides and T cell clones.

C2-220 PRESENTATION OF CARRIER-BOUND PEPTIDE BY MACROPHAGES TO UNPRIMED CYTOTOXIC T CELLS, Marloes L. H. De Bruijn#*, Michael R. Jackson# and Per A. Peterson#, Department of Immunology#, the Scripps Research Institute, La Jolla, CA 92037, USA, and Division of Immunohematology*, University Hospital Leiden, 2300 RC Leiden, The Netherlands.

Strict segregation of the MHC class I and class II loading pathways has been challenged by recent reports that MHC class I molecules can acquire antigen in the phagocytic pathway. We show that this alternative peptide loading pathway can be used to efficiently generate macrophages capable of *in vitro* activation of unprimed antigen specific cytotoxic T cells (CTL). Nanomolar concentrations of latex bead-bound peptide of 8-11 residues were found to be sufficient to induce primary CTL responses in macrophage-containing cultures, whereas longer peptides were ineffective. Bead delivery of these longer peptides did however render macrophages susceptible for lysis by antigen-specific CTL clones, indicating that proteolysis in the portion of the phago(lyso)somal pathway accessed by MHC class I does occur, but has limited capacity. By use of brefeldin A or cytochalasin D, we found that presentation of bead-bound peptide was drastically reduced, indicating that newly synthesized molecules and the phagocytic machinery were involved in presentation of bead-bound peptide. We propose a model for class I loading in the phagocytic pathway consisting of direct trafficking of nascent MHC class I from the trans-Golgi network to the phagosome, prior to appearance at the cell surface, and the use of the narrow cavity between bead and phagosomal membrane as a peptide exchange/loading compartment.

C2-221 UNDERLYING T CELL RESPONSE TO HEN EGG LYSOZYME (HEL) BY NON-RESPONDER C57BL/6 (B6) MICE CAN BE REVEALED BY MUTATING CRITICAL RESIDUES IN THE PUTATIVE SUPPRESSOR DETERMINANT. H-K. Deng, F.V. Henderson, J. Ohmen, C. T-P. Kao, D. Y-T Tsai, H-J. Bang, A. Miller and E. Sercarz. Dept. Microbiology & Mol. Genetics, UCLA, Los Angeles, CA 90024.

Previous studies in our laboratory (J. Exp. Med. 150:293-306) indicate that the T-cell non-responsiveness of B6 mice to i.p. immunization of HEL can be attributed to dominant T suppressor (Ts) cells specific for a determinant at the N-terminus. Additional experiments have implicated phenylalanine at position 3 (F3) as a critical residue in this determinant. In this work we made two HEL mutants by site-directed mutagenesis, F3V and F3Y, to study the importance of F3 for suppression induction. B6 splenocytes primed with HEL gave no recall response to HEL. However, cells primed with F3Y or F3V proliferated in response to both mutant and native HEL. Additionally, B6 splenocytes primed with HEL gave no recall response with a panel of singly overlapping peptides (15-mers) spanning the length of HEL. However, splenocytes primed with F3V or F3Y, proliferated in response to all of the determinants previously characterized in responder H-2^b mice. We also compared stimulation of HEL 74-88/A^b specific T-hybridomas by HEL and mutants. No significant difference was noted, hinting that the mutant antigen unlike REL is not preferentially processed or presented. Response to all of the previously characterized determinants by mutant-primed splenocytes indicates that there are no important processing differences between native and mutant HEL. We therefore conclude that an underlying T-cell response to HEL can be revealed by altering the "suppressor determinant" at its N-terminus, and that a similar pattern may exist with other antigens. We are currently mapping this determinant and exploring the mechanism of antigen-specific suppression. (Supp by grants from the NIH and the Juvenile Diabetes Foundation International)

C2-222 LOSS OF CD28 EXPRESSION IS ASSOCIATED WITH REPLICATIVE SENESCENCE OF T CELLS IN VITRO AND IN VIVO, Rita B. Effros, Francois Schachter, Nathalie Boucher, Carolyn Spaulding, Xiaoming Zhu,& Verna Porter, UCLA School of Medicine, Dept. of Pathology & Laboratory Medicine, Los Angeles, CA 90024, & Centre d'Etude du Polymorphisme Humain (CEPH), Paris, France.

The immune deficiency of aging is characterized by a dramatic decline in functions requiring T cell proliferation. We have studied the long-term proliferative potential of normal human T lymphocytes which are repeatedly activated with antigen and cultured in the continuous presence of Interleukin-2. Experiments on more than 200 individual cultures derived from 10 different donors have shown that T cells, like all other normal cells, have a limited replicative lifespan before reaching a state of senescence. The senescent T cells, though unable to expand in number either in response to antigen, IL-2, or anti-CD3+CD28 stimulation, are nevertheless capable of antigen-specific cytotoxicity. In addition they retain expression of all the characteristic cell-surface markers, activation antigens and adhesion molecules, with the sole exception of the CD28 costimulatory molecule. The expression of CD28 was shown to decline progressively (from > 90% to < 10%) during the proliferative lifespan of the culture with no change in expression following restimulation with antigen. Examination of peripheral blood T cells from young and centenarian donors showed that in vivo aging was also associated with a significant decline in the percentage of T cells expressing CD28, with some individuals showing only 50% CD28+ T cells. A dramatic decline in CD28 expression has also been reported in T cells from individuals in the advanced stage of AIDS. We hypothesize that the immune deficiency associated with aging, HIV infection, and other chronic or latent viral infections may, in part, be the result of repeated stimulation of memory T cells until they reach a state of "exhaustion" or senescence, with the concomitant loss of CD28 expression. The senescent cells become functionally anergic since they are incapable of transmitting the requisite costimulatory signal. (supported by AG00427, AG05309, AG00424, UCLA Center on Aging, and, CEPH Chronos Project).

C2-223 **STUDIES ON THE MODULATION OF THE *IN VIVO* T CELL IMMUNE RESPONSE USING T CELL RECEPTOR PEPTIDE AND PROTEIN.** **F.J. Eidelman and S.M. Hedrick, Department of Biology and Cancer Center, University of California, San Diego, La Jolla, CA 92093**

The immune response to certain antigens can be modulated *in vivo* so as to diminish the T cell immune response. In experimental allergic encephalomyelitis, for example, successful downregulation of the immune response has been accomplished by administration of antibodies specific for the β chain of the predominantly used T cell receptor (TCR) or by injection of T cell clones, antigenic peptide analogs, or TCR peptides. Despite these successes, the mechanism of the downmodulation remains obscure, largely because it is difficult to follow a small population of T cells with a specific TCR *in vivo*. In order to explore the mechanisms involved in immunomodulation, we have used αβ TCR transgenic(TG) mice, in which > 90 % of the T cells are $CD4^+$ and bear the same cytochrome *c*-specific antigen receptor (Vβ3 Vα11), and thus have a large population of T cells which can be followed *in vivo* . *In vivo* responses of the mice depend on the route of administration and the vehicle. Subcutaneous injection of PCC in CFA leads to sustained activation whereas intraperitoneal injection in IFA gives rise to initial activation followed by a state of relative anergy. Pre-immunization of the mice with a peptide corresponding to the CDR2 of Vβ3 leads to a modest decrease in T cell activation as measured by CD69 levels. In order to further elucidate the mechanism underlying this effect we are currently trying to augment this downmodulation using purified proteins corresponding to the whole V region and to the entire polypeptide chain of Vβ3 produced in a baculovirus system. The results of these studies will prove useful in optimizing ways to manipulate the T cell immune response in autoimmune disease.

C2-224 ELIMINATION OF ANERGIC B CELLS BY ANTIGEN-SPECIFIC T CELLS AND SURVIVAL IN THE PRESENCE OF THE BCL-2 TRANSGENE, Ursula Esser*, William Y. Ho*, Michael P. Cooke*#, Christopher C. Goodnow*#, Mark M. Davis*#, *Dept. of Microbiology and Immunology, #Howard Hughes Medical Institute, Stanford University School of Medicine, Stanford, CA 94305

We find that anti-HEL (Hen Egg Lysozyme) Ig transgenic B cells are stimulated when adoptively transferred into a soluble HEL expressing recipient together with HEL-specific TCR transgenic T cells whereas anergic B cells (derived from an animal transgenic for both anti-HEL Ig and soluble HEL) rapidly disappear from the spleen. This T cell mediated function is antigen-specific, as it does not occur when either normal resting T cells or T cells from an irrelevant (Moth Cytochrome c specific) TCR transgenic animal are transferred together with the B cells. Introduction of the bcl-2 transgene and presumptive high expression of its gene product prevents B cell disappearance in this system and thus it appears to result from apoptotic cell death. This specific T cell induced B cell death appears to be a new mechanism for the maintenance of tolerance and the prevention of autoimmunity by eliminating self-reactive B cells.

C2-225 ALTERNATIVE PROCESSING EVENTS IN PROTEINS EXPRESSED INTRACELLULARLY AND PRESENTED IN THE MHC CLASS II PATHWAY, Elizabeth E. Eynon and Ada M. Kruisbeek Division of Immunology, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands

Myelin Basic protein (MBP) is an intracellular protein associated with the myelin sheath in the central nervous system. While there is some evidence that some form of myelin basic protein is present in the thymus in neonatal animals, MBP reactive T cells can be detected in adult mice upon exposure to exogenous sources of MBP. Tolerance to this cytoplasmic protein has been hypothesized to be due to immunological ignorance, that is, the lack of availability of the antigen to the immune system. An alternative explanation for the presence of MBP reactive T cells in the periphery of unprimed mice would be that tolerance to epitopes derived from endogenously presented MBP has been established, while no tolerance exists for exogenously generated epitopes. We are therefore investigating whether MBP present intracellularly is processed into T cell epitopes different from those generated through processing of MBP exogenously.

Sequences 2.0 kb 5' of the MHC class II promoter have been shown to confer tissue specific expression for the MHC class II gene product. We have inserted this 2.0 kb sequence upstream of an MBP minigene construct and transfected it into a B cell lymphoma LS102.9. This minigene has also been placed under the control of an immunoglobulin enhancer/promoter from an Ig/myc translocation. An additional construct provides targeting to the ER and endosomal vesicles by adding sequences encoding the cytoplasmic tail and transmembrane domain of the MHC class II associated invariant chain, which has been shown to target to endosomal vesicles. Cells transfected with these constructs are being tested with T cell hybridomas generated using either exogenous (whole MBP in CFA) or endogenous MBP (injection of MBP transfected cells) as antigen. Using these hybridomas we can screen for processing of different epitopes of MBP when the protein is from endogenous versus exogenous sources.

C2-226 CROSSLINKING CELL SURFACE CLASS II MOLECULES STIMULATES B CELL ANTIGEN PROCESSING. Anne E. Faassen and Susan K. Pierce. Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, IL 60201

Antibody responses to a wide variety of antigens require the collaborative interaction of antigen-specific B and helper T lymphocytes. MHC class II molecules expressed by B cells are required for the presentation of processed antigen to antigen-specific T cells. In addition, class II molecules transduce signals that modulate B-cell function. Crosslinking MHC class II molecules promotes B cell proliferation and differentiation, induces the expression of the costimulatory molecule, B7, and increases cell-cell adhesion. Our results indicate that crosslinking cell surface class II augments the antigen presenting cell (APC) function of the B cell lymphoma, CH27. The processing and presentation of an E^k-restricted antigen to a specific T cell hybrid is increased by the addition of antibody specific for A^k. This augmentation is only observed when processing is initiated by antigen binding to surface Ig and not when antigen is taken up by fluid phase pinocytosis, indicating that simultaneous crosslinking of sIg and class II A^k is required for the effect. Crosslinking of class II molecules does not affect the time required for assembly of processed antigen-class II complexes or the loss of these complexes from the cell surface. Class II signaling appears to affect an intracellular processing event rather than cell surface presentation of antigen. Indeed, crosslinking class II molecules has no effect on the presentation of an antigenic peptide which does not require processing. Moreover, changes in the expression of the adhesion molecule CD11a (LFA-1) and the costimulatory molecule CD80 (B7-1) induced by class II signaling do not appear to account for the augmentation of processing observed here. Thus, the crosslinking of class II molecules on B cell surfaces, as presumably occurs during the initial encounter with antigen-specific T cells, augments the B cell's APC function, enhancing the B cell-T cell interaction.

C2-227 IDENTIFICATION OF SOLUBLE α/β T CELL RECEPTORS AS IMMUNOREGULATORY MOLECULES Robert Fairchild and John Barbo
Dept. of Immunol., Cleveland Clinic Fdn, Cleveland, OH 44195

The chains of the α/β T cell receptor expressed on most peripheral T cells possess hydrophobic transmembrane domains and are non-covalently asssociated with a complex of the CD3 and ζ proteins on the cell membrane. Soluble molecules produced by $CD8^+$ T cells from dinitrobenzene sulfonate-primed mice inhibit DNP-specific contact sensitivity in a class I MHC-restricted manner. A T cell hybridoma, MTs 79.1, constitutively produces a DNP-specific/K^d-restricted suppressor molecule that is a disulfide-linked dimer and is bound by antibodies specific for T cell receptor (TcR) α and β chain determinants. Our previous results have shown that deletion of either the Vα 14 or the Vβ8.2 gene from MTs 79.1 results in loss of surface receptor expression and the ability to produce the soluble suppressor molecule. Moreover, transfection of cDNAs encoding the parental α or β chain into TcR α or β chain gene deletion mutants, respectively, of MTs 79.1 reconstitutes surface TcR expression and the ability to produce the soluble suppressor molecule. While these results are consistent with our hypothesis that these molecules represent soluble forms of the TcR, the structural basis of these molecules has been elusive. We have recently identified a subclone of MTs 79.1, 79.1.4, that doesn't produce the soluble suppressor molecule while expressing equivalent levels of surface α/β TcR as that of a subclone, 79.1.8, that continues to produce the soluble molecule. The biological activity (i.e. inhibition of DNP-specific contact hypersensitivity) is detectable in the supernatants and soluble cell lysates from the 79.1.8 cells but not from the 79.1.4 cells. Western blot analyses using an anti-TcR α chain antibody as the developing reagent have demonstrated the presence of a 90 Kd disulfide-linked dimer in the soluble supernatants and cell lysates from the 79.1.8 cells. This molecule is not produced by the 79.1.4 cells. The soluble molecule is bound by anti-Vβ8 antibodies but not by anti-Vβ6 or anti-CD3 antibodies. These results indicate the ability of some T cells, and not others, to produce a soluble form of the α/β TcR.

C2-228 ANTAGONISTIC PEPTIDES DIFFERENTIALLY INHIBIT PROLIFERATION, CYTOKINE PRODUCTION AND HELP FOR IgE SYNTHESIS IN DER pI SPECIFIC HUMAN T CELL CLONES, Stephan Fasler, Gregorio Aversa, Patricia V. Schneider, Jan E. de Vries, and Hans Yssel, DNAX Research Institute, Human Immunology Department, 901 California Avenue, Palo Alto, CA 94304.

A crucial event in allergic reactions is the triggering of allergen specific T cells to produce Th2 type cytokines and to give help to B cells for IgE synthesis. In this study, we have evaluated the possibility of blocking the T cell-mediated pathway leading to IgE synthesis, using altered peptides to block T cell activation. These altered peptides, which were derived from two independent T cell epitopes on *Der pI*, the major allergen present in house dust, each contained single amino acid substitutions.

T cell clones were activated either in the presence of wild type peptide, or a combination of wild type peptide and a 50-100 fold excess of altered peptides. Several of these altered peptides strongly inhibited wild type peptide-induced proliferation, as well as IFN-γ and IL-2 production. In contrast, the production of IL-4 and IL-5 was affected to a lesser extent, thereby retaining the Th2 cytokine production profile of these T cells. The selective inhibition of Th1 cytokines was confirmed by the ability of culture supernatants from T cells stimulated with wild type peptide in the presence of antagonistic peptides, to induce IgE synthesis in vitro. Stimulation of T cells with the antagonistic peptides alone, at similar concentrations, did not result in proliferation or cytokine production, indicating that these peptides did not act as agonists. In spite of the strong inhibitory effect of the antagonistic peptides on the production of IL-2, they did not interfere with the up regulation of CD25 expression on the T cell clones induced by the wild type peptide. Taken together, these data show that antagonistic peptides, derived from minimal activation inducing epitopes, signal differentially through the TCR.

C2-229 DENDRITIC CELLS CAN PRESENT ANTIGEN IN A TOLEROGENIC OR STIMULATORY MANNER IN VIVO, Fred D. Finkelman, Andrew Lees, William C. Gause, and Suzanne C. Morris, Departments of Medicine and Microbiology and Immunology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814.

Several reports have suggested that the response of naive T cells to antigen presentation is dependent upon the identity of the antigen-presenting cell, with presentation by B cells inducing T cell tolerance and presentation by dendritic cells inducing T cell activation. We have recently published evidence that antigen presentation by B cells can induce either T cell tolerance or activation in vivo, depending on the B cell's activation state. We now report that antigen-presentation by dendritic cells can also induce either T cell activation or tolerance. Injection of mice with 10 ng - 100 µg of a monoclonal rat IgG2b anti-dendritic cell mAb (33D1) failed to stimulate an anti-rat IgG2b antibody response. 33D1 treatment of (BALB/c x CB20)F1 mice prior to challenge with an immunogenic combination of a rat IgG2b anti-IgD mAb (JA12.5) and an allo-anti-IgD mAb (Hδ^a/1, which is seen as self by these mice) strongly inhibited the cytokine response, the polyclonal IgG1 response, and the IgG1 anti-rat IgG2b response to the anti-IgD mAbs. Similarly, 33D1 pretreatment blocked the IgG1 anti-rat IgG2b response to an immunogenic rat IgG2b anti-CR3 mAb (M1/70). Tolerance induction was antigen specific, and 33D1 treatment did not inhibit the response to a simultaneously injected antigen. 33D1 induced tolerance whether injected i.v., i.p., or s.c., and was >100-fold more potent as a tolerogen than a control rat IgG2b. 33D1 induced an IgG1 anti-rat IgG2b response, instead of rat IgG2b-specific tolerance, if it was injected with IL-1 or with a stimulatory allo-anti-IgD mAb, or if it was aggregated prior to injection. Under these circumstances, 33D1 was 10-100-fold more immunogenic than a control rat IgG2b. These observations suggest that antigen presented by dendritic cells in the absence of co-stimuli induces tolerance, while dendritic cell presentation of antigen in the presence of inflammation-induced co-stimuli activates T cells.

C2-230 **EVIDENCE FOR AN ADDITIONAL LOCUS ON HUMAN CHROMOSOME 6p WHICH MEDIATES THE EXPRESSION OF MHC CLASS II/PEPTIDE COMPLEXES.** Steven P. Fling, Benjamin Arp and Don Pious, Department of Pediatrics, University of Washington, Seattle, WA 98195

We recently identified HLA-DMA and DMB as the affected loci in mutant B-LCLs which are defective in the assembly of MHC class II/peptide complexes. To investigate the possibility that other genes within and synteneic to the MHC are also involved in the expression and assembly of class II molecules with cognate peptides, we carried out EMS mutagenesis and immunoselection on progenitor B-LCL 3.1.0/DR3. This B-LCL contains a ~40 Mb hemizygous deletion in chromosome 6p that begins approximately 10 Mb centromeric of the HLA, removes one entire HLA haplotype, and extends to the end of chromosome 6p. To exploit peptide induced conformational changes in class II as a basis for selection, 3.1.0/DR3 was immunoselected with a mAb which exhibits reduced binding on HLA-DM mutants as a result of loss of cognate peptide binding to DR3.

In addition to the previously described HLA-DMA mutant 2.2.93, we isolated from 3.1.0/DR3 a set of mutants which also display a phenotype indicative of defective class II/peptide complex assembly. These mutants express MHC class II molecules which are conformationally altered. This is indicated by a drastic reduction in cell surface staining by peptide sensitive DP4-, DQ1- and DR3-specific mAbs, and by the expression of SDS unstable class II dimers. Based on the high frequency of isolation of these mutants, the lesion most likely maps to the region hemizygously deleted from their progenitor. The defect superficially resembles that seen in DM mutants. However, these mutants are phenotypically distinct from DM mutants and several genetic analyses indicate that the lesions are not in DMA or DMB.

These results suggest that there is a locus distinct from DM which maps to the region of chromosome 6p hemizygously deleted in 3.1.0/DR3 that mediates the expression of functional MHC class II molecules in these B-LCL. Phenotypic and genetic characterization of these mutants will be discussed.

C2-231 **IDENTIFICATION OF A TAP-DEPENDENT LEADER PEPTIDE RECOGNIZED BY ALLOREACTIVE T CELLS SPECIFIC FOR A CLASS Ib ANTIGEN,** James Forman*, Amy DeCloux†, Amina S. Woods§, Robert J. Cotter§, Mark J. Soloski†, and Carla J. Aldrich*, Department of Microbiology*, University of Texas Southwestern Medical Center at Dallas, Dallas TX, 75235-9048, Department of Medicine† and Department of Pharmacology and Molecular Sciences§, Johns Hopkins University Medical School, Baltimore MD 21205.

Recognition of the class Ib antigen Qa-1 by a portion of alloreactive cytotoxic T lymphocyte (CTL) clones requires that the target cell express a second gene, termed *Qdm* (Qa-1 determinant modifier), which maps to the *D-end* of the MHC. We show that *Qdm* is identical to most *D-allele* genes, excepting D^k, and that a synthetic nonamer peptide derived from D-alloantigens restores CTL recognition on cells that lack the Qdm-encoded determinant. An identical peptide can be acid eluted from target cells indicating that this nonamer is the natural peptide. The equivalent D^k peptide has an ALA->VAL interchange at P3 and requires ~4 logs more peptide than the ALA^{P3} peptide for target cell lysis. Two of five Qa-1 specific CTL clones, not dependent on the *Qdm* gene for target cell recognition, also recognize the Qdm-peptide as well as the variant containing VAL^{P3}. Although the Qdm-peptide spans residues 3-11 from the leader sequence it requires the Tap transporters for recognition by CTL. Thus, the response against this class Ib molecule provides a new tool for dissecting alloreactivity as well as a unique pathway for antigen presentation.

C2-232 NEONATAL TOLERIZATION INDUCES A CD4⁺IMMUNE RESPONSE, Thomas Forsthuber and Paul V. Lehmann. *Dept. of Pathology, Case Western Reserve University School of Medicine, Cleveland, OH 44106*

Here we report that, contrary to the previously held notion, neonatal injection of antigen does not inactivate antigen specific T cells, but activates them in such a way that they become functional memory cells. These neonatally primed T cells are $CD4^+$ and do not express the lymph node homing receptor, L-selectin. Subsequently, these cells can be detected in the spleen but not in lymph nodes. Also consistent with the L-selectin⁻ phenotype, the neonatally primed T cells are not recruited to draining lymph nodes following adult immunization, which results in the apparent unresponsiveness that has been interpreted as being a consequence of clonal inactivation. In contrast, even the injection of soluble antigen, which is barely immunogenic to naive mice, boosts the T cell response in the spleen and triggers a strong, secondary antibody response in adult mice that have been neonatally "tolerized". The prevalent class of antibodies produced was T helper-2 (Th-2) cell dependent IgG1. Moreover, IL-5, a Th-2 cell product, was the dominant cytokine produced by antigen challenged spleen cells of neonatally injected mice. The data show that altered migration patterns of the neonatally primed T cells account for the apparent unresponsiveness of neonatally injected mice when tested in the conventional T cell assay. Furthermore, the data suggest that the mechanism for the state of immune tolerance induced by neonatal exposure to antigen results from induction of a Th-2 type of T cell response that interferes with the ability to generate a pro-inflammatory Th-1 response required for the development of autoimmune disease or graft rejection.

C2-233 PRESENTATION OF ENDOGENOUS ANTIGENS ON CLASS II MHC: REGULATION BY THE INVARIANT CHAIN, Kenneth Frauwirth, Sarah Sanderson and Nilabh Shastri, Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720

The presentation of MHC Class II/antigen complexes to $CD4^+$ T cells by APC's is a critical event in an immune response. One point of regulation is in the selection of peptides for loading. Many endogenous antigens are excluded from the Class II pathway, presumably because the Class II-associated Invariant Chain (Ii) prevents access to the peptide binding groove while the Class II molecule is en route to the endosomal pathway. In addition, the invariant chain is responsible for targeting class II complexes to the endosomal compartment, where antigen processing is believed to occur. We have found that the Ii protein plays a more complex role in peptide loading than the simple exclusion of endogenous peptides. In COS cells transfected with the murine A^k molecule, expression of Ii has little effect on the presentation of exogenous hen-egg lysozyme (HEL), but dramatically reduces the presentation of endogenous HEL, to the T Cell hybridoma KZH (specific for A^k/HEL34-45). However, co-expression of Ii is required for efficient presentation of both endogenously- and exogenously-expressed ovalbumin to the T cell hybridoma KZO (specific for A^k/OVA247-265). To map the domains of the invariant chain responsible for these properties, we are examining the effects on endogenous antigen presentation of mutations in both the cytoplasmic and lumenal regions of the protein. The endosomal targeting signal in the cytoplasmic tail is absolutely critical for Ii function in the presentation of endogenous OVA and HEL, and preliminary results indicate that a region containing the CLIP peptide is also important.

This study was supported by grants from the NIH (AI-26604) and the TRDR Program to NS. KF is a predoctoral fellow of the Howard Hughes Medical Institute. SS is a predoctoral fellow of the Biotechnology Research and Education Program.

C2-234 RECONSTITUTION OF THE MHC CLASS I ANTIGEN PRESENTATION PATHWAY IN *DROSOPHILA MELANOGASTER* CELLS

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Class I major histocompatibility complex molecules present peptides derived from intracellular antigens to cytotoxic T-lymphocytes. Antigen is first degraded in the cytoplasm to peptides, which are subsequently translocated across the membrane of the endoplasmic reticulum (ER) by MHC-encoded transporters (TAP). In the lumen of the ER empty class I molecules bind the peptides prior to their transport to the cell surface. We have attempted to reconstitute this class I antigen processing and presentation pathway in insect cells in order to identify the minimal components required and to analyse whether peptide generation is a house-keeping function of eucaryotic cells. We show that the TAP transporters expressed in these cells function efficiently in an *in vitro* peptide transport assay. Furthermore, we have succeeded in coexpression of class I heavy chain, ß2 microglobulin, TAP1 and TAP2. The presence of peptides in such class I molecules was analysed both biochemically and by using specific T-cells. The results indicate that although TAP-dependent peptide loading of class I molecules was reconstituted in such cells other components are required for antigen processing.

C2-235 SPLIT TOLERANCE INDUCED BY RESTRICTED LOW DOSE TRANSGENE EXPRESSION, Er-Kai Gao, Xin-hong Yu, Hui Zhang, Jonathan E. Silbert and Henry J. Kaplan, Department of Ophthalmology & Visual Sciences, Department of Pathology, Washington University School of Medicine, St. Louis, MO 63110

To examine the mechanism of immunologic tolerance to ocular specific antigens, systemic immunological consequences of a presumed photoreceptor-restricted neoantigen expression were studied with R7 transgenic mice. R7 transgenic mice carry the β-galactosidase gene under the control of human opsin promoter on a B6 ($H\text{-}2^b$) genetic background. Since the CTL response to β–gal is restricted to MHC class I molecule, L^d, R7 were crossed with DBA/2 ($H\text{-}2^d$) mice to produce R7D2 mice which carry the required L^d gene. The tissue distribution of transgene expression was first defined by X-gal staining of frozen tissue sections obtained from multiple organs of R7D2. Except the photoreceptor cells of the retina, no β-gal activity can be detected by X-gal staining on all organs tested. The possible leakage of the transgene expression in bone marrow derived cells was further studied by primary CTL recognition in vivo. We found no detectable β-gal specific priming effect after immunization of B6D2 with R7D2 thymocytes or splenocytes. This finding suggests that mature naive CTL can not recognize any transgene expression leakage on the bone marroe derived thymocytes and splenocytes in vivo. The systemic immune responses to the presumed photoreceptor-restricted transgene product, β-gal, were then examined using three assays: a. ^{51}Cr-release for Cytotoxic T lymphocytes; b. β-gal specific proliferation assay for T_h cells, and c. β-gal specific ELISA for B cells response. We found no tolerance to β-gal in T_h cell and B cell responses in R7D2 mice. However, β-gal specific CTL response was profoundly diminished in the same transgenic mice. The mechanism of this split tolerance is currently under investigation with radiation chimeras. The results and its implication will be discussed at the symposium.

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C2-236 BINDING OF INVARIANT CHAIN TO MHC CLASS II MOLECULES. Anand M. Gautam, Terry Moore, Peter J. Milburn and Sarah Weenink, Human Genetics Group, John Curtin School of Medical Research, The Australian National University, Canberra, ACT 2601, Australia.

Major histocompatibility complex (MHC) class II molecules bind peptides derived from exogenous proteins which have undergone intracellular processing in endosomal and/or lysosomal compartments. Invariant chain (Ii) facilitates folding of MHC class II molecules for their exit from the ER to endocytic compartments where it is released from MHC class II molecules to allow peptide binding. The mechanism which allows this association and dissociation of Ii with MHC class II molecules is unknown. There is also no structural information concerning Ii association with MHC class II molecules. By utilising an Ii peptide 86-104 (referred to as CLIP for class II-associated Ii peptide), we show that CLIP binds to various class II MHC I-A haplotypes utilising essentially the same amino acids. We have also identified regions critical for binding of CLIP and I-A class II molecules. In most cases, the binding of CLIP to a number of I-A molecules is modulated by the steric bulk of methionine residues at positions 93 and 99. The effect of site-specifc mutations at positions 93 and 99 on antigen presentation will also be discussed. Taken together, these results provide an insight into how Ii first associates with and then dissociates from the same MHC class II molecule during antigen processing.

C2-237 SYNTHETIC PEPTIDES CORRESPONDING TO HLA CLASS I INHIBIT IN VITRO IMMUNE RESPONSES AND INITIATE CALCIUM MOBILIZATION IN LYMPHOCYTES, Jodi E. Goldberg, Shu-Chen Lyu, Alan Krensky†, and Carol Clayberger, Departments of Cardiothoracic Surgery and †Pediatrics, Stanford University School of Medicine, Stanford, CA 94305

Previous work in our laboratory characterized *in vitro* effects of peptides synthesized from known sequences of the alpha helix of the α_1 domain of the HLA class I protein. This region of the HLA molecule is comprised of amino acids 60 to 84. Synthetic peptides corresponding to this region from certain HLA-B and -C molecules inhibited both T cell proliferation and CTL-mediated lysis. Moreover the inhibition was observed regardless of the HLA type of the responder or stimulator cells. Here we show that synthetic peptides corresponding to residues 75-84 of HLA-B2702 (B2702.75-84) inhibit T cell proliferation and CTL-mediated target cell killing. Additional peptides were prepared in order to determine optimal peptide length and critical peptide residues. An inverted repeat peptide, B2702.84-75/75-84 showed maximal inhibitory effects, although a shorter inverted repeat consisting of residues 84-79/79-84 also inhibited lymphocyte responses. Substitution of individual residues revealed that the isoleucine at position 80 is critical for the peptide's immunomodulatory function. B2702.84-75/75-84 initiated calcium flux in CTL lines, human PBL, and several cell lines including the T cell tumor Jurkat and B cell lymphomas, MS and Daudi. No change in intracellular calcium was detected in other cell lines including K562 and EBV-transformed B cells. Substitution of the isoleucines also eliminated the peptide's ability to mobilize calcium. These findings delineate a new pathway for inducing T cell unresponsiveness that may have important clinical utility.

C2-238 THE ROLE OF T CELL COSTIMULATOR IN THE INDUCTION AND MAINTENANCE OF PERIPHERAL TOLERANCE, Sylvie Guerder*, Jeffrey Meyerhardt and Richard Flavell*¶, *Section of Immunobiology and ¶Howard Hughes Medical Institute, Yale University School of Medicine, 310 Cedar Street, Box 208011, New Haven, CT 06520-8011

T cell tolerance to peripheral antigens is believed to result mainly from the lack of expression of T cell costimulator(s) by parenchymal cells. To test this possibility, we generated transgenic mice that express the T cell costimulator B7-1 specifically on the β cells of the pancreas. Following expression of the B7-1, β cells become immunogenic for naive T cells in vitro and in vivo. Nonetheless, less than 2% of the B7-1 transgenic mice develop diabetes. By contrast, double transgenic mice co-expressing the B7-1 transgene and high level of MHC class II I-E antigen (B7-1/I-E) developed insulitis at about 8 weeks of age associated with a progressive loss of insulin producing β cells. By grafting experiments, we showed that the destruction of β cells in the B7-1/I-E double-transgenic mice predominantly mediated by T cells specific for the islet peptide presented by the I-E molecule. On the other hand, single transgenic mice expressing the I-E molecule alone are tolerant in vivo and do not reject engrafted islets expressing the B7-1 costimulator and the I-E molecule. We are currently analyzing the mechanisms leading to tolerance in these mice, mainly whether an alteration of the balance between Th1 and Th2 could be correlated to the switch from tolerance to autoimmunity observed.

Recent evidence suggests that the B7-2 molecule might play a role in the early events of T cell activation. B7-2 might then by itself allow the activation of naive autoreactive T cells, a possibility that we are presently testing.

Therefore, in our experimental system, expression of the B7-1 costimulator seems sufficient to impair tolerance induction to peripheral antigens and leads to autoimmunity when tissue express sufficient MHC.

C2-239 INDUCTION OF ORAL TOLERANCE IN TCR TRANSGENIC MICE, S. Hachimura[1], T. Sato[2], S. Hashiguchi[1], A. Ametani[1], Y. Kumagai[3], S. Habu[2] and S. Kaminogawa[1], [1]Department of Applied Biological Chemistry, The University of Tokyo, Tokyo, [2]Department of Immunology, Tokai University School of Medicine, Isehara, [3]PRESTO, JRDC, Yokohama, Japan.

The oral administration of antigen induces a state of immune tolerance, which is termed oral tolerance. Induction of oral tolerance has been shown to be T cell mediated, and is explained by several mechanisms including active suppression and T cell anergy. However, the molecular and cellular process has not been fully described. In the present study, we examined the effects of feeding antigen to TCR transgenic (Tg) mice, using Tg mice expressing αβ TCR specific to ovalbumin (OVA) 323-339 + I-A^d. Spleen cells from non-immunized Tg mice proliferate and produce cytokines to *in vitro* stimulation of OVA. When the Tg mice were fed with OVA, the antigen-specific production of IFN-γ in splenic cultures was reduced. On the other hand, the production of IL-4 was rather enhanced. The reduction of IFN-γ was reversed by preculture with IL-2. The results were consistent with observations in other systems that anergy was induced in the Th1 compartment, while Th2 type responses were unaffected, or enhanced. An interesting finding was that the spleen cells from the OVA-fed Tg mice proliferated more strongly compared to the spleen cells from control Tg mice in the first 48 h of culture. This shows that a population of primed cells exists in the spleen of OVA-fed animals.

C2-240 ACTIVATION AND REACTIVATION POTENTIAL OF T CELLS RESPONDING TO STAPHYLOCCAL ENTEROTOXIN B,

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Superantigens (SAs) have in common biological features that distinguish them from conventional antigens: SAs induce vigorous proliferative responses in all T cells expressing particular Vβ chains, both *in vitro* and *in vivo*. *In vivo* these responses are followed by partial clonal deletion and induction of nonresponsiveness in mature peripheral T cells.

To elucidate the parameters that lead to superantigen induced nonresponsiveness, an *in vitro* model for studying primary and secondary responses to the bacterial superantigen Staphylococcal Enterotoxin B (SEB) was established. *In vitro* SEB primed T cells show upon reactivation with SEB an early proliferative response that "quenches" in time and is severely impaired three days after restimulation. Despite their overall impaired proliferative capacity and IL-2 production, these T cells are able to produce IFNγ and to upregulate activation markers CD69 and IL-2Rα upon restimulation with SEB, suggesting that SEB-nonresponsiveness is not absolute. Our results demonstrate that SEB-induced nonresponsiveness is not simply the result of presentation in the absence of costimulation via B7 and B7-related molecules, since presentation of SEB on dendritic cells during the primary response did not prevent the induction of nonresponsiveness. As previously shown, SEB induces a Th1 phenotype in responding $CD4^+$ T cells. Skewing towards a Th2 phenotype by adding IL-4 and antibodies to IFNγ did not prevent the induction of nonresponsiveness by SEB. Interestingly, also T cells pretreated with plate-bound anti-CD3ε and anti-Vβ8 were nonresponsive to SEB restimulation. Thus, nonresponsiveness to SEB seems to reflect an intrinsic inability of previously activated T cells to respond to SEB, probably reflecting differences in signal transduction pathways used in naive versus memory T cells. Whether these differences in responsiveness are related to the differential expression of isoforms of the membranebound tyrosine phosphatase CD45 on naive and memory T cells is currently under investigation.

C2-241 DELETION OF HIGH-AVIDITY T CELLS BY THYMIC EPITHELIUM IN A TCR TRANSGENIC MODEL

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Using embryonic day 10 (E10) branchial clefts that contain the thymic anlage before its colonization by cells of haemopoietic origin, we have previously shown that thymic epithelium (TE) induced a state of split tolerance to a model MHC class I antigen, H-$2K^b$ (K^b). Recipients of K^b TE were tolerant to K^b skin grafts but showed strong *in vitro* anti-K^b CTL responses. The aim of the present study was to identify the toleragenic mechanism in an anti-K^b T cell receptor (TCR) transgenic model. To this end these mice were crossed onto a nude background, and these mice were grafted with E10 branchial clefts of B10.BR (syngeneic control), (C57BL6xB10.BR)F1, or Eμ-K^b origin that expressed K^b on medullary thymic epithelium only. Subsequently, lymph node cells and splenocytes of these mice were analyzed by three-colour flow cytometry for the presence and phenotype of anti-K^b TCR transgenic cells. Cells were stained with a clonotype-specific monoclonal antibody, termed Désirée (Des), as well as with monoclonal antibodies to CD4, CD8, and the TCR β-chain. Whereas the level and proportion of Des^+CD8^+ T cells in B10.BR grafted mice was identical to normal TCR transgenic mice, there was a subtle but very distinct and reproducible absence of $CD8^+$ T cells bearing the highest density of the Des clonotype in mice grafted with (B6xB10.BR)F1 and Eμ-K^b thymic epithelium. In addition, there was a complete absence of Des^+CD4^+ T cells in recipients of K^b-bearing grafts. Double staining using a pan-TCR monoclonal antibody and the Des clonotype showed that the reduction of Des expression represented an overall lower level of TCR expression, since the overall density of the TCR was also decreased. Preliminary data from skin grafting experiments show that TCR transgenic recipients of K^b-bearing TE grafts showed prolonged survival of K^b skin grafts. These data suggest that the split tolerance induced by thymic epithelium reflects the deletion of T cells bearing the highest avidity for antigen, whereas lower avidity T cells persist that are able to give rise to *in vitro* T cell responses.

C2-242 NATURAL KILLER CELLS IN β_2M DEFICIENT MICE: WHAT CAN THEY RECOGNIZE AND WHAT EDUCATES THEM?,

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Mice deficient in β_2m gene expression (β_2m -/-) have greatly reduced levels of MHC class I molecules and a compromised NK cell repertoire. In contrast to normal (i.e. β_2m +/- or β_2m +/+) mice, β_2m -/- mice are unable to reject β_2m -/- bone marrow grafts. NK cells from β_2m -/- mice are also unable to kill β_2m -/- Con A blasts in vitro, although the latter are killed by NK cells from normal mice. This suggested that β_2m -/- NK cells are regulated to maintain nonresponsiveness towards their own self, and thus secure self tolerance. Furthermore, the self tolerance is determined by the bone marrow as demonstrated in bone marrow chimeric mice. However, this tolerance does not extend to all target cells tested. We have obtained evidence that NK cells from β_2m -/- mice are not universely nonreactive against β_2m deficient targets. They can distinguish between MHC class I expressing and deficient cells in three lymphoma models. These include the TAP-2 mutant RMA-S (sensitive) and its control RMA (resistant) as well as the two β_2m deficient mutants C4.4-25$^-$ and A.H-2$^-$ (sensitive) and their β_2m expressing controls EL-4 and E49.3 (resistant). We are now extending these studies aiming to investigate the target cell repertoire of β_2m -/- NK cells, performing in vivo rejection experiments transplanting bone marrow from various allogeneic donors to β_2m -/- mice. Preliminary experiments suggest that certain bone marrow grafts are rejected by β_2m -/- mice while others are accepted. These results will be discussed within a concept of development of NK tolerance in β_2m -/- mice and in bone marrow chimeras between β_2m -/- and β_2m +/+ mice.

C2-243 TAP-1-DEPENDENT PRESENTATION OF A LEADER-PEPTIDE DERIVED EPITOPE YIELDS NO EVIDENCE FOR EFFICIENT PEPTIDE-TRIMMING IN THE ENDOPLASMIC RETICULUM

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Peptides presented by MHC class I molecules are derived from intracellular antigen. Proteins apparently are degraded into peptides within cytosolic proteasome complexes, and peptides are then translocated via the TAP1/2 transporters into the lumen of the ER, were they bind to newly synthesized MHC class I molecules. Although TAP-transporters have already a size preference for peptides of 7-15 animo acids length, peptide-trimming within the ER has been postulated. Accordingly, longer precursor peptides would be allowed to bind to MHC class I, and would then be cleaved to a best-fitting size of 8-9 amino acids. In the ER, one source of peptides of heterogenous length are the leader peptides derived from signal sequences. These peptides enter the ER in a TAP-independent manner, since they are cleaved from nascent polypeptides by the ER-luminal signal peptidase. Indeed, peptides derived from signal sequences have been eluted from MHC class I molecules in TAP mutant cell lines. In this study we functionally analyze the presentation of a defined leader epitope from the signal sequence of the lymphocytic choriomeningitis virus (LCMV) glycoprotein in TAP -/- cells. We show that this H-2^b restricted epitope cannot be presented by LMCV-infected cells from TAP-/- mice. Thus, although the major LCMV MHC class I epitope is located within the leader peptide, it cannot be presented in an TAP-independent manner. These data suggest that the trimming process in the ER is limited and argue against a major role of a second, TAP-independent pathway of antigen presentation.

C2-244 FUNCTIONAL EXPRESSION OF MHC CLASS II ON A CYTOCHROME C-SPECIFIC MOUSE T CELL CLONE.

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Human T cells express MHC class II molecules, and they can act as APC *in vitro*. In order to investigate the immunoregulatory function of the antigen-specific T-to-T cell interactions that could arise *in vivo* as a result of antigen presentation between T cells, class II expressing mouse T cells are required. Hitherto, MHC-class II expression has not been conclusively demonstrated on mouse T cell clones. From the I-E^k-restricted, cytochrome C-specific B10 T cell clone we have now isolated sublines which constitutively express cell-surface class II antigens. The parental B10 clone was propagated *in vitro* in medium containing rIL-2 for several months without restimulation with antigen or APC. This was done to ensure the complete absence of residual APC or membrane fragments bearing class II molecules which we have shown to persist *in vitro*. We have reproducibly observed that these B10 cells can respond to the pigeon cytochrome C peptide in the absence of APC. Moreover, the APC-independent peptide response can be blocked by Mab specific for either the B10 TCR clonotype (8G2) or I-E^k (14-4-4s), but not with a Mab specific for I-A^k (11-5.2). Indirect immunofluoresence staining revealed that a distinct proportion (15-20%) of these B10 cells reacted with the 14-4-4s and 11-5.2 antibodies. In addition, 2-color immunofluoresence confirmed the cell surface co-expression of TCR and MHC class II on a subpopulation of B10 cells. Removal of anti-MHC class II reactive cells from the B10 culture by antibodies and anti-Ig columns yielded cells which lacked APC-independent responsiveness. In contrast, enrichment of the B10 population for I-E and I-A by panning for anti-MHC class II reactive cells produced a more vigorous APC-independent response. Fractionated B10 cells cultured in rIL-2 maintained their class II phenotype suggesting that I-E and I-A expression was constitutive. These B10 cells exhibited functional class II expression inasmuch as peptide pulsed, glutaraldehyde fixed cells could stimulate either B10 or the I-A^k-restricted D10 T cell clone in an antigen-specific manner. MHC class II^+ B10 cells could also bind biotinylated SEA and SEB. The response of class II^+ B10 cells to intact cytochrome C could only be detected in the presence of APC, suggesting that these cells are deficient in the endocytosis and/or processing of antigen. Furthermore, B10 cells did not appear to express the B7 molecule required for co-stimulation via CD28, indicating a distinction from activated human T cells.

C2-245 Vß TCR SPECIFIC CD8+ T CELLS THAT DELETE CD4+ CELLS EXPRESSING Vß8 TCR: THE ROLE OF QA-1.

Hong Jiang*, Randle Ware**, Alan Stall*, Lorraine Flaherty***, Leonard Chess** and Benvenuto Pernis* Columbia University, College of P&S, New York, NY and the Axelrod Institute for Public Health***, Albany, NY.

In the present studies we demonstrated that CD8+ T cells participate in the "in vivo" regulation of CD4+,Vß8+ T cells following SEB administration. We first confirmed that deletion of 30-40% of CD4+,Vß8+ cells 7-14 days after a single injection of SEB in mice. We then showed that the downregulation of CD4+,Vß8+ T cells below baseline is not observed in mice depleted of CD8+ cells either by treatment with monoclonal anti-CD8 antibody or in CD8+ T cell deficient ß2 M-/- knockout mice. Moreover, we observed that following SEB administration splenic and lymph-node CD8+ T cells preferentially recognizing CD4+,Vß8+ T cells can be specifically expanded in vitro. These CD8+ T cells were cytotoxic to autologous CD4+,Vß8+ T cells but not to autologous CD4+,Vß8- T cells. The evidence that CD8+ T cells arise which are specific for CD4+ cells expressing particular Vß TCRs was demonstrated by showing that CD8+ T cells obtained by stimulation with SEB activated CD4+,Vß8+ T cells only kill SEB activated CD4+,Vß8+ T cells but not CD4+,Vß8- T cells even though SEB activates Vß7+ and Vß17+ T cells as well as Vß8+ T cells in Balb/C mice. We assayed the destruction of CD4+ cells by a 51Cr release assay as well as by measuring the CD8+ effector cell induced change in the ratio of Vß8+/Vß8-cells in mixed T cell culture by FACS. The killing observed is not related to T cell activation alone because Vß8+ and Vß8- targets cells were both activated. Further, we showed that the killing is not dependent on the particular superantigen used or to superantigen carryover. Furthermore, we demonstrated that this autologous TCR Vß specific cytotoxicity is dependent on recognition of ß2-microglobulin associated molecules and is inhibited by antisera specific for Qa-1 molecules but not by antibody to classical MHC class I-a molecules. Together these data support the idea that the specificity of immune regulation may be mediated in part by specific recognition by CD8+ T cells of TCR Vß peptides bound to Qa-1 MHC class I-b molecules expressed on the surface of autologous CD4+ T cells.

C2-246 COEXISTENCE OF TRANSGENE POSITIVE AND NEGATIVE CELLS IN A H-2 TRANSGENIC STRAIN WITH A CHIMERIC PHENOTYPE LEADS TO NK TOLERANCE AGAINST "MISSING SELF"

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We have previously shown that a H-2D^d transgene introduced on a B6 background (as in D8 transgenic mice) conveyed rejection of "previously syngeneic" B6-derived (H-2^b) lymphomas after s.c. and i.v. challenge. Transfection studies showed that the lymphomas were rejected because they lacked H-2D^d. This finding was consistent with the "missing self" hypothesis for NK mediated rejection of MHC disparate grafts. In order to investigate which regions of the H-2D^d molecule that were necessary to convey lymphoma resistance, we created transgenic mice carrying an exon-shuffled H-2 transgene containing the $\alpha1/\alpha2$ domains from H-2D^d coupled to the $\alpha3$ domain from H-2L^d. T62UL mice, in which the exon-shuffled transgene was expressed in a similar fashion as the H-2D^d gene in D8 mice, were as efficient as D8 mice in rejecting H-2^b lymphomas. In contrast, in the T62D6 mice (generated from another founder) no rejection was seen. Upon close analysis of the T62D6 mice we found that the transgene was not uniformly expressed as would have been expected considering that the transgene is regulated by its endogenous promoter. Surprisingly, the transgene was expressed in only a fraction of a given cell population. This fraction varied (10 - 80 %) between animals. It was however consistent within the animal, meaning that in a given mouse the same percentage of i.e. T cells, B cells and NK cells expressed the transgene. Regardless of the percentage of transgene positive cells, none of the mice challenged so far have been able to reject H-2^b lymphomas in the in vivo assay measuring NK cell mediated elimination. The data will be discussed within a concept for development of "NK tolerance".

C2-247 **BCL-2 PREVENTS THE CLONAL DELETION OF PHOSPHOCHOLINE-SPECIFIC B CELLS IN μκ BUT NOT IN μ-ONLY M167 TRANSGENIC XID MICE**

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We have previously shown (J. Immunol.146:2568,1991) that the combined expression of the M167 μκ anti-phosphocholine (PC) transgenes (TG) with the x-linked immunodeficiency gene, xid, results in an almost total failure to develop B cells in the peripheral lymphoid organs of such male mice, whereas, PC-specific B cells develop normally in their bone marrow. In F1 progeny of B6.xid x μ-M167 mice, the phenotypically normal females produce large numbers of PC-specific B cells, while the immunodeficient males lack PC-specific B cells. Thus, the lack of peripheral PC-specific B cells in M167 μκ and μ-only xid mice appears to be due to an Ig-receptor-mediated clonal deletion of these B cells, whereas, PC-specific B cells are positively selected and expanded in mice expressing a normal X-chromosome. M167 μκ and μ-only TG^+ female mice homozygous for the xid gene were crossed to Bcl-2 transgenic mice to determine whether or not over-expression of Bcl-2 would prevent the apparent clonal deletion of PC-specific B cells in the Bcl-2^+:V_H1^+ double transgenic F1 male progeny. The number of PC-specific splenic B cells increased greatly in M167μκ xid mice in the presence of Bcl-2 going from < 2 x 10^6 B cells in the Bcl-2^-:V_H1^+ males to ~ 50 x 10^6 in the Bcl-2^+:V_H1^+ male mice, and these double TG^+ B cells respond in vitro to stimulation with the TI-antigen PC-Sepharose but not to PC-dextran. However, PC-specific B cells did not develop in the immune deficient M167 μ-only F1 male mice. Since over-expression of Bcl-2 had no effect on the bone marrow pre-B cell pool in any of the F1 progeny, these data suggest that Bcl-2, which is down regulated in pre-B cells, cannot overcome clonal deletion which occurs in pre-B or very immature B cells, but can prevent negative selection if the B cell skips these early stages of B cell development by the simultaneous surface expression of both μ and κ transgene products.

C2-248 COREGULATION OF HLA CLASS II EXPRESSION AND ANTIGEN PRESENTING CELL FUNCTION.

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The absence of HLA class II gene expression in bare lymphocyte syndrome (BLS) is due to a defect in the transcriptional activation of class II genes encoded within the MHC. Previously we showed that DR-transfected BLS-1 cells exhibit defects in class II-mediated presentation of exogenous antigens and alterations in class II$\alpha\beta$ structure, suggesting coregulation of genes required for functional antigen presentation and class II structural genes. Genetic studies have revealed that distinct defects in multiple trans-acting factors result in the class II null phenotype of BLS cells. To determine if the genes required for antigen presentation are regulated by the same set of trans-acting factors as the class II structural genes, we studied APC function in BLS cells derived from four complementation groups. All DR-transfected B cell lines displayed the same defective APC phenotype: an inability to participate in the class II-mediated presentation of exogenous native protein antigens yet efficient presentation of preprocessed peptides, as well as structurally abnormal class II $\alpha\beta$ dimers that lack specific conformational mAb epitopes and show reduced stability in SDS. Expression of the HLA class II-like genes DMA and DMB, required for appropriate antigen presentation, was reduced or absent in BLS cells. Fusion of BLS cells with the HLA class II region deletion mutant 721.174, which is also a defective APC, restores class II DR and DM gene transcription and a wild-type APC phenotype. These results indicate that non-MHC linked factors in 721.174 can complement each of the molecular defects in BLS cells. Whether additional non-MHC linked genes potentially required for APC function are coregulated by these class II gene transactivators is being addressed. Thus, each of the molecular defects that silences class II structural gene transcription also results in a defective APC phenotype, providing strong evidence for coregulation of these pathways.

C2-249 **BACTERIAL SUPERANTIGEN BINDING TO CLASS II MHC IS INFLUENCED BY THE MHC BOUND PEPTIDE,**

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The bacterial superantigens, staphylococcal enterotoxin A (SEA) and toxic shock toxin 1 (TSST-1) bind to class II molecules. The class II molecules involved also bear peptides of many different sequences in their peptide binding grooves. We predicted that the peptides bound to the MHC molecule might affect superantigen binding. Nine different class II molecules each covalently bound to single peptides were produced. Surface plasmon resonance was used to detect real time interactions. IE molecules bearing different peptides showed diverse binding kinetics to the superantigens. The kinetics were peptide sequence specific. Furthermore, binding competition with anti-class II antibodies showed SEA inhibited the binding of both anti-class II α chain and anti-class II β chain antibodies. This suggests that SEA may engage both the α-chain and β-chain of class II. These also indicate that SEA may contact some of the residues of the peptide bound to class II or it may contact class II epitopes whose conformation is affected by peptide amino acids.

C2-250 CHARACTERIZATION OF INTERLEUKIN-1β-CONVERTING ENZYME-DEFICIENT MICE.

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The mammalian interleukin-1β–converting enzyme (ICE) has a homology to ced-3 which is known to be a cell death gene in C. elegans. Overexpression of ICE causes fibroblast cells to undergo programmed cell death. Mutaions in ced-3 prevent cell death in C. elegans.

In order to determine the role of ICE in programmed cell death in general, we have generated mice carrying a targeted mutation in the ICE gene. A 1.5 kb fragment containing exon 6 and 7 was replaced by the neomycin resistance gene, resulting in a homologous deletion of the active site of ICE. An another construct which has the hygromycin resistance gene was used for making a double knock-out ES cell line which enables us to analyze ICE chimera mice. We will present data on our characterization of homozygous ICE-deficient mice and double knock-out chimera mice.

C2-251 **TCR PEPTIDE-SPECIFIC CD4 T CELLS PARTICIPATE IN THE MAINTENANCE OF TOLERANCE TO THE PATHOGENIC SELF.**

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Self reactivity is now thought to be important for the normal functioning of the immune system. T cells recognizing self determinants capable of causing autoimmunity exist in the periphery. We have asked if self TCR-peptide-specific T cells are involved in regulating predominantly Vβ8.2-expressing, MBP Ac1-9-specific T cells which mediate experimental autoimmune encephalomyelitis (EAE) in B10.PL or (B10.PLxSJL)F1 mice. Regulatory T cells (Treg) recognizing a determinant from the framework 3 region on the TCR Vβ8.2 chain become activated in vivo during the recovery from EAE. Experimental evidence indicates that the immunodominant FR3 peptide is processed and presented physiologically. These CD4 cells predominantly utilize the TCR Vβ14 or Vβ3 gene segments and are capable of protecting mice from EAE. Treating mice with anti-Vβ-specific monoclonal antibodies resulted in an increase in the severity and duration of the disease, affecting both the course and recovery from EAE. Antibody-treated mice have more relapses which are still predominantly mediated by the Vβ8.2 cells. Possible mechanisms for the in vivo priming of Treg, as well as, the down-regulation of responses to MBP, are being investigated. Observations in the EAE model have been extended to the collagen II-induced arthritis in DBA/1 LacJ mice to generalize some of the findings. Thus, it appears that TCR-peptide-specific Treg are hard-wired into a cellular circuitry designed to maintain peripheral tolerance to the dangerous self. Supp. by funds from ACS, NMSS and NIH.

C2-252 FLEXIBILITY OF THE T CELL RECEPTOR REPERTOIRE ON TOLERANCE INDUCTION.
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Alternative T cell receptor gene usage between mice of different Mls alleles has been demonstrated in a number of T cell responses. A clear illustration of a flexible TCR Vβ usage in the same strain of mice after tolerance induction remains to be established. By using model system where I-E^k-restricted T cells recognizing λ repressor cI 12-26 and pigeon cytochrome c (pcc) 81-104 predominantly use Vβ3 in B10.A and B10.BR mice, and Vβ1 in Mls-2^a-bearing A/J and C3H mice, we have first demonstrated that the hierarchy of TCR Vβ usage can not be inferred from one strain of mice to the other. The presumed flexibility of Vβ3 to Vβ1 did not exist in B10.BR mice in these given responses after $V\beta3^+$ populations were tolerized. Instead, a switch of dominant TCR from Vβ1/Vβ3 to Vβ8 was identified in C3H and B10.BR mice. In contrast, there was an absolute rigidity in TCR repertoire usage in mouse strain such as A/J. The lack of flexibility was not due to slow generating kinetics of replacing T cells, A/J mice treated with SEA since neonate still responded poorly to cI 12-26 and pcc 81-104. Therefore, whether TCR Vβ usage in a T cell response would be flexible or rigid is highly dependent on each strain of mice. But even the plasticity seen in B10.BR mice is very limited, further tolerance of $V\beta8^+$ population resulted in non-responsiveness toward the given antigens.

C2-253 THE ANTIGENIC LOAD CORRELATES WITH TOLERANCE PHENOTYPE (IGNORANCE, FUNCTIONAL INACTIVATION, OR DELETION) IN IMMUNOGLOBULIN TRANSGENIC MIXED BONE MARROW CHIMAERAS.
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Mechanisms for the maintenance of B-cell tolerance have been well-studied in immunoglobulin transgenic mouse systems. Using anti-class I (3-83:anti-K^k,K^b,D^k) immunoglobulin transgenic mice, we have previously shown deletion of the autoreactive B-cells upon encounter with membrane-bound $K^{k,b}$ molecules in the bone marrow. However, others have shown induction of an anergic state in the B-cells with exposure to a soluble, non-membrane-bound antigen. Here we describe functional inactivation of B-cells following encounter with a membrane-bound antigen. Using mixed bone marrow chimaeras in which limiting amounts of cells were derived from H-2 K^k or D^k mice, we studied the effects of antigenic load on autoreactive, transgenic B-cells. Correlating with the amount of antigen present we saw a range of tolerance phenotypes from ignorance to partial deletion accompanied by functional inactivation in the remaining B-cells to complete deletion of the autoreactive B-cells. We propose that the duration of antigen exposure is critically important for the tolerance phenotype.

C2-254 INDUCTION OF MHC-CLASS I AND MHC CLASS II-RESTRICTED T CELL RESPONSES USING HYBRID Ty-VIRUS-LIKE PARTICLES,
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In our attempts to develop improved vaccines, we have been evaluating particulate antigen presentation systems, one of which utilises the p1 protein encoded by the TYA gene of the yeast retrotransposon (Ty). Nucleotides encoding antigens of interest are fused to the 3' end of a truncated TYA gene. The over-expression of these genes in yeast leads to high level production of p1 fusion proteins which assemble into hybrid virus-like particles (VLPs) of between 60-100nm in diameter depending on the size of the antigen fused to p1.
VLPs carrying the V3 loop of HIV-1 gp120 (V3-VLPs) were found to be potent inducers of CD8+ V3-specific CTL responses in mice. We have now demonstrated that hybrid VLPs carrying sequences from several other microbial proteins can induce rapid and potent CTL responses; for example, Sendai and influenza virus nucleoproteins and Malaria circumsporozoite protein.
The use of alum adjuvant was found to inhibit CTL induction but augment T helper cell priming. This indicates that alum inhibits the access of particles into the intracellular MHC class I processing pathway of the antigen presenting cell (APC) and facilitates access into the class II pathway. Also, different APCs may handle alum-adjuvanted and non-adjuvanted VLPs. In this regard, splenocytes or peritoneal macrophages were found to process VLPs in vitro for presentation to CD4+ T helper cells (class II) but not CD8+ CTL (class I). Both cell populations were able to present peptide, but not VLPs, for CTL activation. This suggests that the macrophage may not be the APC responsible for CTL induction in vivo. The mechanisms underlying the induction of MHC class I responses by hybrid VLPs are under investigation.

C2-255 **INDUCTION OF ANERGY IN NAIVE T CELLS BY ANTI-CD3 ANTIBODIES IS INDEPENDENT OF COSTIMULATORY FUNCTIONS,**
Oberdan Leo, Marcelle Van Mechelen, Erika Baus, Jacques Urbain and Fabienne Andris, Department of Molecular Biology, Université Libre de Bruxelles, Brussels, BELGIUM.
TcR occupancy by Ag/MHC complexes leads to functional responses such as lymphokine production, new surface receptor expression and proliferation. Under certain experimental conditions, TcR ligation can also induce functional unresponsiveness (or anergy), as demonstrated by the potent in vivo immunosuppressive properties of anti-CD3 mAbs. In order to better understand the regulation of T cell responsiveness to extracellular ligands, an in vitro model of anergy induction by anti-CD3 mAbs has been developed. Following a prolonged activation by anti-CD3 mAbs, purified $CD4^+$ cells become refractory to a subsequent stimulation despite a normal TcR complex surface expression. Unresponsive cells produce IL-2 and proliferate in the presence of pharmacological agents bypassing the early steps of signal transduction. A reduced calcium influx was observed in these cells in response to TcR stimulation, suggesting that unresponsiveness results from defective signal transduction. The presence of APC expressing B7.1 and B7.2 molecules (dendritic cells) or the addition of anti-CD28 mAbs during the primary in vitro stimulation were unable to affect anergy induction in this model, suggesting that unresponsiveness induced by anti-CD3 mAbs occurs independently of costimulatory functions. These observations indicate that in contrast to previously characterized models of T cell anergy, T cell unresponsiveness induced by anti-CD3 mAbs occurs despite the delivery of costimulatory signals. Data supporting the in vivo relevance of this model will be presented and briefly discussed.

C2-256 INTRAVENOUS INJECTION OF HIGH-DOSE SOLUBLE ANTIGEN INDUCES THYMIC AND PERIPHERAL T CELL APOPTOSIS, Roland S. Liblau, Roland Tisch, Kevan Shokat, Xiao-dong Yang, Nicole Dumont, Christopher C. Goodnow and Hugh O. McDevitt, Department of Microbiology and Immunology, Sanford University School of Medicine, Stanford, CA 94305.

Systemic injection of a high-dose of antigen induces a state of long-lasting antigen-specific T cell unresponsiveness. The mechanisms by which this unresponsiveness is established are still unclear, mainly because of the low frequency of a given antigen-specific T cell population and the absence of appropriate markers to follow the fate of these cells. We have assessed the mechanisms of high-dose tolerance induction using mice transgenic for a T cell receptor specific for the 126-138 peptide of influenza hemagglutinin (HA). After intravenous (iv) injection of 750 (but not 75) µg of HA peptide, a state of hyporesponsiveness was rapidly induced which lasted for more than 15 days as judge by calcium flux experiments and T cell proliferation assays. Following HA peptide iv injection, a synchronous and massive deletion of $CD4^+CD8^+$ thymocytes was observed within 24 hours. We utilized the terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL) reaction on tissue sections to assess cellular apoptosis. In the thymus, intense apoptosis was detected as early as 6 hours after iv injection of HA peptide, peaked at 12 hours and was essentially complete by 24 hours. The majority of cells in the thymus cortex and at the cortico-medullary junction showed evidence of apoptosis, with only a few cells in the medulla appearing to stain positive. The apoptotic cells were frequently seen grouped as clusters possibly within phagocytic cells. In secondary lymphoid organs, HA-reactive T cells were initially activated as judge by increased size and numbers, and elevated surface expression of CD4, IL-2Rα chain and CD69. However, these activated T cells were hyporesponsive at the population level in T cell proliferation assays and at the single cell level in calcium flux experiments although they responded vigourously to IL-2. After three days, those cells were rapidly deleted through *in situ* apoptosis in lymph nodes and spleen. Therefore, in our system, both thymic and peripheral apoptosis, in addition to peripheral anergy, contribute to high dose tolerance.

C2-257 THE MHC CLASS II MOLECULE H-2M IS TRANSPORTED TO AN ENDOSOMAL COMPARTMENT INDEPENDENTLY OF INVARIANT CHAIN, Ragnar Lindstedt, Monika Liljedahl, Per A. Peterson and Lars Karlsson. The R.W. Johnson PRI at the Scripps Research Institute 10666 North Torrey Pines Road, La Jolla,CA 92037

MHC class II ab heterodimers are formed in the ER shortly after synthesis. In the presence of invariant chain (Ii) the class II molecules are transported out of the ER to an endosomal/lysosomal compartment. Two leucine based targeting motifs in the cytoplasmic tail of invariant chain direct the invariant chain/class II complex to this compartment. The nonpolymorphic class II molecule H-2M in mouse (HLA-DM in man) facilitates formation of SDS stable dimers of other class II molecules.

We have investigated the targeting of H-2M molecules to endosomes in transiently transfected HeLa cells. H-2M molecules were transported to a CD63 positive endosomal compartment in the absence of Iip31. A chimeric cDNA construct was made coding for the *H-2A^k beta* extracellular and transmembrane domain with the *H-2M beta* cytoplasmice domain. HeLa cells were transfected with this construct together with a *H-2A^k alpha* cDNA. The expressed H-2A^k chimera was transported to an endosomal compartment independently of Ii as was wild type H-2M but not H-2A^k. Alanine substitution of two putative tyrosine based targeting motifs in the H-2Mb cytoplasmic tail revealed that one of these acted as the targeting motif. We have also studied the importance of this tyrosine based targeting motif for H-2M dependent SDS stable dimer formation of H-2A^k heterodimers.

C2-258 IMMUNOTOLERANCE IN TRANSGENIC MICE EXPRESSING A FOREIGN ANTIGEN IN A SEQUESTERED ORGAN, M. C. Lobanoff •, J. C. Lai •, A. Fukushima, E. F. Wawrousek, R. S. Lee, S. M. Whitcup, S. J. Smith-Gill*, and I. Gery, National Eye Institute, *National Cancer Institute, NIH, and •Howard Hughes Medical Institute Bethesda, MD.

Transgenic (Tg) mice expressing foreign antigens in tissues readily accessible to the immune system usually develop immunotolerance to these antigens. The present study examined whether immunotolerance also develops in Tg mice in which a foreign antigen is expressed in the lens, an avascular and encapsulated organ. Five lines of Tg mice were generated in which hen egg lysozyme (HEL) was expressed under the control of the αA lens crystallin promoter. In four of the lines HEL was membrane-bound, whereas in the other line HEL was expressed in a soluble form (The KLK and pMTH plasmids generously provided by C. Goodnow, Stanford University). Tg mice of the five lines developed immunotolerance to HEL, demonstrated by their failure to mount either cellular or humoral immune responses following immunization with HEL (25 µg/mouse, in CFA). Moreover, no inflammation was detected in eyes of any of the immunized Tg mice, even in those of the two lines in which the expression of membrane-bound HEL caused marked lens disruption. High levels of HEL were measured in eyes of all Tg mice, whereas no HEL could be detected in their sera, using the sensitive particle concentration fluorescence immunoassay (sensitivity limit ~1.0 ng/ml). Yet, the possible expression of HEL in other organs was suggested by our finding that immunotolerance was retained in Tg mice even at 2 months following enucleation of both eyes. Furthermore, HEL mRNA was detected in the thymus of the Tg mice using the RT-PCR technique. Our data thus suggest that immunotolerance does develop against a foreign antigen expressed in the lens, possibly due to the presence of minute amounts of the antigen in the thymus.

C2-259 ANTIGEN PRESENTATION MEDIATED BY RECYCLING OF SURFACE HLA-DR MOLECULES, Eric O. Long, Oddmund Bakke*, and Valérie Pinet, Laboratory of Immunogenetics, National Institute of Allergy and Infectious Diseases, NIH, Rockville, MD 20852, and *Department of Biology, University of Oslo, Oslo, Norway.

Class II molecules of the major histocompatibility complex associate with peptides derived from antigens that are processed in endocytic compartments. Antigen presentation to class II-restricted T cells generally requires newly synthesized class II molecules1, associated invariant chain, and HLA-DM. Exceptions to these rules have been reported but without description of an underlying mechanism. Here we show that presentation of an immunodominant epitope in the hemagglutinin protein of influenza virus correlates strictly with recycling of surface HLA-DR molecules. Truncation of either one of the α or β cytoplasmic tails virtually eliminated internalization of HLA-DR molecules and presentation of hemagglutinin from inactive virus particles. In contrast, the invariant chain-dependent presentation of matrix antigen from the same virus particles was unaffected by these truncations. Thus, HLA-DR cytoplasmic tails are not required for the conventional presentation pathway but jointly contribute a signal for an alternative pathway involving internalization of HLA-DR molecules.

C2-260 **ANTIGEN-SPECIFIC IMMUNOSUPPRESSION: ABROGATION OF ANTI-SULFAMETHOXAZOLE (SMX) ANTIBODY RESPONSES BY SMX-DEXTRAN ARRAYS.**

Catherine McCall, Anna Gallegos, Jane Kehl, Don McLeod, and Claire Coeshott. Cortech, Inc., 7000 N. Broadway, Denver, CO 80221.

We have developed an antigen-specific immunosuppressant which can suppress ongoing IgG and IgE antibody responses to sulfamethoxazole (SMX) in mice. SMX is widely used in HIV+ patients undergoing prophylaxis or treatment for *P. carinii* pneumonia, but many of these patients experience hypersensitivity reactions which limit their use of the drug. We immunized Balb/c or BDF1 mice with SMX-carrier *protein/adjuvant* to induce IgG or IgE responses. Once antibody responses were established, mice were treated subcutaneously with constructs consisting of SMX covalently coupled to a dextran backbone. The size (less than 100 kD) of these constructs was designed to be suppressive according to the criteria defined by Dintzis et al.* Treated mice showed a rapid and sustained fall in serum antibody IgG or IgE anti-SMX titers, and mice remained suppressed after a boost given 28 days after the initial treatment. The suppression observed was antigen-specific, in that antibody responses to the immunizing protein carrier were unaffected. The mechanism of the suppression at the cellular level is not defined and currently under investigation.

*Dintzis R.Z., M.H. Middleton and H.M. Dintzis. 1983. J. Immunol. 131:2196.

C2-261 THE SELECTION OF IMMUNODOMINANT EPITOPES IS DIFFERENTIALLY REGULATED WITHIN B-CELLS AND MACROPHAGES, Michelle T. McMurry, Carol Fang, *Phyllis E. Whiteley, and Janice S. Blum, Immunology and Diabetes Programs, Virginia Mason Research Center and the University of Washington, Seattle, WA 98101 and *Syntex Discovery Research, Palo Alto, CA 94304

Processing reactions within APC regulate the selection of antigenic epitopes displayed in the context of class II histocompatibility antigens. In human B-lymphoblastoid cell lines, processing of the IgG kappa light chain yielded dominant and subdominant peptides which were presented by class II DR antigens. Dominant peptide:class II complexes were preferentially displayed verses subdominant peptide:class II complexes in B-cells using either exogenous or endogenous IgG as the source of antigen. In contrast, the hierarchy of IgG peptides processed and presented by a human monocytic/macrophage tumor cell line differed such that primarily the subdominant IgG kappa epitope was bound to DR antigens. T-cell responses to synthetic kappa peptides were similar using macrophages or B-cells as the presenting cell. Differences in antigen uptake, intracellular sorting, proteolytic processing and denaturation may be responsible for this distinction between B-cells and macrophages. Fc-receptor mediated internalization of IgG was not required for the preferential presentation of the subdominant peptide in monocytic cells, as identical data was obtained with both $F(ab')_2$ fragments and purified kappa light chains. Studies are underway to evaluate the role of distinct intracellular proteases in the generation of immunodominant epitopes within monocytes and lymphocytes.

C2-262 **RECOGNITION OF NONPEPTIDE ANTIGENS BY HUMAN γδ T CELLS,**

Craig T. Morita*, Yoshimasa Tanaka‡, Jack F. Bukowski*, Edward Nieves‡, Hamid Band*, Barry R. Bloom‡, and Michael B. Brenner*, *Department of Rheumatology/Immunology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115 and ‡Howard Hughes Medical Institute, Albert Einstein College of Medicine, Bronx, NY 10461.

Human γδ T cells are a distinct population of T cells differing from αβ T cells in cell surface phenotype, in limited combinatorial diversity of their T cell receptors (TCR), and in selective anatomical localization. Since human γδ T cells expressing Vγ2Vδ2 TCRs expand in patients with certain infections (up to 45% of all T cells in the peripheral blood) and since γδ T cells respond to bacterial extracts *in vitro*, they are likely to be involved in the host response to infection. To elucidate the molecular basis for this recognition, we have determined the structure of natural and synthetic γδ antigens and have studied the recognition and presentation of these γδ antigens. We find that mycobacterial γδ antigens are protease resistant, small (<500 D), and phosphatase sensitive suggesting that they are not peptides. Strikingly, synthetic monoalkyl phosphate compounds precisely mimicked the biological properties of the natural antigens. Nonpeptide antigen recognition was restricted to T cells expressing Vγ2Vδ2 TCR and this recognition could be transferred to TCR^- Jurkat cells by transfection of Vγ2 and Vδ2 cDNA constructs. Recognition of the nonpeptide antigens was independent of APC for some γδ T cell clones and for transfectants and did not require classical MHC class I, class II, or CD1 molecules. Thus the Vγ2Vδ2 subset of human γδ T cells may have been selected to respond to antigens entirely distinct from the peptide antigens that αβ T cells respond. Presentation of these nonpeptide monoalkyl phosphate antigens to γδ T cells is different from presentation of peptide antigens to αβ T cells and may have a unique molecular basis.

C2-263 MAPPING OF ANTIBODY AND T CELL EPITOPES OF INVARIANT CHAIN DERIVED CLIP PEPTIDE BOUND TO MOUSE MHC CLASS II MOLECULE I-A^b.

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CLIP peptide, a proteolytic fragment of the invariant chain (Ii) spanning residues 80-106, is frequently found in the association with MHC class II molecules in normal APC as well as in antigen processing deficient B cell lines. In order to study CLIP : MHC class II complexes in detail, we have generated a monoclonal antibody (Ab) 30-2 and a panel of T cell hybridomas recognizing complex of MHC class II molecule I-A^b and human CLIP. Both 30-2 Ab and T cells recognize synthetic as well as naturally processed CLIP peptides. Surprisingly, the T cell recognition site is separate from the 30-2 Ab epitope. However, we found both epitopes expressed on the same I-A^b molecule. Moreover, both T cells and Ab recognize 10-12 kD fragments of Ii associated with I-A^b. A number of truncation and single aa substitution variants of human CLIP peptide were used to study its binding to I-A^b as well as T cell and Ab recognition of this complex. Our experiments suggest that CLIP peptide as well as the corresponding region of Ii is localized within or in a close proximity of the peptide binding groove of I-A^b molecule. The model of CLIP binding to MHC class II will be discussed.

C2-264 **THE ROLE OF ANTIGEN GLYCOSYLATION IN THE T CELL RESPONSE**, S. Mouritsen[*], L. Galli Stampino[*], K. Frische[¤], T. Jensen[#], O. Werdelin[#] and M. Meldal[¤]. Inst. Med. Microbiol. and Immunology[#], University of Copenhagen, Carlsberg Laboratory, Department of Chemistry[¤], Valby, Copenhagen and M&E A/S[*], 40 Lersø Parkallé, Copenhagen, Denmark.

Glycoproteins are present on many microorganisms and a change in the carbohydrate structure on tumor cells is a characteristic trait of the malignant phenotype. Therefore, it is of considerable interest to study whether glycopeptides can be recognized by T cells. Also it would be interesting to know whether the naturally processed peptides on MHC molecules contain carbohydrates. We have studied whether different types of glycopeptides can bind to MHC class II molecules. HEL(81-96)Y, which binds to the murine E^k molecule, was synthesized in 5 different glycosylated forms. An N-terminal serine of HEL(81-96)Y was derivatized with D-glucose, maltotriose, and a branched D-glucose pentasaccharide. Furthermore, HEL(81-96)Y was synthesized with a central serine or asparagine derivatized with the branched D-glucose pentasaccharide and a GlcNAc, respectively. Only the N-terminally modified glycopeptides were able to bind - some even better than the non-modified peptide - to the E^k molecule. The increased binding to was found to be due to an increased association rate. The immunogenicity of the peptides corresponded exactly to the binding data.

In order to examine whether a carbohydrate moiety could be accepted at a central position, a serine-GalNac peptide-scan of the E^k restricted self-peptide, Hb(64-76) was examined. This was compared to peptide-scans using either serine, threonine, tyrosine or alanine, and the T-cell immunogenicity of some of the Hb(64-76) glycopeptides were examined. Finally, the naturally processed self-peptides derived from four different murine MHC class II molecules were purified and the contents of carbohydrates were studied. Data indicating that these peptides may contain GlcNac moities are presented.

C2-265 **ACTIVATION OF MURINE T CELLS BY BACTERIAL SUPERANTIGENS REQUIRES B7-MEDIATED COSTIMULATION,** E. Muraille, T. De Smedt, M. Van Mechelen, F. Andris, K. Thielemans, J. Urbain, M. Moser, and O. Leo. Laboratoire de Physiologie Animale, Université Libre de Bruxelles, BELGIUM.

Although the role of non-TcR associated costimulatory receptors during antigen-specific T cell stimulation has been clearly established, the involvement of costimulatory activity in T cell activation by bacterial superantigens (SAgs) has been the matter of controversy. The aim of this study was to evaluate the role of the costimulatory-receptor ligand molecules CD28/B7 on bacterial SAg-mediated activation of naive murine T cells. We demonstrate that a combination of monoclonal antibodies (mAbs) to murine B7.1 and B7.2 molecules inhibits the *in vitro* response of naive murine T cells to various SAgs (SEA, SEB and TSST-1) and the *in vivo* T cell activation following SEB administration. The inhibition of T cell responses *in vitro* and *in vivo* required simultaneous blocking of B7.1 and B7.2, suggesting that either B7.1 or B7.2 is sufficient to provide costimulatory signals to naive T cells in response to SAgs. Inhibition of T cell activation *in vitro* by mAbs to B7-related molecules can be overcome by mAbs to CD28, a finding in agreement with the hypothesis that CD28-mediated signals participate in T cell activation by bacterial SAgs. Moreover, as the data described here indicate a strict requirement for B7-mediated costimulation in response to SAgs, we postulate that *in vivo* production of T cell-derived cytokines observed early following SEB administration results from SAg-presentation by APC constitutively expressing B7.1 and/or B7.2 molecules, such as dendritic cells. These results suggest that an anti-B7-based therapy could be of benefit in the treatment of toxic shock syndrome involving SAgs.

C2-266 *Abstract Withdrawn*

C2-267 **CONSEQUENCES OF NEGATIVE SELECTION: APOPTOSIS, ENGULFMENT AND PRESENTATION OF SELF ANTIGEN.** Stephan Oehen and Stephen Hedrick. Univ. of California, San Diego, Dept. of Biology, 9500 Gilman Drive, La Jolla, Ca-92093-0063.

Self reactive thymocytes are eliminated during negative selection through apoptosis. DNA fragmentation has been used as a major landmark to monitor apoptosis. However we have previously shown that DNA fragmentation can be uncoupled from apoptosis and negative selection. In *in vitro* thymocyte deletion cultures we find that antigen presenting cells (APC) are highly phagocytic and rapidly engulf negatively selected thymocytes. Using fluorescent dyes we can visualize engulfed thymocytes within macrophages and fibroblasts that serve as APCs. Engulfment of thymocytes may thus represent a crucial and highly effective step during negative selection. However, when resting B cells, which are not phagocytic, are used as APCs, they can also promote negative selection, even when DNA fragmentation is inhibited with Cyclosporin A. This suggests that engulfment may not be necessary for clonal deletion. In a deletion culture using non-phagocytic APCs, thymocytes may however "disappear" by the formation of apoptotic bodies and disintegration. To evaluate the fate of negatively selected thymocytes *in vivo* , we generated transgenic mice that express pigeon cytochrome *c* (PCC) in thymocytes. In TCRxPCC transgenic mice PCC-specific thymocytes are deleted. This may be due to the engulfment of apoptotic thymocytes by thymic APCs followed by the presentation of PCC peptide on MHC. By using the TUNEL method to detect dying thymocytes and by creating bone marrow chimeras, we are able to monitor the fate, not only of whole thymocytes, but also of their self antigens and to investigate the role of this mechanism in the shaping of the T cell repertoire to self antigens.

C2-268 DEFINITION OF A PEPTIDE EPITOPE FOR HUMAN SUPPRESSOR T CELLS, Tom HM Ottenhoff, Tuna Mutis and René RP de Vries, Dept. Immunohematology and Bloodbank, University Hospital, PO Box 9600, 2300 RC Leiden, The Netherlands.

Mycobacterium leprae induced suppressor T (Ts) cell clones have been previously isolated from peripheral blood and skin lesions of lepromatous leprosy patients and were shown to downregulate mycobacterium specific Th responses. Despite considerable effort the antigens recognized by these Ts cells have thus far not been identified. Here we report that all HLA-DR2 restricted CD4+ Ts cell clones derived from a borderline lepromatous leprosy patient recognize an epitope that maps between amino acid residues 439-448 of the *mycobacterial hsp65*. This peptide was presented to the Ts cells by HLA-DRB1*1503, a recently discovered HLA-DR2 variant. Non-Ts cell clones from this patient recognized antigens other than the hsp65 and were stimulated by other HLA-DR2 variants. In independent cloning experiments peptide 435-449 and r-hsp65 induced exclusively Ts cells in this lepromatous leprosy patient. The Ts cell clones recognizing this particular epitope were derived from at least 7 different progenitors as they expressed different TCR-α and -ß chains. Thus, our data indicate that a specific peptide-HLA class II combination may exclusively activate Ts cells.

C2-269 **ANTIGEN-SPECIFIC IMMUNOSUPPRESSION: KINETIC STUDIES OF ANTIBODY SECRETING CELLS AFTER TREATMENT WITH SULFAMETHOXAZOLE-DEXTRAN CONJUGATES,**

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A large fraction (30%) of the HIV-positive human population will develop hypersensitivity to the frequently prescribed antibacterial drug, sulfamethoxazole (SMX). The ability to manipulate the immune response by eliminating undesirable allergic or hypersensitive reactions has been investigated by Dintzis et al., 1983 (J. Immunol. 131:2196). Treatment of animals with multivalent constructs consisting of antigenic determinants arrayed on a dextran (dex) scaffold results in significant, long-term reduction of the specific antibody response provided that the constructs are of subthreshold size or valence. Using a defined SMX-dex conjugate, CI-694, SMX-specific antibody suppression has been successfully demonstrated in several animal models. The mechanistic basis of this regulation has not yet been described. Our approach has been first to characterize the responses in treated animals by monitoring the presence of SMX-specific antibody secreting cells (ASC). In SMX-KLH immunized mice treated with the suppressive conjugate, the number of ASC in spleen and bone marrow has been determined by ELISA-SPOT analysis. ASC of IgG isotype disappear rapidly after treatment (within 3-10 days) and the loss is long-lasting (monitored up to 60 days). The kinetics of ASC disappearance may point to the nature of the regulation at the cellular level which results in the observed elimination of SMX-specific antibodies.

C2-270 **SOLUBLE ANTIGEN CAUSES APOPTOSIS OF GERMINAL CENTER CELLS: A MODEL FOR CLONAL DELETION WITHIN THE GERMINAL CENTER?** Bali Pulendran, George Kannaroukis and GJV. Nossal, The Walter and Eliza Hall Institute of Medical Research,PO Royal Melbourne Hospital, Melbourne,Victoria 3050,Australia.

Germinal centers are dynamic microenvironments, within which activated B lymphocytes proliferate rapidly and introduce point mutations in their immunoglobulin variable region genes. Frequently B lymphocytes acquire mutations , which bestow them with the ability to bind antigen with a higher affinity. Such B cells are preferentially selected and expanded, resulting in affinity maturation.
Given the fast rate of mutation, and the large number of self-antigens, it is likely that B lymphocytes frequently acquire mutations which confer self-reactivity. It has been proposed that germinal center cells have a special "window of tolerance susceptibility," during which self-reactive mutants can be eliminated. The experiments described here were designed to test this hypothesis. C57BL/6 mice were immunized with NP-HSA (4-hydroxy-3-nitrophenyl acetate, coupled to human serum albumin), precipitated in alum. Fourteen days later, at the peak of the response, they were injected with a soluble, deaggregated form of NP-HSA, a few hours before being killed. Mice which received soluble antigen showed markedly enhanced apoptosis within the germinal centers, in contrast to mice which received no injections or those which received an irrelevant protein, such as transferrin. Apoptosis was detected by the TUNEL technique. Hapten-carrier experiments showed that this apoptosis acts directly on the B cells. These results may reflect a form of clonal deletion within the germinal center.

C2-271 VARIABILITY IN THE LEVELS OF CLIP AND ENDOGENOUS PEPTIDE ASSOCIATED WITH MURINE CLASS II EXPRESSED IN HUMAN B LYMPHOCYTES.
Lakshmi Ramachandra and Alexander Rudensky, HHMI and the Department of Immunology, University of Washington, Seattle, WA 98195.

In processing deficient mutant cells a high percentage of class II molecules have been found associated with a fragment of the invariant chain CLIP, suggesting that this complex represents an important intermediate in the MHC class II processing pathway. To understand the relationship between the generation of MHC class II complexes with CLIP and 'conventional' endogenous peptides we have generated a monoclonal antibody, 30-2, which recognizes human CLIP in the context of I-A^b. The 30-2 MAb and a previously characterized MAb YAe , which recognizes the endogenous peptide I-Eα/DRα52-68 also in the context of I-A^b were employed to analyze formation of these complexes in human B cell line Sweig transfected with I-A^b. Although the individual transfectants expressed similar levels of mouse class II, an inverse correlation between levels of DRα52-68:I-A^b and CLIP:I-A^b complexes was observed i.e. transfectants having high levels of the DRα 52-68:I-A^b complex had low levels of the CLIP:I-A^b complex and vice versa. Some transfectants, however, had intermediate levels of both YAe and 30-2 epitopes. All the transfectants expressed comparable levels of DR and invariant chain as demonstrated by antibody staining. Since the CLIP : I-A^b complex is an intermediate in the antigen processing pathway, these transfectants demonstrate the balance existing between this intermediate and the formation of endogenous class II complexes. The potential factor(s) which control this balance are under investigation.

C2-272 **PROBING T CELL RECOGNITION WITH CLASS II MHC/PEPTIDE-SPECIFIC ANTIBODIES**

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We have generated three antibodies which are specific for IE^k molecules bound to a peptide representing residues 95-103 of moth cytochrome c (MCC) as assessed by FACS analysis of peptide pulsed cells, ELISA on soluble IE^k bound to different peptides, and immuno-precipitation. The affinities of these reagents for IE^k/MCC, determined by surface plasmon resonance using the Biacore™, are >1000-fold higher than for unbound IE^k. Epitope mapping shows that the binding of these antibodies is selectively sensitive to changes in the sequence of either the peptide or the IE^k, allowing an orientation of the peptide in the complex to be derived. Competition studies demonstrate that none of these reagents recognize peptide alone.

These antibodies were used to correlate the number of IE^k-MCC complexes per cell (formed after pulsing with different peptide concentrations) with T cell stimulation capacity. Maximal T cell stimulation is achieved by cells expressing c. 5000 complexes. Although an average of 200 complexes per cell is sufficient to provoke a detectable bulk culture T cell response, it appears that an individual presenting cell must express many more (>2000) to stimulate a single T cell.

One of these antibodies (G-35) specifically inhibits the in vitro response of T cells recognizing IE^k/MCC. This prompted us to investigate the effect of this antibody on the development of T cells with the same specificity. We find that G35 specifically blocks the positive selection of transgenic T cells expressing a receptor recognizing IE^k/MCC. This indicates that the IE^k molecules mediating positive selection of this TCR must be occupied with peptide, and that these selecting peptides are structurally related to the nominal MCC antigen sequence. We are currently using this antibody to select the endogenous peptides that are responsible for mediating positive selection in vivo.

C2-273 LOW MOLECULAR WEIGHT ANTIGEN ARRAYS TARGET SELECTIVELY THE HIGH AFFINITY MEMORY B CELL COMPARTMENT.

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In transgenic and non transgenic model systems antigen concentration and valence, together with the developmental state of the B cell and the availability of T cell help, have been confirmed as crucial parameters for immune regulation. While the transgenic system elucidated parameters of tolerance induction for mature, primary recirculating B cells , the fate of higher affinity memory B cells could not be addressed with this model system. Our previous results suggested that suppressive antigen arrays act to suppress antigen-specific B cells by induction of either anergy or clonal deletion.

Here we adress how suppressive antigen arrays eliminate high affinity antibodies from the peripheral circulation at a cellular level. The questions addressed are 1) do antigen arrays selectively target the B cell compartment or do they affect T and B cell compartments? 2) are high affinity memory B cells rendered anergic or are they deleted ?

After establishing a high affinity anti-FL response, tolerance was induced by chemically and physically defined fluorescinated antigen arrays. Adoptive cell transfer experiments were used to follow the functional status of suppressed cell populations in the absence of suppressive polymer. In order to distinguish functionally between anergy and deletion, adoptively transferred B cells were (1) provided with optimal T cell help; (2) treated with LPS and (3) analysed with flow cytometry for anti-FL sIg positive B cells. Our findings indicate preferential elimination of the high affinity memory B cell compartment by suppressive antigen arrays. Carrier specific T cell help remains functionally intact and suppressive antigen arrays do not induce actively suppressive (non-B) cells.

C2-274 **DEVELOPMENT OF A HLA DR51 SPECIFIC, HIGH AFFINITY BLOCKING PEPTIDE.**

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Inhibiting T cell reactivity to HLA-DR-self peptide complexes by either MHC-binding competition or TCR antagonism, is of major interest in studying autoimmune responses and for developing potential therapies. For this reason, the DR binding of a set of alanine-substituted analogues of the peptide HA 307-319,S309 (PKSVKQNTLKLAT) was analyzed to design a non-self peptide with strong and specific interaction to the DR2 isotype DRB5*0101 (DR2a). This peptide has a biased interaction with this allele in opposition with the promiscuity of the HA 307-319 peptide. When assayed with L cells transfected with DR51 *(DRA,DRB5*0101)*, DR15(2) *(DRA,DRB1*1501)* or DR1 *(DRA,DRB1*0101)*, different analogues bound with increased or decreased affinity, reflecting structural requirements of the peptide-allele interaction. Interestingly, alanine substitutions at 310 and 315 resulted in a more restricted DR2a-binding. Additional analogues, polyalanine substituted at either the N or the C terminus, demonstrated that the minimal region with key anchor side chains (core) was contained within the seven central amino acids of the peptide. Based on these results, a new peptide, HA#20, was designed to increase both affinity and DR51 binding specificity. This peptide contains two repeated, alanine substituted core sequences. When assayed, HA#20 bound DR51 15-fold higher than the S309 analogue and 2-10 times that of the promiscuous peptides MBP 152-170 and SWM 132-151. Importantly, transfectants expressing other DR alleles bound HA#20 at either extremely low or undetectable levels. Comparison with a DR51 binding motif recently reported indicates that HA#20 contains alternative binding subsites. Interestingly, it doesn't substantially fit any of the binding motifs described so far for other DR alleles. This synthetic peptide is therefore an outstanding candidate for inhibiting in vitro T cell responses.

C2-275 INTRACELLULAR TRAFFICKING OF MHC CLASS II ANTIGENS:CO-LOCALIZATION OF INVARIANT CHAIN DISSOCIATION AND PEPTIDE BINDING IN B CELLS.

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The intracellular binding of peptides to MHC class II antigens temporally follows the dissociation of the newly synthesized complexes of MHC class II αβ dimers with the invariant chain, a chaperone for class II molecules. A cysteine protease sensitive to the inhibitor leupeptin, is required for invariant chain processing and MHC class II release in a murine B-cell lymphoma. Subcellular fractionation of leupeptin-treated murine B-cells, demonstrated that invariant chain cleavage is initiated in late endosomes. Processing of the invariant chain proceeded in a stepwise fashion with intermediate fragments LIP (21kDA) and SLIP (10-14kDA) co-precipitating with class II antigens in late endosomes 1-2 hrs after biosynthesis. Only complexes of class II antigens and SLIP were transported into the dense organelle MIIC after 4 hrs. Dissociation of invariant chain fragments occurs rapidly in MIIC such that the binding of a specific endogenous peptide to MHC class II antigens was detected shortly after class II antigen delivery into this subcellular compartment. The binding of peptides with a high affinity for MHC class II molecules confer stability of αβ dimers in the presence of SDS. Subcellular distribution of SDS-stable class II dimers will be presented. Analysis of MIIC vesicles revealed that this organelle is rich in class II *Ma* and *Mb* (DMA/DMB, RING6/RING7) antigens. These results suggest that delivery of MHC class II antigens into MIIC is regulated in part by the rate/extent of invariant chain processing. The final stages of invariant chain processing occur in MIIC confirming the localization of corresponding active proteases in this organelle. Our studies of antigen processing in MIIC also demonstrated that unique fragments of an exogenous antigen were formed in this organelle upon comparison with early and late endosomes. Thus, the proteolytic content of MIIC appears distinct from other endosomal vesicles and maybe important in modulating the accessibility of class II antigens to peptides.

C2-276 CONVERSION OF A MOUSE LYMPHOMA TO A CTL RESISTANT, NK SENSITIVE PHENOTYPE WITH LOW MHC-I EXPRESSION AS A RESULT OF IL-10 TREATMENT OR TRANSFECTION.Flavio Salazar*, Max Petersson*, Lars Franksson*, Masanori Matsuda*, Klas Kärre* and Rolf Kiessling*,&,* Microbiology and Tumor biology Center, Karolinska Institute, S-171 77 Stockholm, Sweden, & Dept. of Experimental Oncology, Radiumhemmet, Karolinska hospital.

Interleukin-10 (IL-10) is a cytokine with a variety of reported effects including: inhibition of monocyte MHC class II dependent antigen presentation, Th1 cytokine production, and inhibition of T cell proliferation. Recently we have shown that IL-10 also inhibits antigen presentation to human tumor- and allo-specific CD8+ cytotoxic T lymphocytes (CTL). Here we studied the effect of IL-10 on tumor- and minor-histocompatibility antigen specific CTL- and NK cell-mediated cytotoxicity against the mouse lymphoma RMA (H-2 ^{b}). Pre-treatment of RMA lymphoma cells with recombinant IL-10 (rIL-10) resulted in an inhibition of lysis by antigen specific CTL's, whereas pre-treatment or transfection of RMA cells with IL-10 induced a dramatic increase in their susceptibility to lysis by NK cells. The RMA-10 transfectants which produced high levels of IL-10 had levels of H-2 ^{b} expression as low or even lower than those found on the RMA-S mutant line, and treatment of RMA with rIL-10 also gave a partial reduction of H-2 expression. The levels of the adhesion molecules ICAM-1 and CD11a, however, were unaltered. In addition, relative to untreated target cells, IL-10 producing transfectants were unaltered in their capacity to affect CTL-mediated lysis by cold target inhibition, demonstrating that the effect of IL-10 is unrelated to the initial binding of CTL's to their targets. The expression of H-2 ^{b} was partially restored by coculturing RMA-10 transfectants or RMA-S cells with class I binding peptides. Taken together, these results indicate that IL-10 exerts a post-transcriptional effect on H-2 expression, compatible with an IL-10 induced decrease in the access of peptides to the MHC class I complex , thereby affecting both CTL and NK cell-mediated cytotoxicity.

C2-277 **RECOGNITION OF UNPROCESSED ANTIGEN BY A HERPESVIRUS SPECIFIC TCRγδ CELL,** Roger Sciammas, Raymond M. Johnson, Anne I. Sperling, William Brady*, Peter S. Linsley*, Patricia G. Spear#, Frank W. Fitch, & Jeffrey A. Bluestone, Ben May Institute and the Committee on Immunology, University of Chicago, Chicago, IL; *Oncogen Division, Bristol-Myers Squibb, Seattle, WA; #Department of Microbiology and Immunology, Northwestern University, Chicago, IL

The immunological roles of T cell receptor γδ (TCRγδ) cells is recently gaining more attention. The advent of Cα null mice has allowed immunologists to investigate the ability of TCRγδ cells to protect against infections and have demonstrated that they do indeed possess immunoregulatory functions. This implies that TCRγδ cells are responding to antigenic stimulus; however, in most instances, the nature of the antigenic ligands for TCRγδ cells remains to be determined. Interestingly, there is an emerging notion that TCRγδ cells can bypass classical antigen processing and presenting pathways and may recognize whole antigen in its native conformation. We have isolated a TCRγδ clone, TgI4.4, that is specific for a Herpes Simplex Virus (HSV) Type 1 glycoprotein, gI. TgI4.4 can recognize gI on β_2m negative cells, xenogeneic COS cells, and on TAP-2 deficient RMA-S cells. Antigen recognition can be inhibited by anti-gI antisera. Most importantly, a soluble form of gI, gI-Ig, can be used to crosslink and activate TgI4.4 thus demonstrating that recognition of gI occurs in the absence of processing or accessory cell function. The nature of gI recognition is dependent on conformation; however, it is independent of N-linked glycosylation. We are further characterizing the gI epitope recognized by TgI4.4. TgI4.4 expresses a heterodimer composed of a Vα2 containing delta chain and a Vγ1.2 containing gamma chain; both of which encode for a high degree of junctional diversity. Therefore we are characterizing the role of the TCR junctions in determining antigen specificity. In conclusion, TgI4.4, has enabled us to investigate the nature of antigen recognition by TCRγδ cells and suggests that TCRγδ cells recognize antigen in a manner akin to immunoglobulin recognition. In light of isolating TgI4.4 from a HSV infected mouse, this population of cells might serve to react to antigens expressed on tissues devoid of MHC expression such as the CNS.

C2-278 The invariant chain p31 and p41 transgenic mice

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During biosynthesis, class II molecules of the major histocompatibility complex are associated with the invariant chain (Ii), which facilitates folding of the class II molecules and their exit from the endoplasmic reticulum, interferes with their association with peptide and directs their post-Golgi transport. The murine Ii gene encodes two polypeptide chains, one of relative molecular mass of 31,000 (p31) and another less abundant 41,000 (p41). The role of these two isoforms has been studied only in transfected cells which are inappropriate APC types. To circumvent this problem and to study the role of these isoforms *in vivo*, we have generated invariant chain deficient mice which express exclusively p31 and p41 isoforms. As observed previously, the absence of Ii caused significant reduction in class II molecules surface expression and the α and β chains retained in high molecular weight aggregates characteristic of misfolded proteins. Low level expression of p31 or p41 is not sufficient for rescuing high level of cell surface class II expression. However, low levels of typical compact conformation dimers indicative of tight peptide binding are observed. Thus, both p31 and p41 participate in class Ii folding and assembly.
Furthermore, it was shown previously that invariant chain deficient mice have a deficient $CD4^{+}CD8^{-}$ thymocyte compartment and have very few $CD4^{+}$ T cells in the periphery and increased $CD8^{+}$ T cells population. Both p31 and p41 retrieve the $CD4^{+}$ population while $CD8^{+}$ cells level is reduced. Therefore, we can suggest that although the MHC class II level on the cell surface is low, those complexes that are expressed, are probably in the compact form and are loaded with peptides.

C2-279 ANTIGEN PRESENTATION AND CONFORMATION OF MURINE CLASS II MOLECULES IN ANTIGEN PROCESSING MUTANT CELL LINES IS SPECIES AND ALLELE DEPENDENT,
Christopher C. Stebbins, G. Loss, A. Chervonsky and A.J. Sant, Committee on Immunology and Department of Pathology, University of Chicago,Chicago, IL 60637.

Currently, the molecular events involved in the maturation, trafficking, and peptide loading of class II major histocompatibility complex (MHC) antigens are poorly understood, as is the role of protein co-factors in these processes. Recent studies on several cell lines that are defective in MHC class II restricted antigen presentation and that exhibit changes in class II conformation have identified a MHC encoded protein co-factor, DM, which participates in antigen presentation. In order to examine whether the defects seen in these cell lines were global or dependent on the allele or species of class II tested, we developed models involving murine class II molecules expressed in normal antigen presenting cells (APC) or mutant APC that lack expression of DM. We analyzed the conformation of class II molecules in these cells by assessing expression of monoclonal antibody epitopes and the ability of class II to form SDS-stable dimers. We also examined these cell lines for their ability to present a wide array of antigens to a large panel of class II restricted T cell hybridomas. We have found that specific alleles of class II are able to present antigen and adopt SDS-stable conformations in the absence of DM. Our results thus indicate that different alleles of class II may differ in their requirement for these protein co-factors and that different cell lineages may differ in their ability to provide or utilize these co-factors.

C2-280 SUPERANTIGEN INDUCED HYPORESPONSIVENESS *IN VIVO*, Anette Sundstedt, Gunnar Hedlund, Terje Kalland and Mikael Dohlsten. Pharmacia Oncology Immunology, Scheelev. 22, S-223 63 Lund, Sweden and Department of Tumor Immunology, The Wallenberg Laboratory, University of Lund.

Staphylococcal enterotoxins (SE) activate a high frequency of T cells, which has led to their designation as superantigens. Administration of SE to adult mice results in cytokine secretion and expansion of $CD4^+$ and $CD8^+$ T cells expressing the appropriate TCR Vβ-chains. Cytotoxic T cells are generated in the $CD8^+$ but not in the $CD4^+$ compartment. The initial activation phase is followed by a state of hyporesponsiveness, characterized by clonal deletion and anergy in the remaining T cells.

We have investigated how administration of SEA influences the balance between activation and hyporesponsiveness in $CD4^+$ and $CD8^+$ T cells. Repeated injections of SEA to mice resulted in $CD4^+$ T cell deletion and a reduced capacity to produce IL-2 and TNF in the remaining $CD4^+$ cells, while IFN-γ production and cytotoxicity in the $CD8^+$ T cells were relatively intact. The failure to produce IL-2 was further examined at the transcriptional level using the EMSA technique. Distinct different patterns of transcription factors were found in anergized and activated T cells.

Superantigen induced hyporesponsiveness has mainly been demonstrated in $CD4^+$ T cells. Surprisingly, we found that suboptimal but not optimal doses of SEA induced profound anergy of the cytotoxic function in $CD8^+$ T cells in the presence of retained capacity to proliferate, express IL-2R and produce IFN-γ. These findings suggest that there are distinct stimulus dependent thresholds for aquiring responsiveness or anergy in $CD4^+$ and $CD8^+$ T cells.

C2-281 IN SITU DETECTION OF T-CELL APOPTOSIS DURING POSITIVE AND NEGATIVE SELECTION IN THE THYMUS. Charles D. Surh and Jonathan Sprent. Department of Immunology, IMM4, The Scripps Research Institute, 10666 N. Torrey Pines Road, La Jolla, California 92037.

Because of positive and negative selection to MHC molecules, only a small proportion of the massive numbers of T cells generated in the thymus are selected for export. Immature thymocytes have a rapid turnover, and it has long been assumed that most thymocytes die in situ, presumably from apoptosis. This has yet to be proved, however, and conventional staining techniques have shown only minimal evidence of cell death in the normal thymus. Using a method for detecting cells with DNA strand breaks, we present direct evidence for apoptosis in the normal thymus. In sections of thymus from adult mice, apoptotic cells are scattered throughout the cortex and are engulfed locally by $F4/80^+$ macrophages. Apoptosis in the cortex is not reduced in MHC-deficient mice, which suggests that T cell death is primarily a reflection of lack of positive selection rather than negative selection. Direct evidence for apoptosis due to negative selection was obtained by crossing a Vβ5 transgenic (TG) line to $I\text{-}E^+$ vs $I\text{-}E^-$ mice; $I\text{-}E^+$ mice are known to eliminate $V\beta5^+$ T cells in the thymus whereas $I\text{-}E^-$ mice do not. In marked contrast to $I\text{-}E^-$ mice, the medulla of $I\text{-}E^+$ Vβ5 TG mice contains dense aggregates of apoptotic cells; these cells are engulfed by a distinct population of $F4/80^-$ $MAC\text{-}3^+$ macrophages. Negative selection of $V\beta5^+$ cells is thus restricted to the medulla.

C2-282 TOLERANCE INDUCTION IS DETERMINED BY CONCOMITANT RECOGNITION OF DIFFERENT CROSSREACTIVE SELF-PEPTIDES, Robert C. Tam, Eugenia Fedoseyeva, Patricia L. Orr, Marvin R. Garovoy and Gilles Benichou, Dept of Surgery, UCSF Medical School, San Francisco, CA 94143-0508.

Although current dogma of T-cell recognition stresses its exquisite specificity, recent evidence shows that T-cell clones selected for a given peptide can recognize other sequentially or structurally related peptides. It is clear that such crossreactive events occurring between self peptides within the thymus could have a drastic impact on the selection of the T-cell repertoire. Here, we have examined the immunogenicity of various sequentially related self- peptides (residues 61-80) derived from different MHC class I proteins co-expressed in the same mouse. Following immunization with self-L^d 61-80 peptide, B10.A (K^k, A^k, E^k, L^d, D^d) mice mounted vigorous MHC class II-restricted T-cell proliferation not only after restimulation by the immunogen but also with self-K^k 61-80. No T-cell response was observed after challenge with self-D^d 61-80. Similar results were obtained in other mouse strains with different combinations of self-MHC I peptides indicating that this represents a general phenomenon. Furthermore, adult B10.A mice, tolerized i.v. with 500 μg L^d 61-80 12 days prior to immunization with L^d 61-80 did not respond to an *in vitro* challenge with L^d 61-80 or the crossreactive K^k 61-80. However, following K^k 61-80 immunization, L^d 61-80-tolerized mice while unresponsive to *in vitro* challenge with L^d 61-80, mounted a strong proliferative response to K^k 61-80. Thus tolerance induction to self-L^d 61-80 resulted in the elimination/inactivation of L^d 61-80-reactive T-cells including the subpopulation which crossreacted with K^k 61-80. However, T-cells that recognize K^k 61-80 exclusively were preserved. Finally, B10.A (4R) mice (K^k, A^k, L^d, D^b) when immunized with self-K^k 61-80 mounted vigorous T-cell responses to *in vitro* challenge with both the immunogen and surprisingly, with D^b 61-80 (B10.A (4R) mice are normally tolerant to self-D^b 61-80). Therefore, immunization with K^k 61-80 resulted in the breakdown of tolerance to the crossreactive, dominant self-peptide, D^b 61-80. Taken together, these results demonstrate that both the selection of the autoreactive T-cell repertoire during development as well as induction of T-cell responses in adults are determined by the concomitant recognition of different crossreactive self-peptides within the same individual. The implications of this finding for elucidating the mechanisms involved in autoimmunity will be discussed.

C2-283 QUANTITATIVE EXPRESSION OF CLASS II WITHIN ENDOCYTIC COMPARTMENTS IS POSITIVELY AFFECTED BY INVARIANT CHAIN AND CAN BE POSITIVELY OR NEGATIVELY AFFECTED BY SINGLE AMINO ACID CHANGES IN THE 80-84 REGION OF THE CLASS II β CHAIN, L.J Tan, A. Chervonsky, J.M. Rodriguez-Paris, T.L. Steck and A.J. Sant, Depts. of Pathology and Biochemistry and Molecular Biology, University of Chicago,Chicago, IL 60637.

Fundamental to the understanding of major histocompatibility class II-restricted antigen (Ag) presentation is the elucidation of the mechanisms by which the class II molecules traffic to and localize in the endocytic pathway within the antigen presenting cell (APC). A number of studies have shown that the invariant chain (Ii) plays an important role in the targeting of the MHC class II/Ii complex into the proper endocytic compartments (E.C). More recent studies have shown that even in the absence of Ii, class II is detectable in E.C. In order to clarify the role that Ii- and class II-derived sequences have in localization of class II in the E.C, we have developed a technique to specifically isolate the endocytic vesicles of L cells transfected with wild type $I\text{-}A^d$, or several different mutant forms of $I\text{-}A^d$, each expressed in the absence or presence of invariant chain. Our studies show that in the absence of Ii, MHC class II molecules can localize in the endocytic pathway and that the class II molecules isolated from the endocytic compartments are stable to SDS, an indication of peptide loading. Supertransfection of Ii into L cells bearing mutant class II that show differential endosomal localization has varying effects. For example, in one mutant, Ii enhanced endosomal localization while in another, Ii had no effect. We also show that following an external feed of antigen, class II molecules can be isolated from the endocytic pathway that is loaded with the corresponding antigenic peptide and functional for antigen presentation to the appropriate T cell hybridoma. Taken together, these data suggest a role for both MHC class II-borne and Ii-borne signals that ultimately target class II into the appropriate endosomal compartment for functional association with antigenic peptides.

L.J Tan is the recipient of an Arthritis Foundation Post-doctoral Fellowship.

C2-284 T HELPER CELLS LACKING CD4 IN *LEISHMANIA*-SPECIFIC T CELL RECEPTOR (TcR) TRANSGENIC MICE, Jeannie F. Tseng*, Nigel Killeen#, Steven L. Reiner* and Richard M. Locksley#, Univ. of California, San Francisco (#) and Univ. of Chicago (*).

TcR transgenic mice (TcR+) were generated using the rearranged TcRα and TcRβ chains from an I-Ad-restricted, *Leishmania*-specific CD4+ T cell clone which expresses the Vα8 and Vβ4 gene products. Greater than 95% of the peripheral CD3+ T cells express Vβ4, the lymphocytes and thymocytes proliferate briskly to the peptide that stimulates the parent clone and during experimental infection the T cells are capable of Th1 or Th2 effector functions. Curiously, the phenotype of cells in the periphery that express the clonotype is predominantly CD4-CD8- or CD4-CD8+ as assessed by surrogate studies performed in the absence of an anti-clonotypic antibody (exclusion of endogenous TcRα, upregulation of IL-2Rα after peptide/MHC stimulation, phenotype of Rag-1 -/- TcR+ T cells). The atypical lineage of this class II-restricted TcR might be due to overexpression of TcR as a consequence of transgenesis, with high affinity and avidity requiring the downregulation of CD4 to escape deletion or tolerance. Consistent with this is the fact that Rag-1 -/- TcR+ mice have "rescue" of the CD4+CD8+ thymic population but no CD4+CD8- cells in the periphery and that mice congenitally deficient in CD4 (CD4-/- TcR+) have clonotype populating the periphery. Alternatively, positive selection may occur prior to the double-positive stage of thymic maturation such that peripheral clonotype has never expressed CD4 during its development with attendant deletion of the CD4+CD8+ cells in the thymus. Consistent with this are the facts that constitutive CD4 expression neither deletes nor tolerizes the clonotype and that the thymi of TcR transgenic mice are smaller than non-transgenic littermates. Another possible explanation may be an unusual regulatory event initiated by transgenesis has allowed promiscuous thymic maturation or even thymic-independent maturation. Consistent with this is the presence of peripheral clonotype in both MHC-mismatched (H-2b TcR+) and class II -/- TcR+ mice. We are currently performing the other crosses and thymectomies necessary to delineate this unusual lineage of T helper cells.

C2-285 A SEQUENTIAL MODEL FOR PEPTIDE BINDING AND TRANSPORT BY THE TAP PEPTIDE TRANSPORTERS. P.M. van Endert, S. Daniel, S. Caillat-Zucman and J.F. Bach. INSERM U25, 161 rue de Sèvres, 75743 Paris, France.

The TAP proteins translocate antigenic peptides into the endoplasmic reticulum. We have developed an overexpression system in which binding of peptides to the TAP substrate binding site and peptide transport by TAP can be quantified separately. Efficiency of peptide accumulation in the ER parallels affinity for the TAP substrate binding site, but can be modified by interaction with the glycosylation system and, probably, peptide efflux. Random peptide mixtures of 9 to 16 amino acids display significantly higher affinity for the binding site than mixtures of shorter or longer peptides. Formation of the substrate binding site requires both TAP subunits. Binding occurs in the absence of ATP and is reversed by non-metabolizable ATP analogues. These findings suggest a substrate driven process of peptide translocation in which "empty" TAP complexes display high affinity for peptide and low affinity for ATP. Peptide binding can be predicted to increase the affinity for ATP, whose subsequent binding and metabolism mediates peptide release and translocation. Because of the relatively high levels of TAP expression in this system, the ease of expression of mutants and the possibility to introduce additional proteins involved in antigen processing the presented system should be useful in the further characterization of TAP-mediated peptide transport. First results of the outlined studies will be presented.

C2-286 DOWNREGULATION OF CLASS II MHC MOLECULES ON ANTIGEN PRESENTING CELLS AFTER INTERACTION WITH HELPER T CELLS, Damir Vidovic´, Fiorenza Falcioni, David R. Bolin*, and Zoltan A. Nagy, Departments of Inflammation & Autoimmune Diseases, and *Molecular Sciences, Preclinical Pharmaceutical Research, Hoffmann-La Roche, Inc., Nutley, NJ 07110-1199

The recognition of major histocompatibility complex class II/peptide complexes by $CD4^+$ helper T cells (Th) is demonstrated here to result in a dramatic reduction of class II molecules on the antigen presenting cells. The reduction is selective to the class II isotype presenting the antigen, but it affects both allelic forms of the same isotype in heterozygous APC. The class II downregulation requires direct contact between Th and APC, T cell antigen receptor (TCR)-peptide-MHC interaction, and involvement of CD2 molecules. These findings have important implications for the regulation of immune response, self tolerance, and autoimmunity.

C2-287 PARTIAL TOLERANCE TO SELF MHC CLASS I IN CLASS I DEFICIENT MICE, Stanislav Vukmanović and Kanchan G. Jhaver, Department of Pathology, NYU Medical Center, New York, NY 10016.

In order to understand mechanisms of tolerance to self we have been studying $CD8^+$ cytotoxic T cells from β2-microglobulin (β2M) deficient mice. Normally, these mice have very few $CD8^+$ cells due to the very low levels of MHC class I expressed and consequent poor positive selection in the thymus. Repeated immunizations with tumor cells bearing allogeneic, or high levels of syngeneic MHC class I, result in the appearance of some cytotoxic $CD8^+$ cells. Curious feature of these cells is that they are biased for the recognition of syngeneic MHC class I (cells raised against allo-MHC kill syngeneic cells as well), and yet are not able to protect the host specifically against syngeneic tumors. We have found that this is due to the fact that these $CD8^+$ cells do not proliferate when stimulated with self MHC class I, even in the presence of exogenous IL-2, although they are activated to kill the same cells. Thus H-2^b β2M-/- $CD8^+$ line cells, raised against H-2^d tumors, kill and proliferate when stimulated with the same H-2^d tumor, and kill, but fail to proliferate when stimulated with H-2^b β2M+/+ tumors. This is not due to the induction of apoptosis or TCR antagonism induced by self MHC as H-2^d tumors transfected with H-$2K^b$ and H-$2D^b$ still stimulate proliferation. Further, when H-2^b β2M-/- mice were immunized with H-2^b β2M+/+ spleen, or tumor cells, $CD8^+$ cells were generated that, again, kill but do not proliferate upon stimulation with syngeneic stimulator cells. At the same time, both cytotoxicity and proliferation are induced with allogeneic cells (H-2^d). Thus, irrespective of the H-2 haplotype used for in vivo immunization, $CD8^+$ cells from the H-2^b β2M-/- mice respond to H-2^d stimulators with both proliferation and cytotoxicity, while only cytotoxic response can be induced with syngeneic, H-2^b, stimulators. These findings indicate that: 1) T cell receptors from β2M-/- $CD8^+$ cells probably react with MHC class I determinants shared by several alleles; and 2) partial tolerance to syngeneic MHC class I expressed at much higher levels is maintained in β2M-/- mice. The studies looking at the mechanism(s) underlying this partial tolerance to self MHC class I is maintained.

C2-288 SUPPRESSION OF SPECIFIC ANTIBODY RESPONSE WITH LOW MOLECULAR WEIGHT ARRAYS OF PEPTIDE ANTIGEN, Douglas C. Watson*, Johannes Reim** and Howard Dintzis**, Departments of *Pediatrics and **Biophysics and Biophysical Chemistry, Johns Hopkins U., Baltimore, MD 21205.

The peptide ^{24}AWCSDEALPLGSPRCD^{39} includes the second disulfide loop of human platelet GPIIIa (integrin β_3) and the ^{33}L↔P polymorphism associated with neonatal alloimmune thrombocytopenia. Mice were injected with two doses of oxidized peptide coupled at the N terminus to ovalbumin. The peptide was also coupled to dextran to yield arrays with M_w = 51,000, of which 27,000 is dextran; on average, each array has 14 copies of the peptide. Four weeks after the second immunization, mice were injected with 200 µg (as dextran) of the array. High affinity IgG to peptide immediately disappeared and then partially recovered, but two months after suppression, animals given peptide array had only 50% of the level of antibody in controls given dextran. Immunized animals given a high molecular weight (M_w = 615,000) array of the same peptide density also had an immediate drop in specific IgG, but by two weeks recovered to the level of controls. When 200 µg of low molecular weight peptide arrays was given two weeks prior to initiating immunization, three out of four mice did not produce specific IgG after two immunizations. Spleen cells or splenic B cells (plus ovalbumin-primed spleen cells) were transferred into irradiated recipients from immunized mice given low molecular weight peptide arrays two weeks previously. Three days after transfer, the recipients were boosted with peptide coupled to ovalbumin. Recipients of spleen cells or B cells from suppressed mice had about one-tenth the IgG response to the immunization, as compared to recipients of cells from immunized mice given dextran. These data demonstrate that injection of a low-molecular weight array of a peptide antigen can suppress the production of high affinity IgG to the peptide or induce tolerance to subsequent immunization. The phenomenon is a function of the molecular weight of the array and appears to be associated with deletion of specific B cells.

C2-289 TCR/SEB INTERACTIONS INFLUENCE THE MHC SPECIFICITY OF SEB RECOGNITION BY T CELL HYBRIDOMAS, Renren Wen*, Marcia A. Blackman*‡, David L. Woodland*‡ *Department of Pathology, University of Tennessee, Memphis, Memphis, TN18163 ‡Department of Immunology, St. Jude Children's Research Hospital, Memphis, TN38105

Several studies have suggested direct interactions between the MHC class II molecule and the TCR influence the reactivity of some T cells to superantigen. For example, the recognition of the bacterial superantigen SEB by $V_{\beta 14}^+$ T cell hybridomas is strongly influenced by MHC polymorphism. However, the recognition of SEB by $V_{\beta 8.2}^+$ hybridomas appears to be independent of MHC polymorphism. One possible explanation for this difference is that the affinity of $V_{\beta 8.2}$ for SEB is much greater than that of $V_{\beta 14}$ and therefore overrides the influence of TCR/MHC contacts. To test this hypothesis, we compared $V_{\beta 8.2}^+$ T cell hybridoma responses to either wild type SEB or mutant SEB(Y_{61}/C_{61}) thought to have decreased affinity for TCR. Our data show that whereas $V_{\beta 8.2}^+$ T cell hybridomas are not influenced by MHC/TCR interactions when recognizing wild type SEB, they are strongly influenced by MHC/TCR interactions when recognizing mutant SEB. These data support the hypothesis that MHC-specific recognition of superantigen is a characteristic of T cells that have intrinsically low avidity for the superantigen.

C2-290 **PEPTIDE RESIDUES OUTSIDE THE ANTIGEN BINDING GROOVE OF MHC CLASS II MOLECULES CONTRIBUTE TO T CELL RECOGNITION**

Emmanuel Wiertz 1,2, Jacqueline van Gaans-van den Brink 1, Esther Donders 1, Humphrey Brugghe 1, Hidde Ploegh 2 , Ada Prochnicka-Chalufour 3 , Peter Hoogerhout 1 and Jan Poolman 1.
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To investigate the contribution of terminal peptide residues on MHC class II-restricted T cell recognition, all 65 possible peptide analogues with a size between 3 and 12 residues, derived from a 13-residue T cell epitope, were prepared. When the peptides were tested for recognition by a panel of HLA-DR2 restricted T cell clones, a dynamic interaction of MHC and TCR contact residues was deduced. Sometimes, the addition of a C-terminal amino acid residue was found to compensate for the truncation of an important N-terminal residue, and vice versa. Moreover, a significant response was observed to a peptide containing the N-terminal four residues of the parental epitope, extended with an additional 14 residues at the N-terminus, according to the sequence of the parental protein. Based on the premise that most of these additional residues occur outside the open-ended antigen binding site, it is tempting to conclude that MHC anchoring may not be limited to the peptide residues present within the antigen binding groove of MHC class II molecules. The results are discussed in the context of a three-dimensional model of the HLA-DR2 - peptide complex.

C2-291 PERIPHERAL TOLERANCE IN T CELL RECEPTOR TRANSGENIC MICE.

Matthew Wikstrom, Kate Scott, Matthew Cook and Barbara Fazekas de St. Groth. *Centenary Institute of Cancer Medicine and Cell Biology, Locked Bag No. 6, Newtown, NSW, AUSTRALIA, 2042.*

We are using transgenic mice expressing a class II-restricted T cell receptor derived from the 5C.C7 cell line, which recognises residues 87-103 of moth cytochrome *c* in association with I-E^k, to study the cellular mechanisms which regulate primary T cell responses. Since intravenous administration of antigen is a well established means of inducing tolerance in normal animals, we have characterised the response to intravenous antigen in T cell receptor transgenic mice in order to follow the process of tolerance induction at the single cell level.

Up to 80% of CD4+ T cells expressing the transgenic T cell receptor (CD4+TgTCR+ cells) expressed CD69 2 hours after intravenous peptide administration. By 24 hours, the number of CD4+TgTCR+ cells in the spleen, lymph nodes and blood had declined by 50%, leaving a population that was only 50% positive for CD69. This implies that a large number of activated cells disappeared from the peripheral lymphoid organs during the first 24 hours of the response. The remaining cells exhibited enhanced proliferation and secreted large amounts of IL-2 and IFN-γ, but no IL-4 upon re-stimulation with peptide *in vitro*.

Three days after iv immunisation, the number of CD4+TgTCR+ cells had expanded by 2-3-fold and the population expressed a phenotype typical of late activation, namely $CD69^-$ $CD44^{bi}$. Proliferative responses of spleen and lymph node cells were no longer enhanced, nor did the cells secrete more IL-2 or IFN-γ than naive cells. The number of CD4+TgTCR+ cells returned to normal by day 7, then continued to decline to half that in control mice by 6 weeks after immunisation, provided the animals had been thymectomised. Dose response experiments revealed that there was a clear-cut threshold dose for i.v.-induced deletion and that no activation (independent of deletion) could be detected at lower doses of antigen.

In other experiments, transgenic mice tolerised by a single dose of intravenous peptide exhibited a reduced response per cell when challenged with a second intravenous dose. Three weekly doses removed about 75% CD4+TgTCR+ cells from the periphery, and the remaining cells were resistant to further deletion by repeated doses of antigen. Spleen and lymph node cells harvested after 5 weekly doses exhibited a 20-50% reduction in proliferation and secreted 10-fold less IL-2 per cell *in vitro* in comparison to naive cells.

C2-292 CELL SURFACE EXPRESSION OF CRYPTIC MINOR HISTOCOMPATIBILITY ANTIGENS.

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T cell responses against complex antigens are often directed against a few immunodominant epitopes. The existence of other, so called cryptic epitopes, have been shown in several systems. A cryptic epitope have been defined by Sercarz et al, for class II restricted epitopes, as immunogenic when present alone, but not in context of its complex antigen. We have previously shown that the CTL response of C57BL/6 mice against BALB.B mice, a minor histocompatibility barrier consisting of at least 40 different antigens, is directed against 3 immunodominant epitopes. In addition, epitopes associated to the BALB.B alleles H-8^c, H-19^c and H-25^c were found to be immunogenic when present alone in spleen cells from C57BL/6 congenic mice, but not immunogenic in BALB.B spleen cells, although they were found to be processed and loaded in MHC class I molecules of BALB.B spleen cells; they were hence termed cryptic. The aim of the present study was to investigate whether the crypticity of those three epitopes was due to lower levels of presentation of the epitopes in the BALB.B cells, than in the cells from the congenic strain. All three cryptic epitopes were found in surface eluates from BALB.B spleen cells; preliminary data suggests that they are present in the same amounts as in surface eluates from the congenic cells. BALB.B spleen cells were also able to in vitro reactivate CTL primed in vivo with congenic cells carrying the cryptic epitopes, indicating that the cryptic epitopes are present on BALB.B cells responsible for T cell activation. These data are in line with our previous suggestion that the reason for crypticity of the epitopes associated to H-8^c, H-19^c and H-25^c does not reside in the antigen presenting cell, but on the responding T cell level.

C2-293 **ANALYSIS OF SELF TOLERANCE TO AN ENGINEERED IMMUNOGLOBULIN IN TRANSGENIC MICE,** Elias T. Zambidis and David W. Scott, Immunology Department, Holland Laboratory, American Red Cross, Rockville, Md. , and University of Rochester School of Medicine, Rochester, N.Y., U.S.A.

Although transgenic mouse models have established the role for both thymic and peripheral deletion/anergy in inducing B and T cell tolerance to soluble and membrane-bound self antigens, the cellular mechanisms for these processes are still poorly defined. For example, the nature of the antigen presenting cell (APC) in peripheral T cell tolerance is not well described. Since self Ig is a potent tolerogen, and resting B cells are defective costimulatory APC, uptake of self-antigen by B cells and presentation to peripheral T cells may be an efficient mechanism of extrathymic tolerance. To address some of these issues, we have generated transgenic mice which express an engineered self-Ig construct in the peripheral B cell compartment. This engineered murine IgG_1 H chain has been "grafted" with a model immunodominant T and B cell epitope (residues 12-26 from bacteriophage λ cI repressor). The sequence for this class II-restricted 15-mer has been fused in frame to the N-terminus of the H chain, and the peptide is shown to be readily processed and presented to T cells, even in the context of an IgG scaffold. Transgenic fusion IgG_1 H chain readily combines with endogenous light chains, and expression can be detected in serum by ELISA and Western blotting at physiological levels (~10-10,000 ng/ml, depending on the line). Endogenous serum levels of IgM and IgG are unaffected. Unlike transgenic mice which express rearranged IgM constructs, the engineered IgG_1 does not appear to block endogenous Ig rearrangements: flow cytometric analysis shows similar levels of endogenous surface IgM^+ B cells as nontransgenic control littermates. More importantly, 12-26-IgG_1 transgenic mice are shown to be profoundly tolerant to the 12-26 peptide at the humoral and cellular level as assessed by 12-26-specific antibody and cytokine responses. This unique model system is well suited for studying the role that B cells and self-Ig may play in tolerogenesis. Additionally, these mice serve as a model for studying the potential of expressing gene-transferred epitopes on engineered Ig for the elimination of undesirable immune responses in allergy, autoimmunity, and AIDS. (Supported by AI-29691 and American Red Cross funds.)

C2-294 DOWN-REGULATION OF CD8 ON MATURE ANTIGEN REACTIVE T CELLS AS A MECHANISM OF PERIPHERAL TOLERANCE,

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Previously we have shown that intravenous injection of male B6 lymphocytes containing $CD8^+$ cells into B6 female anti-HY TCR transgenic mice results in the disappearance of the majority of male antigen reactive T cells from the periphery. In the present studies, we investigated the underlying mechanism leading to this disappearance of antigen reactive T cells. B6 female anti-HY TCR transgenic mice were intravenously injected with viable lymphocytes from male B6 normal, $CD8^{-/-}$ or CD8 tailless mice and the fate of HY specific T ($TCR^{hi}CD8^+$) cells was followed in vivo. It was found that there was always a large expansion of HY specific T cells in the periphery 4 days after encountering male antigen. The fate of activated male antigen specific T cells differed, however, depending on whether the injected male lymphocytes contained normal $CD8^+$ cells. If the injected male lymphocytes contained normal $CD8^+$ cells, there was a down regulation of CD8 (but not TCR) on the anti-HY cells. If the injected male lymphocytes lacked the CD8 molecule or expressed tailless CD8 (moleculae with the extracellular and transmembrane portions of CD8 but without a cytoplasmic domain), no down regulation of CD8 on anti-HY T cells could be observed. Moreover, we found that after down regulation of CD8, the HY specific cells, although carrying high levels of the male specific TCR, no longer reacted to male antigen and their ability to respond in anti-CD3 induced activation, which does not require CD8 as a co-receptor, was also significantly impaired. Our data suggest that down regulation of CD8 on the mature antigen reactive T cells accounts, at least partially, for the disappearance of HY reactive T cells from the periphery and that signals through the cytoplasmic domain of CD8 on the injected antigen-bearing cells is important for the induction of peripheral tolerance.

C2-295 HUMAN MHC CLASS II GENE TRANSCRIPTION DIRECTED BY THE CARBOXYL TERMINUS OF CIITA, ONE OF THE DEFECTIVE GENES IN TYPE II MHC COMBINED IMMUNE DEFICIENCY, Hong Zhou* and Laurie H. Glimcher, Department of Cancer Biology, Harvard School of Public Health, 665 Huntington Ave., Boston, MA 02115, USA

Type II major histocompatibility complex combined immune deficiency (Type II MHC CID or bare lymphocyte syndrome) is a primary severe immunodeficiency disease characterized by absent MHC class II expression. It is due to largely unknown defects in transcriptional regulation of MHC class II genes. Four distinct complementation groups and two typical *in vivo* promoter phenotypes have been identified. Recently, the wild type cDNA of the defective gene in group II Type II MHC CID has been isolated *via* complementation cloning and termed CIITA. Upon transfection into group II mutant cell lines, CIITA restores the expression of MHC class II molecules. However, the mechanism by which it regulates MHC class II gene transcription, like those of other previously isolated MHC class II promoter-binding factors or complexes, remains to be elucidated. In this report, we demonstrate that the intact CIITA protein is an MHC class II gene specific transcription activator. The general transcription activating activity is provided by its amino-terminal acidic domain (a.a.25~135) which is experimentally exchangeable with the heterologous transcription activating domain of Herpes simplex virus 1 alpha trans-inducing factor, while the specificity of the transcription activation of at least three major MHC class II genes, DR, DQ and DP, is mediated by its remaining carboxyl terminal residues (a.a.317~1130). The proline-, serine-, and threonine-rich stretches between these two domains are required only for optimal activity. The separation of CIITA into two essential domains with distinct functions establishes a critical link between general eukaryotic transcription activation and the specific regulation of the MHC class II gene family. It also defines specific targets for developing therapeutics to downregulate the expression of MHC class II alleles s associated with autoimmune disorders.

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Lymphocyte Development and Subsets; Identification of Autoantigens

C2-300 EARLIER ONSET OF COLLAGEN-INDUCED ARTHRITIS BY A TYPE II COLLAGEN PEPTIDE AND DEVELOPMENT OF ANTIBODY RESPONSE TO A REGION DISTANT FROM THIS PEPTIDE, A. Ametani, T. Matsumoto, S. Hachimura, A. Iwaya, J.-H. Kim, T. Nakagami,* Y. Sato,* K. Fujita* and S. Kaminogawa, Department of Applied Biological Chemistry, The University of Tokyo, Tokyo 113, and * Research and Development Center, Nippon Meat Packers, Tsukuba, Japan

CIA (collagen-induced arthritis) can be a disease model for human rheumatoid arthritis and useful for analysis of autoimmunity. We investigated CIA and antigen-specific immune response in DBA/1 mice injected with bovine type II collagen (bCol II) and its peptide. bCol II peptides 245-270 and 316-333 which are known to contain a dominant T cell and B cell determinant, respectively, were synthesized. Mice were immunized with these peptides and complete Freund adjuvant before injection with bCol II. Immunization only with p245-270 or p316-333 induced antibody response to each peptide, but not CIA. CIA developed faster in mice injected sequentially with p245-270 and bCol II than those only with bCol II. The onset of CIA in mice injected with p316-333 and bCol II was earlier than or the same as that in mice with only bCol II, dependent upon the number of injection times with p316-333. These indicate that the autoimmune reaction was not induced, but enhanced by injection with the synthetic peptides containing the dominant determinants. Immunization with p245-270 and bCol II elicited IgG2a and IgG2b response to both regions 245-270 and 316-333 more strongly than that only with bCol II. Thus, immunization with p245-270 induced earlier onset of CIA as well as stronger antibody response to the region distant from p245-270. These suggest that such antibody response to the particular region other than the peptide used for injection is the cause or the effect for induction of the autoimmune reaction, perhaps accompanying T cell help for B cell activation with an unexpected-specificity coupling between these T and B cells.

C2-301 B CELL REPERTOIRE PARTICULAR IN MOUSE PEYER'S PATCHES Michiko Ametani, Yuri Inoue, Aya Kawada, Chouemon Kanno, Noriko M. Tsuji *, Jun-ichi Kurisaki *, Department of Applied Biochemistry, Utsunomiya University, Tochigi, and *National Institute of Animal Research, Japan

The Peyer's patch is a unique lymphoid organ where germinal centers are well organized because of the successive stimulation by antigens in the intestine. Previous studies have shown that immunoglobulin genes of most of B cells in Peyer's patches are class-switched to IgA. To investigate how these unique features are reflected to development of B cell repertoire in Peyer's patches, we established IgA-producing B cell hybridomas from Peyer's patches in normal BALB/c mice. RT-PCR analysis showed that most of the clones expressed a significant amount of J-chain mRNA, whereas detectable message of J-chain was not amplified from Peyer's patch cells. On the other hand myeloma cells used as a fusion partner express only a low level of J-chain mRNA. These suggest that expression of J-chain in Peyer's patches is competent, but blocked by an inhibitor(s) or lacking in an indispensable factor(s), which characterizes Peyer's patches as an inductive site, but not an effector site. Analysis of antigen specificity of monoclonal IgA showed that antibodies from about 30 % clones slightly reacted with dsDNA, suggesting that immunoglobulin genes of some of Peyer's patch B cells encode IgA having a characteristics as natural antibody which can react with multiple antigens. Further we examined immunoglobulin genes of the hybridoma clones. The results showed that somatic mutation already occurred in those genes from both clones producing dsDNA-reactive and nonreactive antibodies, suggesting that they were selected by some antigens other than dsDNA. We demonstrate that antibodies encoded by the genes of some B cells in Peyer's patches in normal mice have both of features of the primary IgM-like natural antibody and the secondary mature antibody.

C2-302 CD4 TS AND TA CELLS REGULATE COGNATE HELPER FUNCTION OF TH CELLS, Yoshihiro Asano, Department of Immunology, Faculty of Medicine, University of Tokyo, Tokyo 113, Japan.

Cognate Th cell-dependent B cell activation is regulated by CD4 T suppressor (Ts) cells and CD4 T augmenting (Ta) cells. CD4 Ts cells inhibited the increase of intracellular Ca^{2+} of Th1 and Th2 clones induced by antigen and APC but not by Con A in an MHC -restricted manner. Ca^{2+} response of CD4 Ts clones was not inhibited by other CD4 Th and Ts clones. Activation of Ts clones overcame the MHC-restriction indicating the suppressive activity is mediated by soluble factor(s). Ts clones released soluble immunosuppressive factor(s) of 60-70kd by stimulation with immobilized anti-TCR or anti-CD3. The factor is different from any known cytokines including IL10, IFNγ, TNFαβ and TGFβ. The factor could inhibit the Th cell-dependent antibody formation and the IL-production. The factor could inhibit neither phosphorylation of TCRζ, proliferation of Th nor expression of IL2R, CD28 or CD69 of anti-TCR-activated Th cells. The addition of helper type lymphokines did not reverse the inhibitory effect of Ts clones. These results indicate that Ts clones inhibit the early signal transduction of Th cells by producing soluble factor(s) upon stimulation via TCR and thus suppressing specific immune responses. In addition, it is suggested that Ts clones can directly inhibit B cells as well.

CD4 Ta cells augmented the Th cell-dependent B cell activation. A population of B cells producing low affinity antibodies was involved in the augmented response. The presence of Th clones was required for the induction of the augmentation. The interaction between Th clones and Ta cells appeared to be mediated by soluble factor(s). T cells prestimulated with anti-TCR were effectively activated to proliferate by the soluble factor. The present study demonstrates that the cognate Th cell function is positively and negatively regulated by the cell interaction among CD4 T cells subsets, i.e., Th cells, Ts cells and Ta cells.

C2-303 **T CELL RECEPTOR SPECIFIC BLOCKADE OF POSITIVE SELECTION**. Kristin K. Baldwin[1] Philip A. Reay[2], Andrew G. Farr[3] and Mark. M. Davis[1,4]. [1]Program in Immunology, [4]Department of Microbiology and Immunology and the Howard Hughes Medical Institute, Stanford University, Stanford, CA 94305. [2]Immunochemistry Unit, Nuffield Department of Medicine, John Radcliffe Hospital, Headington, OX3. [3]Department of Biological Structure and Immunology. University of Washington, Seattle, WA 948195.

Immature T cells bearing the 5C.C7 T cell receptor (TCR) require the presence of the class II MHC molecule I-E^k for functional maturation. Mature 5C.C7 transgenic T cells respond to I-E^k in complex with the peptide moth cytochrome C(MCC) 88-103. In order to study these processes, we have generated monoclonal antibodies that bind preferentially to I-E^k-MCC complexes but not to "empty" I-E^k. One such antibody, G35, inhibits 5C.C7 T cell activation by MCC-I-E^k complexes. In addition, G35 stains thymic sections as well as cultured thymic stromal cells. These short-term heterogeneous stromal cell cultures support T cell development *in vitro*, including both positive and negative selection of 5C.C7 transgenic thymocytes. Furthermore, G35 treatment of these cultures blocks transgenic T cell development at the immature, double-positive stage while non-transgenic thymocytes develop normally. We conclude that G35 blocks positive selection of a specific TCR by binding to the naturally occurring I-E^k- peptide complexes that are required for thymocyte maturation. Further studies will employ the antibody G35 in combination with the thymic stromal cell cultures to characterize the naturally-occurring peptides that are responsible for positive selection of this class II restricted TCR.

C2-304 **THE PREDOMINANT EXPRESSION OF HEAT- SHOCK PROTEINS (Hsp)70 FAMILY IN THE MOUSE GUT PARALLELS THE PREDOMINANT DISTRIBUTION OF INTRAEPITHELIAL TcRγδ T LYMPHOCYTES (IEL)** Clara G.H. Bell, U.I.C., Department of Micro./Immuno., College of Medicine, Chicago, IL. 60612

Hsps were initially recognized in Drosophila after exposure to higher temperature & in bacterial cells exposed to heat, as elements induced rapidly & transiently to cope with increased protein damage. That transient induction of at least certain Hsps occurs in prokaryotes & eukaryotes under non-heat stress (such as under DNA-damaging agents & amino acid analogues) suggests Hsps to be important biological molecules. The delineation that the transiently induced Hsps exhibit highly conserved primary structures & may be subjected to conserved mechanism of regulation to exhibit conserved function, also suggests that transient induction of Hsps represent an important protective/homeostatic mechanism to cope with physiological & environmental stress. In bacteria, induction of Hsps in order to protect proteins (i.e., chaperons, that assist correct folding & assembly of proteins & protein transport, whose function is either to prevent inactivation of cellular proteins or to reactivate denatured proteins or to degrade cellular proteins that have been altered beyond repair), is primarily at the level of transcription. The transcription may be controlled by heat-shock transcription factors, which may regulate the transient activation of Hsp, as needed for adjusting the levels of Hsp required for optimal growth & function.

In Western immunoblotting dissections of murine tissues, I delineated Hsps members of the Hsp68/Hsp70 family (that share sequence similarity to the Drosophila, to the yeast & to the Escherichia coli Dnak bacterial Hsps) present in multiple copies within the mouse, as proteins constitutently expressed in murine tissues & amenable to upregulation upon temperature upshifts or stress. That both the constituent & inducible, transient expression of the Hsp70 family members was delineated in gut IEL tissues --irrespective as to whether the tissues were derived from mice maintained under conventional or under bacteria-free barrier condition-- may suggest a requirement within the gut for Hsps, irrespective of bacterial regulation & requirement. A parallel cytofluorometric dissection of the same murine tissues for T- & B-lymphocytes showed the predominant expression of the Hsp68/70 within the gut to be coincident with the highest representation of T TcRγδ in gut-associated IEL. Unlike the spleen & lymph node TcRγδ, which were of the $CD4^-CD8^-$ phenotype, gut-associated IEL TcRγδ were essentially of the $CD4^-CD8^+CD5^-$ phenotype (~80% of the IEL TcRγδ) & exhibiting the unique $CD4^-CD8\alpha\alpha(Lyt2)^+\beta(Lyt\text{-}3)^-$ homodimers, presumed to be associated with the thymus independent TcRγδ set. That these TcRγδ IEL were mostly $Pgp\text{-}1^+$, i.e., exhibiting a marker of memory T, suggesting an in vivo antigen (Ag) exposure, & that they were reactive with bacterial Hsp65 members, might suggest the Hsp68/70 family members function as targets for the TcRγδ T resident within the gut IEL.

While central to the function of the immune system, to protect the host from exogenous invaders & endogenous aberrations, are the TcRαβ, that differentiate intrathymically from fetal liver & from bone-marrow stem cell migrants, via a series of steps that require the thymus to lead: (i) to the expression of the TcRαβ -that convey the clonal specificity to recognize foreign Ags & discriminate them from self; (ii) to the expression of the CD4 & CD8 skewed to recognition of the foreign Ag in the context of the self MHC gene; & (iii) to the selection & maturation of the useful $TcR\alpha\beta^+CD4^+CD8^-$, $TcR^+CD4^-CD8^+$ repertoires, the potential selection & function of the TcRγδ that fail to exhibit the classical TcRαβ MHC-restricted recognition of foreign Ag, might be related to the reactivity against damaged self cells. The TcRγδ predominant distribution in the IEL & the high frequency of the Hsp-responsive TcRγδ T cells there, suggestive of a first line of defense at the site of first invasion, may argue that the Hsp expression --in itself fundamental in normal cell physiology & protection from denaturation-- may also contribute multiple selective functions that may differentially affect the TcRγδ selection, however, whether Hsp expression is only causal in promoting TcRγδ, or whether TcRγδ is an independent consequence of Hsp elevated expression is unclear.

C2-305 **The ligand of mouse $NK1.1^+$ T cells.** By Albert Bendelac (Princeton University) and Randy Brutkiewicz (National Institutes of Health).

Several lines of studies have shown that mouse $NK1.1^+$ TCR α/β^+ thymocytes constitute a separate T cell-lineage, which includes $CD4^+$ and $CD4^-8^-$ (double-negative) mature T cells. Their thymic selection depends upon expression of β2-microglobulin but not MHC class II, and is affected by the the level of expression of CD8, suggesting that their MHC ligand is an MHC class I molecule that binds CD8. We recently showed that they use a TCR repertoire made of a single, invariant TCR α chain, Vα14-Jα281, and of a restricted set of TCR β chains (Vβ-8, -7, and -2). In addition, we showed that this restricted TCR repertoire is identical in mice of different MHC haplotypes, and that similar cells using a homologous invariant TCR α chain, Vα24-JαQ, are found in humans. We therefore suggested that the $NK1.1^+$ T cell-ligand is a non-polymorphic MHC class I molecule shared by mouse and humans.

We have now identified this ligand, and we will report its sequence, as well as its tissue distribution pattern.

C2-306 ACTIVATION AND REGULATION OF HUMAN DJCμ GENE SEGMENT REARRANGEMENT PRIOR TO SURFACE EXPRESSION OF THE B CELL MARKER CD19. Fred E. Bertrand III, Linda G. Billips, Peter D. Burrows, G. Larry Gartland, Hiromi Kubagawa and Harry W. Schroeder, Jr. Division of Developmental and Clinical Immunology, Departments of Medicine and Microbiology, University of AL at Birmingham, Birmingham AL 35294-3300.

The stages of human B lineage development can be distinguished on the basis of surface expression of the stem cell marker CD34, the pan B cell marker CD19 and IgM. In order to determine the stages of bone marrow B lymphopoiesis during which IgH rearrangement and transcription occurs, we have sorted fetal and adult bone marrow into B lineage subpopulations on the basis of surface expression of these lineage associated molecules. Each subpopulation was examined for IgH transcription and rearrangement. The IgH locus is transcriptionally active prior to surface expression of CD19, as indicated by the presence of Iμ, Cμ, and DHQ52 germline transcripts in $CD34^+CD19^-$ cells. DJCμ transcripts were also detected in the $CD34^+CD19^-$ subpopulation. Within this subpopulation, fetal cells exhibited low levels of DJ transcripts containing members of the DHDXP family, and abundant DHQ52 DJCμ transcripts. In contrast, DHQ52 DJCμ transcripts were not detected in $CD34^+CD19^-$ cells derived from adult bone marrow. A low level of DHDXP DJ transcription persisted. These data suggest that 1) activation and initial IgH DJ rearrangement steps occur in the absence of surface CD19 expression during normal bone marrow B cell development and 2) transcription of the IgH locus is regulated differently between fetal and adult B lymphopoiesis during the earliest stages of B lineage commitment.

C2-307 THE IKAROS GENE IS REQUIRED FOR THE DEVELOPMENT OF ALL LYMPHOID LINEAGES Michael Bigby, Arlene Sharpe, Jin-Hong Wang, Arpad Molnar, Paul Wu, Susan Winandy and Katia Georgopoulos, Cutaneous Biology Research Center, Mass General Hospital, Harvard Medical School, Charlestown, MA 02129

The Ikaros gene encodes a family of early hemopoietic and lymphocyte-restricted transcription factors. We have tested and verified the hypothesis that the Ikaros gene plays a pivotal role in lymphocyte specification. We targeted a deletion in a region of the Ikaros gene that encodes the DNA binding domain of its protein products by homologous recombination in embryonic stem (ES) cells. Mice homozygous for this Ikaros mutation lack not only mature T and B lymphocytes, but also their earliest described progenitors. Natural killer (NK) cells, which are proposed to arise from a common T cell precursor, are also absent in Ikaros mutant mice. However, the Ikaros gene is not essential for the production of totipotential hemopoietic stem cells, erythrocytes, myelocytes, monocytes, dendritic cells, megakaryocytes and platelets. Together, the erythroid and myeloid lineages comprise almost 100% of the spleen and the bone marrow cell populations in this Ikaros mutant mouse. Extensive extramedullary hemopoiesis was detected in the spleens of these mutant mice which were larger in size than those in wild type littermates. In contrast, the bone marrow in these animals was hypocellular. Ikaros mutant mice lack specific immune responses mediated by T and B cells and the first line of defense mediated by NK cells. These severely immunocompromised animals rapidly succumb to opportunistic infections.

These results demonstrate that Ikaros proteins are required for the development of all cells of the lymphopoietic system. This is the only known transcription factor whose expression is essential for development of all three lymphoid lineages, but not for the development of the erythroid and myeloid lineages.

C2-308 STABILITY OF CD45 ISOFORMS AS MARKERS FOR NAIVE AND MEMORY CD4 T CELLS, Tamar E. Boursalian and Kim Bottomly, Section of Immunobiology, Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, CT 06510.

Little is known about the nature of immunological memory and the differences between naive CD4 T cells and effector and memory CD4 T cells. The differential expression of CD45 isoforms is thought to differentiate between naive and memory CD4 T cells. Cells that have high expression of the CD45RB isoform are said to be naive cells, and upon encounter with antigen, they have been shown to switch to low expression of the CD45RB isoform. However, it is not clear whether this change in CD45 phenotype is reversible or how consistently this phenotype correlates with function or stage of differentiation. We have addressed this question using an adoptive transfer approach in which T-deficient mice were reconstituted with donor-marked cells that were either CD45RBhi or CD45RBlo CD4 T cells or a mixture of the two cell types. We took advantage of mice transgenic for a known T cell receptor to donate naive or CD45RBhi CD4 T cells. In this way we could determine whether conversion was due to a loss of donor type cells or a change in the isoforms expressed. The CD45RBlo CD4 T cells were from normal mice. The CD45 phenotype of the transferred cells was followed over a period of several months by analysis of peripheral blood lymphocytes. This FACS analysis showed that the CD45 phenotype for both donor populations remained stable over several weeks. However, after two months, while the CD45RBlo donor population maintained its phenotype throughout the analysis period, the CD45RBhi donor population began to shift towards CD45RBlo. Currently we are investigating whether this shift is due to a loss of T cells expressing the transgene or due to a change in isoform expression.

C2-309 DEFINING THE CELLULAR COMPONENTS NECESSARY FOR TH2 DEVELOPMENT IN MURINE LEISHMANIASIS, Daniel R. Brown*, Richard M. Locksley# and Steven L. Reiner*, University of Chicago (*) and University of California, San Francisco (#)

Infection of inbred mice with *Leishmania major* serves as a model for the in vivo generation of antigen-specific helper cells. C57BL/6 mice develop a Th1 response while BALB/C mice mount a Th2 response. *In vivo* and *in vitro* experiments implicate IL-12 and IL-4 as critical in the early induction/priming of Th1 and Th2 responses, respectively. In several microbial disease models, the significant IL-12 is apparently derived from pathogen-induced macrophages. The cellular source responsible for the significant early IL-4 production remains much less well-defined and a primary role for a cell type within the innate immune system such as the mast cell or basophil has yet to be documented. IL-4 production by popliteal lymph node cells after footpad infection with *Leishmnaia* reaches a transitory peak at 4 days *in vivo* and the mRNA is transcribed predominantly by CD4+ cells (Reiner et al, J. Exp. Med. 179, 447). CD4+ subpopulations, however, exhibit broad heterogeneity. CD4+NK1.1+ cells are class I-restricted (Bendelac, *et al.*, Science 263,1774. Coles and Raulet, J. Exp. Med. 180, 395) and in one model of in vivo T cell activation contribute most of the early wave of IL-4 (Yoshimoto and Paul, J. Exp. Med. 179, 1285). To better understand the requirements for stimulating the early IL-4 burst seen in murine leishmaniasis we have infected class I- (β2m -/-), or class II-deficient mice. The early peak in IL-4 mRNA was intact in the β2m-/- mice as compared to wild-type controls but entirely absent in the class II-deficient mice. These results suggest that the priming levels of IL-4 required to initiate a Th2 response in murine leishmaniasis are not derived from the CD4+NK1.1+ cells but rather from conventionally class II-restricted CD4+ cells. *In vivo* immunization experiments in β2m-/- mice to generate IL-4-secreting effectors and back-cross of the β2m-/- defect onto the BALB/c background followed by *Leishmania* challenge should confirm wether the CD4+NK1.1+ population is necessary or dispensible for development of some Th2 responses.

C2-310 IL-4 DOWN-REGULATES IFNγ- INDUCED FcγR1 (CD64) EXPRESSION IN HUMAN MONOCYTIC CELL LINE, U937, Xiao-Yan Cai, Carl P. Gommoll Jr., Tracey A. Waters, Jay S. Fine, Linda D. Hamiltonl, Satwant K. Narula, and Michael J. Grace, Department of Immunology, Schering-Plough Research Institute, Kenilworth, N.J. 07033

The Th1 cytokine, interferon γ (IFN-γ), transcriptionally activates several early responsive genes in monocytes that are important for the phenotype of the activated macrophage. IL-4 has been the target of many recent studies as a Th2 cytokine that can antagonize many of the responses induced by IFN-γ, however little is known about the mechanisms involved. We have used a well defined system, the transcriptional activation of an early responsive gene encoding the high-affinity Fc receptor for IgG (FcγR1 or CD 64) by IFN-γ, for studying the mechanism of action by IL-4 on IFN-γ-induced gene expression. Pretreatment of a monocytic cell line, U-937, with IL-4 resulted in inhibition of IFN-γ-induced mRNA level for the CD 64 gene. The inhibitory effect of IL-4 on the steady state level of CD 64 mRNA in U-937 cells was blocked by chclohexamide (CHX), an inhibitor of protein synthesis, suggesting that newly synthesized factor(s) were involved in the regulation of CD 64 expression. In addition, the mRNA stability of IFN-γ + IL-4 treated cells was indistinguishable from IFN-γ alone treated cells. Furthermore, it was shown by EMSA that the inhibitory effect of IL-4 on IFN-γ-induced CD64 mRNA levels was not due to inhibiting the binding of the IFN-γ-induced transcription factor, STAT 91, to its responsive region of the CD 64 gene. These results suggest that factors other than STAT 91 may be involved in the regulation of IFN-γ-induced CD 64 expression by IL-4.

C2-311 INHIBITORY EFFECT OF HIGH AFFINITY INTERACTION BETWEEN TCR AND MHC.PEPTIDE COMPLEX ON THE PRIMARY RESPONSE OF CD8+ T CELLS, Zeling Cai and Jonathan Sprent, Department of Immunology, The Scripps Research Institute, La Jolla, CA 92037

The influence of the affinity between the TCR and MHC-bounded pep on the activation of resting CD8+ T cells was investigated with the aid of resting CD8+ cells from the 2C TCR transgenic mouse. Other workers (Sykulev et al.) have shown that the 2C TCR has high affinity ($2x10^6$ M^{-1}) for allo L^d molecule plus the wild-type p2Ca peptide, and even higher affinity ($1\text{-}2x10^7$ M^{-1}) for L^d plus a variant peptide, QL9. The QL9 peptide has much higher affinity for L^d than the p2Ca peptide, and we have found that only the QL9 peptide can stabilize the L^d expression on RMA-S cells at 37°C. Primary responses of purified 2C cells to the two peptides were examined using L^d-transfected RMA.S cells (RMA.S-L^d) as APC. In the absence of exogenous cytokines, both QL9 and p2Ca peptides presented by RMA.S-L^d can stimulate the proliferation of resting 2C cells. The proliferative response of 2C cells to QL9 peptide is independent of both B7 and CD8. Significantly at high concentrations of peptide (10μM and above), the proliferative response of CD8+ 2C cells to the QL9 peptide is inhibited. Moreover, the inhibitory effect can be overcome by adding anti-CD8 mAb or the CTLA4Ig fusion protein to the culture. The inhibitory role of CD8 in high affinity TCR-MHC.pep interactions is further supported by the finding that CD8- 2C cells proliferate better in response to a high concentration of QL9 peptide than do CD8+ 2C cells. Collectively, the data suggest that costimulation resulting from CD8/MHC class I and CD28/B7 interactions can lead to over stimulation when the affinity of TCR/peptide/class I interaction exceeds a certain threshold.

C2-312 **INDUCTION AND MODULATION OF IgE SYNTHESIS IN THE SCID-hu MOUSE BY IL-4 AND AN IL-4 RECEPTOR ANTAGONIST.**

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SCID-hu mice were constructed by subcutaneous implantation of fetal bone and fetal thymus. These mice develop human bone/thymus structures capable to generate both human T and B lymphocytes as well as myeloid cells. Furthermore, human IgM, IgG, IgE and IgA can be detected in the serum of these SCID-hu mice. This indicates that productive human T-B cell interactions, resulting in Ig isotype switching and production, occurs *in vivo* and therefore opens a possibility for experimental manipulation of these processes. Indeed, the ongoing human IgE responses could be suppressed following *in vivo* administration of an IL-4 mutant protein which acts as an IL-4 receptor antagonist. Moreover, *in vivo* treatment with rhIL-4 induces human IgE synthesis in those SCID-hu mice that were IgE seronegative prior to the treatment. The IL-4 treatment also results in an enhanced expression of B cell maturation-activation antigens such as CD23 and CD40 and increases sIg expression on human bone marrow B cells. These results indicate that the SCID-hu mouse is a suitable model to study the generation and regulation of human IgE responses *in vivo*.

C2-313 A COMPARISON OF LOCAL AND SYSTEMIC CYTOKINE PROFILES IN MICE AFTER VACCINATION WITH ADVANCED ADJUVANTS, Julia R. Carlson and Gary Van Nest, Chiron Corporation, Emeryville CA 94608

Cytokines are major mediators of the specific immune response to vaccine adjuvants. Previous studies (Valensi et al. J. Immunol., in press) have shown a qualitative and quantitative difference in the serum cytokine profiles of mice after immunization with influenza antigens combined with two different types of adjuvants; MF59-0, an oil-in-water emulsion, and Quil A LTC, a plant-derived glycoside. Balb/c mice vaccinated with influenza antigens and the above adjuvants were bled at three, six or twelve hours post third injection and assayed for IL-2, IL-4, IL-5, IL-6 and IFNγ by commercial ELISA kits. Mice showed high serum levels of IL-2 and IFNγ in response to Quil A, with lesser amounts of IL-5 and IL-6. MF59 generated a high serum IL-5 level, some IL-6, and little IL-2 and IFNγ. There was little or no IL-4 detected in the serum with either adjuvant. We then used an *ex vivo* model to monitor post-vaccination cytokine profiles at the local draining lymph node. Animals were immunized subcutaneously at the base of the tail three times and inguinal lymph nodes were harvested three hours after last injection, teased apart and cultured in 96-well plates without further stimulation. At 2 or 24 hours after plating media were harvested and assayed by ELISA. Cytokine profiles measured at the draining lymph node were different than those detected in the serum for both adjuvants. Both adjuvants stimulated measurable IL-4 locally, in contrast to the systemic model. MF59-0 induced much greater levels of IL-2 and IFNγ relative to its IL-5 and IL-6 levels at the lymph node; Quil A induced higher levels of IL-5 and IL-6 locally compared to those it generated in the serum. Additionally, the type of antigen combined with these adjuvants appeared to influence the cytokine profile seen, as did the age of the mouse. The effect of the strain of mouse used was also investigated.

C2-314 THE ROLE OF BCL-X_L UPON THYMOCYTE DEVELOPMENT IN A TRANSGENIC MODEL, Debra T. Chao, Gerald P. Linette, and Stanley J. Korsmeyer, Howard Hughes Medical Institute, Division of Molecular Oncology, Department of Medicine and Pathology , Washington University School of Medicine , St. Louis, Missouri 63110

Bcl-x, a Bcl-2 family member, in its long form with BH1 and BH2 domain, represses apoptosis following factor withdrawal in an IL-3-dependent cell line. In order to study the role of Bcl-x_L in thymocyte development, we generated transgenic mice that overexpress Bcl-x_L in thymocytes under the control of the lck-proximal promoter. While overexpression of Bcl-x_L does not substantially increase the number of total thymocytes, it promotes thymocyte maturation with an increase in the $CD3^{int/hi}$ population. Bcl-x_L also alters the distribution of T cell subsets by increasing the ratio of CD8 SP to CD4 SP cells within the thymus and the periphery. Moreover, Bcl-x_L transgenic thymocytes demonstrate improved in vitro survival and protection from radiation, glucocorticoid and in vivo anti-CD3 induced apoptosis. These findings in the Bcl-x_L transgenic mice are strikingly similar to that of the Lck^{pr}-Bcl-2 transgenic mice. Furthermore, endogenous Bcl-2 is down regulated in Lck^{pr}-Bcl-x_L mice and so is the expression of endogenous Bcl-x_L in Lck^{pr}- Bcl-2 mice. When Lck^{pr}-Bcl-x_L transgenic mice were crossed to Bcl-2 deficient animals, Bcl-x_L compensated for the loss of Bcl-2 in restoring viability to the T cell lineage. Both Bcl-x_L and Bcl-2 have been shown to require heterodimerization with Bax to rescue cells from apoptosis. The evidence here suggests that Bcl-x_L and Bcl-2 work through a common pathway in T cell development that involves Bax.

C2-315 THE MURINE HEAT STABLE ANTIGEN-SPECIFIC ANTIBODY M1/69 ALTERS THE IN VITRO DIFFERENTIATION OF B LYMPHOCYTES, Suzanne Chappel, Margaret Hough, Robert Kay and Keith Humphries. The Terry Fox Laboratory, British Columbia Cancer Agency, Vancouver, British Columbia, Canada.

The murine Heat Stable Antigen (HSA) is widely expressed throughout hematopoeiesis, however, its biological role in differentiation remains unresolved. To more specifically assess potential function of HSA we have assayed the effects of the HSA-specific monoclonal antibody M1/69 on B cell development *in vitro*. Total murine bone marrow was cultured for six weeks on the adherent S17 feeder cell line in combination with M1/69 and/or anti-VLA-4, either in the presence or in the absence of secondary crosslinking antibodies. All antibodies were added by a single addition at culture inititiation and were not maintained in the cultures thereafter. The B cells derived from these cultures were quantitated and analyzed by three-colour FACS for the expression of B220 , CD43, HSA and Thy 1. Approximately $3x10^5$ $B220^+$ cells were obtained from cultures grown in the absence of any antibodies. Similar cellular yields were obtained from cultures grown in the presence of M1/69 and anti-VLA-4, either alone or in combination, however, the presence of M1/69 resulted in a significant, $B220^+$-specific down-regulation of HSA expression within all $CD43^+$ and Thy 1^+ subpopulations. The cross-linking of HSA with a secondary antibody completely abolished B cell development, whereas the crosslinking of anti-VLA-4 had no effect. Cells cultured in the presence of isotype-matched control antibodies yielded FACS profiles which were indistinguishable from those of cells cultured in the absence of any antibodies. Taken together, these data suggest that the engagement of HSA by M1/69 may alter the developmental program of B lymphocytes either through the modification of stromal cell interactions or by modulating selection mechanisms. Moreover, these data offer the possibility that HSA may participate in signaling pathways involved in B lymphopoeisis. The target cells of the M1/69 antibody and the functional properties of the HSA^{lo} B lymphocytes derived from cultures exposed to M169 are currently under investigation. In addition, a model for the role of HSA in B cell development is currently being formulated in the context of transgenic animals which overexpress HSA and which similarly show significant perturbations in B lymphopoeisis.

C2-316 A CELL SUSPENSION MODEL FOR *IN VITRO* POSITIVE SELECTION.

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To investigate the identity of stromal cells and molecules involved in adult murine positive selection, a cell suspension model for *in vitro* positive selection was developed. T cell precursors from mice transgenic for MHC Class I restricted αβ TCR specific for the Lymphocytic Choriomeningitis Virus (LCMV), bred on a non-selecting MHC background, were co-cultured with freshly purified adult thymic stroma of a selecting and non-selecting MHC type, with and without the nominal LCMV peptide. After five days in culture, transgenic cells alone remained as $CD4^+CD8^+$ or $CD4^-CD8^-$ cells. In the presence of non-selecting stroma no differentiation into single positive T cells occurred however a slight increase in viability of the transgenic cells was evident. When co-cultured with selecting stroma approximately 20% of the transgenic T cells differentiated into $TCR^+CD4^-CD8^+$ cells. With the addition of 10^{-5}M LCMV peptide and to a lesser extent 10^{-6}M LCMV, this population increased to ~40-50%. This positive selection was accompanied by the expression of CD69. Approximately 20% differentiation occurred at 10^{-10}M; at a higher concentration of 10^{-4}M there was evidence of apoptosis. We are currently using this model to examine the cellular and molecular basis of adult positive selection.

C2-317 A MOUSE MODEL TO INVESTIGATE THE ROLE OF TH2 CELLS IN AIRWAY INFLAMMATION AND HYPERRESPONSIVENESS, Lauren Cohn and Kim Bottomly, Section of Immunobiology and Section of Pulmonary and Critical Care Medicine, Yale University School of Medicine, New Haven, CT 06520

Asthma is a chronic inflammatory disease. The airway hyperresponsiveness (AHR) and bronchospasm that are characteristic of asthma are believed to be the result of an inflammatory infiltrate in the bronchial wall which consists predominantly of lymphocytes and eosinophils. Activated CD4 T lymphocytes have been identified in bronchial biopsies and lung lavage fluid from asthmatics and they have been shown to express mRNA for the cytokines IL-4 and IL-5. High serum levels of IgE and eosinophil accumulation in the bronchial mucosa are characteristic of asthma and these features are dependent on the secretion of IL-4 and IL-5. Thus, Th2 cells appears to play a critical role in the development of reactive airways disease, yet definitive documentation of the part that these cells play is lacking.

To define the relationship between induction of Th2 cells and the development of AHR and inflammation, we have designed a model in which Th2 cells are activated in the respiratory tract. We have looked at the effect of transfer of Th2 or Th1 cells into sublethally irradiated syngeneic hosts. Th1 and Th2 cells were generated in vitro from naive, TCR transgenic CD4 T cells in the presence of inductive cytokines (IL-4 for Th2, IL-12 for Th1) and specific peptide, moth cytochrome c (MCC). After CD4 T cell transfer, mice were aerosolized with MCC peptide and the local lung-draining lymph nodes were assessed for cytokine production. Mice having received Th2 or Th1 cells and secreting these cytokines in the local lymph nodes were then compared for differences in AHR by body plethysmography and airway inflammation by histology. These results will be discussed. Thus, this model will help to define the role of Th2 cells in the development of inflammation and AHR in an antigen-specific system.

C2-318 mAb MTS 32 DEFINES A SUBSET OF CD4+8- T CELLS

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In the mouse thymus mAb MTS 32 reacts with the majority of thymocytes and a subset of cortical epithelium, in the periphery it reacts with the majority of T cells. An important feature of MTS 32 expression is that in both thymus and lymph node the CD3+CD4+CD8- subset can be divided into MTS 32+ and MTS 32- populations. The MTS 32-CD4+CD8- thymocytes have a more mature phenotype and cytokine secretion pattern than the MTS32+CD4+CD8- cells (Vicari et al., 1994). MTS 32 expression on CD4+ thymocytes and peripheral T cells was down-regulated in IL-2 gene knockout mice, and IL-4 transgenic mice, but was unaffected in IL-4 knock-out and IL-2/IL-4 double knockout mice, suggesting a role for IL-4 in MTS 32 Ag down regulation. In an analysis of MTS 32 Ag expression during the late embryonic and neonatal period, the MTS32-CD4+CD8- thymocytes are first detected at day 2 post-birth, a critical timepoint in the production of immunocompetent T cells.

From biochemical analysis the MTS32 antigen is a 26-28 kDa glycoprotein linked to the membrane by a GPI-anchor. mAb crosslinking results in Ca flux.

When added as purified Ig to deoxyguanosine treated E15 foetal thymus organ culture (FTOC) reconstituted with foetal liver, MTS 32 (200ug/ml) caused a reduction in total cell yield and block of differentiation at the CD3-CD4-CD8- stage. This decrease was seen for the total number of all αβ- &γδ TcR, CD4, CD8 defined subsets however the proportion of TcR-CD4-CD8-, γδTcR+CD4 CD8+ and αβTcR+CD4-CD8+ cells was increased. Current analysis involves Rag-1 and TcRβ RNA hybridisation of control and treated FTOC in order to determine whether the block at the CD3-CD4-CD8- stage involves the disruption of TcR gene rearrangement.

Vicari, A., Abelsira-Amar, O., Papiernik, M., Boyd, R.L., & Tucek, C.L. (1994) J. Immunol. 152: 2207-2213.

C2-319 POSITIVE SELECTION OF CD8-LINEAGE T CELLS IN THE ABSENCE OF CLASS I MOLECULES,

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The constitutive expression of CD4 by thymocytes allows the positive selection of CD8+ T cells that express class II-restricted TCRs. It remains formally possible, however, that cross-reactions between the class II-restricted TCR and class I molecules could allow CD8 to contribute to signals through the TCR, and that such signals may be required for commitment to the CD8 lineage. Transgenic mice expressing a class II-restricted TCR specific for pigeon cytochrome c (PCC) in the context of constitutive CD4, which show substantial positive selection of CD8+ T cells bearing the PCC TCR, were backcrossed to the β2-microglobulin (β2m)-deficient mouse line. The β2m-deficient, double transgenic progeny of this mating rescued CD8+, PCC TCR-expressing T cells comparably to the β2m-expressing littermates. Whereas CD8+ T cell lines expanded from PCC TCR single transgenic mice killed alloreactive targets in the absence of cytochrome c peptide, the rescued CD8+ T cells from the double transgenic mice, whether expressing β2m or not, required cytochrome c peptide to lyse the same target cells. The rescued cells thus appear to recognize only class II molecules. The cytolytic activity of the rescued CD8+ T cell lines was less sensitive to inhibition by cycloheximide than CD4-lineage T cell lines, indicating that the cytolytic activity was characteristic of the CD8 lineage. The rescued CD8+ T cells from the β2m-deficient double transgenic animals failed to upregulate CD40 ligand in response to stimulation by PMA/ionomycin, indicating that the failure to upregulate CD40 ligand did not depend on class I molecules. Both positive selection and functional maturation of the rescued CD8-lineage, PCC TCR T cells are therefore independent of class I molecules.

C2-320 **DIFFERENTIATION OF HUMAN NEONATAL CD4 T CELLS INTO TH-2 LIKE EFFECTOR CELLS IN THE ABSENCE OF EXOGENOUS IL-4.**

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Highly purified neonatal CD4 T cells were submitted to repetitive cycles of stimulation (for 3 days) and IL-2 expansion (for 4 days). When anti-CD3 mAb was immobilized on mouse L fibroblasts transfected with both CD32 ($Fc\gamma RII$) and B7, neonatal cells could be restimulated up to 8 times, resulting in a 10^8 fold cellular expansion. Cells primed and restimulated in the absence of exogenous IL-4 acquire a typical Th-2 phenotype after a few cycles of anti-CD3 activation and IL-2 expansion. These cells induce IgE synthesis and release high levels of IL-4 and IL-5 but little IFN-γ and IL-2. Cells primed in the presence of a neutralizing anti-IL-4 mAb develop into effectors producing low levels of IL-4 and high levels of IFN-γ, implicating that IL-4 is released during primary activation of naive cells. The Th2 cell lines obtained after repetitive stimulations on CD32-B7 L transfectants remain IL-2 and B7-dependent inasmuch as their proliferation and cytokine production are blocked by an anti-IL-2 Ab or the CTLA-4 Ig fusion protein.

C2-321 FAS-LIGAND - FAS MEDIATED LYSIS OF CD4+ TH2 AND TH0 CELLS BY CD4+TH1 CELLS: A POTENTIAL DOWNMODULATORY MECHANISM, Peter Erb, Sinuhe Hahn, Thomas Stalder, Marion Wernli and Diana Buergin, Institute for Medical Microbiology, University of Basel, CH-4003 Basel, Switzerland

The $CD4^+$ T cell population can be divided into Th1, Th2 and Th0 subsets according to their lymphokine profile and function. Th1 cells preferentially evoke cell-mediated and Th2/Th0 humoral immune responses. The cytolytic activity of Th1 cells was recently shown to be largely mediated via the interaction with Fas on the target cells. The Fas antigen, a member of the TNF-receptor family, triggers apoptosis in the cell after interaction with its ligand or anti-Fas mAb. Fas was reported to be expressed on various cells including cells of the lymphoid and myeloid lineage.

We examined Fas and Fas-ligand expression on $CD4^+$ Th1, Th2 and Th0 clones, and investigated the susceptibility of such cells to cytotoxic attack by $CD4^+$ or $CD8^+$ CTL. Fas mRNA was expressed predominantly on Th2 and Th0 cells, and poorly on Th1 cells. Conversely, Fas-ligand mRNA was strongly expressed on Th1 but not Th2 cells. Fas expression rendered Th2 and Th0 cells sensitive to Fas dependent lysis by both $CD4^+$ Th1 clones and a $CD8^+$ CTL clone, since the addition of soluble Fas-Ig blocked lysis of susceptible target cells by $CD4^+$ or $CD8^+$ CTLs. In contrast, $CD4^+$ or $CD8^+$ CTL did not lyse each other or themselves nor were Th2 or Th0 cells capable of lysing Th1 oder $CD8^+$ target T cells. These results suggest that the cytotoxicity exercised by $CD4^+$ Th1 cells may play an immunomodulatory role, regulating a Th2/Th0 response by Fas mediated lysis. As Th2 predominance can be disease-promoting as demonstrated in several disease models, our mechanism proposed might be of relevance in reverting Th1-Th2 imbalances.

C2-322 IN VITRO POSITIVE SELECTION OF THYMOCYTES INDUCED BY DISPERSED POPULATON OF THYMIC EPITHELIAL CELLS,.Bettina Ernst, Charles D. Surh, and Jonathan Sprent, Dept. of Immunology, The Scripps Research Institute, La Jolla, CA, 92037.

I investigate positive selection with a system in which immature TCR^- $CD4^+8^+$ (double positive, DP) thymocytes are cultured with dispersed population of purified thymic epithelial cells (TEC) in vitro (originally described by Jenkinson et al. J.Exp. Med. 1992. 176:845).

With the in vitro positive selection system I have thus far obtained following results: i) Immature thymocytes undergo distinctive changes in cell surface molecules during positive selection. ii) TEC are unique in their ability to support the maturation of TCR-CD4+8+ thymocytes into CD4+ or CD8+ single positive (SP) T cells. iii) Small non-dividing DP thymocytes can mature into SP thymocytes upon reaggregation with TEC; the kinetics of positive selection of small DP cells are faster than larger DP cells. iv) TEC derived from ß2m- mice lacking detectable class I major hisocompatibility complex molecule (MHC) expression are capable of reducing maturation of CD8+ single positive (SP) T cells; Staining the CD8+ SP T cells for TCR, HSA and CD69, however, shows that majority of these CD8+ SP cells do not have a fully mature phenotype. The immature CD8 SP cells could arise by positive selection on class II MHC molecule, or represent an intermediate stage cells which is possible in a stochastic differentiation scheme. I plan to investigate these two possibilities by using TEC derived from ß2m-IA- double-knockout mice in the future.

C2-323 **SELF TOLERANCE TO T CELL RECEPTOR Vβ SEQUENCES,** **Fiorenza Falcioni, Damir Vidovic, E. Sally Ward, David Bolin, Geeta Singh, Himanshu Shah, Bertram Ober, and Zoltan Nagy. Dep. of Inflammation and Autoimmune Diseases, Hoffmann-La Roche Inc., Nutley, NJ 07110, and Dep. of Microbiology, Univ.of Texas Southwestern Medical Center, Dallas, Texas 75235.** **T cell tolerance to self is achieved by deletion or inactivation of clones recognizing peptides of self proteins presented by MHC molecules. Thus far, it has remained unclear, whether antigen receptors are subject to self tolerance, or on contrary, they engage into network interactions implying immunity rather than tolerance. In this study, we demonstrate self tolerance to synthetic peptides corresponding to the first hypervariable region of the Vβ 8.1 and Vβ 8.2 TCR proteins. We also show that a tolerogenic synthetic peptide corresponds to a fragment produced by processing of the Vβ protein, and conversely, that a Vβ peptide not produced by processing is also not subject to self tolerance. Thus, the rules of tolerance seem to apply to antigen receptors, at least to their germline-encoded portions, in a similar fashion as to other self proteins. This finding has important implications for studies of natural and artificially induced immune networks.**

C2-324 THE INFLUENCE OF ANTIGEN ORGANIZATION ON DEVELOPMENT OF LUPUS AUTOANTIBODIES, Saeed Fatenejad, Michele Bennett, and Joe Craft, Department of Internal Medicine, Yale University School of Medicine, New Haven, CT 06520-8031.

Antibodies against U1 small nuclear ribonucleoprotein particles (U1 snRNP) are specific for both human and murine (MRL) lupus. We have shown that intrastructural help is required for diversification of antibodies from one protein to the rest of the proteins of the U1 snRNP particles (*Proc. Natl. Acad. Sci. USA* 90:12010, 1993). We have also shown that antibodies against the protein components of U1 snRNPs in MRL/*lpr* mice follow a pattern of expansion over time that suggests intact U1 snRNP particles, and not their individual proteins, may be the targets of the humoral immune response (*J Immunol* 152:5523, 1994). To test this hypothesis further, we examined the immune response of normal mice (B10.BR; H-2^k) to either intact or ribonuclease (RNase)-disrupted human snRNPs. Mice immunized with intact snRNPs developed antibodies against the A protein, followed by antibodies to the B and/or 70K proteins of the U1 snRNP, similar to the development of anti-snRNP antibodies in MRL/*lpr* mice (H-2^k). In contrast, mice immunized with RNase-treated snRNPs had random antibody development, *i.e.*, antibodies to B and 70K proteins occurred in the absence of anti-A. To show that the same grouping of antibodies also arise to intact self snRNPs, we took advantage of a novel method to induce such antibodies in normal mice (normal mice are tolerant to immunizations with self [mouse] snRNPs [*J Immunol* 152:1453, 1994]). We transfected a murine B cell line CH27 (H-2^k) with the eukaryotic expression vector pRC/CMV (Invitrogen) carrying the cDNA for human U1 snRNP A protein downstream of the CMV promoter. We next demonstrated that this system allows for the endogenous production of the human A protein in mouse cells and the formation of chimeric human A-mouse snRNPs. B10.BR mice were immunized with biochemically purified intact chimeric snRNPs. Human A in these particles abrogated tolerance of normal mice to self snRNPs, with induction of autoreactive T cells and generation of autoantibodies against snRNPs. Again, we observed that anti-A antibodies were grouped with antibodies against other proteins. In conclusion, these experiments provide more evidence that intact U1 snRNPs are *in vivo* immunogens in MRL/*lpr* mice and possibly in human lupus.

C2-325 TRANSGENIC MODELS OF IMMUNOREGULATION Barbara Fazekas de St. Groth, Matthew Wikstrom and Matthew Cook, Centenary Institute of Cancer Medicine and Cell Biology (University of Sydney), Locked Bag 6, Newtown, NSW 2042, AUSTRALIA. Ph: 61 2 565 6137 FAX: 61 2 565 6105

The ability of peptidic antigen to induce a variety of functionally distinct peripheral immune responses, depending on the dose and route of administration, suggests that factors other than TCR binding to peptide-MHC complexes are crucial in determining the outcome of an encounter with antigen in the periphery. We are using T and B cell receptor transgenic models in which the frequency of lymphocytes responding to cognate antigen is artificially high to examine phenotypic and functional aspects of the primary immune response at the single cell level. Our data suggest that, for T cells, immunogenic encounters with antigen are characterised by a peak of production of IL2, IL3, IL4 and IFNγ which precedes the peak in T cell numbers by 3-4 days. The Th1/Th2 balance, as measured by the ratio of IFNγ to IL4, is a complex function. Once the threshold needed to stimulate a measureable response is reached, higher doses of antigen bias the response in favour of IL4 production whilst depressing IL2 and IFNγ production, consistent with a dominant role for antigen-dependent, primary T cell-derived IL4 in controlling Th1/Th2 ratios by positive feedback. Although the total numbers of antigen-specific T cells return to baseline in the weeks following immunisation, memory cells are selectively retained and represent more than 60% of the antigen-specific cells several weeks after immunisation. At that time, mice show an increased response to rechallenge, consistent with the generation of T cell memory *in vivo*. Thus in this model memory is the result of phenotypic shift rather than an increase in precursor frequency.

We are determining whether APC type or activation state is crucial in regulating Th1/Th2 immune responses and, in particular, which APC's are required for the stimulation of a Th2 response. To examine the role of B cells *in vivo* we have set up a model of B cell antigen presentation using a hybrid antigen (hen egg lysozyme linked to cytochrome C) to allow specific presentation by immunoglobulin transgenic B cells to TCR transgenic T cells.

C2-326 TOLEROGENICITY OF THE SELF-PEPTIDE L^d 61-80 IS CONTROLLED BY FACTORS WHICH MAP TO CLASS II REGION OF THE MHC LOCUS, Eugenia Fedoseyeva, Robert C. Tam, Patricia L. Orr, Marvin R. Garovoy and Gilles Benichou, Department of Surgery, UCSF, San Francisco, CA 94143-0508

Self-peptides can be divided into two main categories: those which are regularly processed and whose efficient presentation induces tolerance *(dominant)* and those which do not reach the threshold of presentation to mediate T cell deletion and therefore are immunogenic in adult animals *(cryptic)*. We have previously shown that the peptide L^d61-80 was cryptic in syngeneic B10.A mice. Here, we report that crypticity is not an intrinsic property of this self-peptide since it was found to be dominant (tolerogenic) in BALB/c mice. the following data show that efficient presentation in BALB/c mice resulted in tolerance induction during ontogeny: i) L^d61-80 binds with high affinity to E^d class II molecule; ii) it causes strong proliferative T cell response in mutant BALB/c-dm2 mice lacking L^d protein; iii) BALB/c APCs naturally present this peptide as they stimulate anti-L^d61-80 T cell lines in the absence of exogenous peptide. In addition, L^d61-80 was immunogenic in different H-2^a mice while it was invariably tolerogenic in H-2^d mice regardless of their background genes. We then investigated which MHC-encoded factors were responsible for the presentation of the self-peptide. We showed that L^d61-80 bound equally well to E^d and E^k MHC class II molecules. Also, no correlation was found between the quantity of self-L^d protein and the tolerogenicity of L^d61-80. Surprisingly, *in vivo* and *in vitro* studies in (H-2^axH-2^d) F1 mice revealed that F1 APCs could not spontaneously present L^d61-80 either in E^k or even E^d class II context. This indicated that the presence of gene(s) located in the MHC locus of H-2^a mice had impaired L^d61-80 peptide processing and presentation. Analyses of T cell responses to the self-peptide in several H-2 recombinant mice revealed that the presentation of L^d61-80 was controlled by gene(s) that mapped to a 170 kb portion of the MHC class II region. This study shows that 1) the phenotype of the T cell response to the self-peptide L^d61-80, whether cryptic or dominant, does not represent an intrinsic property of this peptide 2) a 170 kb portion of the MHC class II region of H-2^a mice encodes factors that abrogate the presentation of L^d61-80 by MHC class II molecules. The implications of this finding for T cell tolerance induction will be discussed.

C2-327 TCR CROSSLINKING IS SUFFICIENT FOR ACTIVATION BUT NOT DELETION OF DOUBLE POSITIVE THYMOCYTES, Galen H. Fisher*, Juan Carlos Zúñiga-Pflücker, Michael Lenardo, NIAID, N.I.H. Bethesda MD 20814 * HHMI-NIH Research Scholar.

During thymocyte development, the process of negative selection is central to the maintenance of self tolerance. Thymocyte negative selection occurs by apoptosis and results in the deletion of thymocytes which bear potentially autoreactive TCRs. It has been observed that in vivo administration of the anti TCR-epsilon antibody, 2C11, causes deletion of double positive thymocytes. To gain insight into the molecular requirements for negative selection I am using an in vitro system where fresh thymocytes are stimulated by plate bound 2C11. In this system I have observed that, unlike the in vivo system, the double positive thymocytes are not killed by 2C11. In this system, the only cells killed are the HSA low TCRhi single positive thymocytes. The cells remaining after 24 hours of 2C11 exposure have however been activated by 2C11, as indicated by the increase in surface expression of activation molecules. I have found that death by TCR ligation is potentiated by ligation of certain cytokine receptors. A model for for signal co-operation in negative selection of double positive thymocytes will be presented.

C2-328 THE POSITIVE SELECTION OF CD8+ T CELLS REQUIRES INTERACTION WITH THYMIC EPITHELIAL CELLS, Madeline M. Fort and Drew M. Pardoll, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21205

The process of positive selection ensures that only T cells with T cell receptors (TCR's) that have low or moderate affinity for self MHC will reach full maturity. Immature T cells with TCR's which have no affinity for self MHC do not mature past the $CD4^+CD8^+$ stage. Some studies indicate that positive selection is mediated exclusively by thymic epithelial cells, while others suggest that bone marrow-derived antigen presenting cells (BM-APC's) can also play a role. In order to determine which cells mediate the positive selection and maturation of a specific class I-restricted T cell, we used mice that were homozygous for a transgenic TCR specific for H-Y (male) antigen presented on D^b. Positve selection of the transgenic TCR only occurs in female mice on a $H\text{-}2^b$ background. By transplanting bone marrow from $H\text{-}2^b$ TCR transgenic females into mice with a $H\text{-}2^{d/d}$ MHC background, chimeras were created that only expressed the positively selecting MHC molecule (D^b) on BM-APC and not on thymic epithelial cells. Our data shows that TCR transgenic $CD8^+$ T cells do not reach full maturity in these bone marrow chimeras, indicating that only thymic epithelial cells are capable of mediating the positive selection of immature T cells.

C2-329 INTRODUCTION OF A PARASITE-SPECIFIC TCR TRANSGENE INTO BALB/C MICE INTERFERES WITH STABLE DEVELOPMENT OF TH2 EFFECTOR CELLS. Deborah Fowell, Steve Reiner, Nigel Killeen and Richard Locksley. Infectious Disease Division, Department of Medicine, Box 0654, University of California San Francisco, San Francisco, CA 94143.

Most inbred mouse strains control *Leishmania major* infection by mounting a $CD4^+$ Th1 cell response. However susceptible BALB/c mice form an inappropriate Th2 $CD4^+$ T cell response and develop fatal disease. The susceptibility of mouse strains on a BALB background to *L. major* suggests that disease is linked to an inappropriate switch in $CD4^+$ effector cell development. In order to study the control of T cell development *in vivo* in genetically resistant or susceptible backgrounds, transgenic animals with an MHC Class II-restricted TCR ($V\beta4/V\alpha8$) recognizing an immunodominant *L. major* antigen were generated. Transgene TCR+ mice on a BALB/c background developed large lesions 3-4 weeks after infection with *L. major* and T cells from these mice generated IL-4 and little IFNγ after stimulation with antigen *in vitro*. TCR+ mice on a resistant B10D.2 background arrested lesions by 4 weeks and controlled infection despite having this single TCR specificity. Stimulation of B10D.2 T cells *in vitro* generated IFNγ but no IL-4 protein. When infection in BALB/c mice was followed beyond 4 weeks however, all animals spontaneously cured. Control of infection was associated with a change in the cytokine profile, with stimulation *in vitro* now yielding IFNγ and little IL-4. Further, recovered BALB/c mice remained immune to a second parasite challenge. Thus, a single immunodominant TCR is sufficient to control infection due to this organism, but establishment of this TCR in susceptible BALB/c mice abrogates stable Th2 development that otherwise occurs. The ability of allelic exclusion to abrogate susceptibility suggests a necessary role for other α/β TCR+ T cell populations for stable Th2 development.

C2-330 **THYMIC ONTOGENY AND FUNCTION OF $CD8^+$ T CELLS IN CD8ß KNOCKOUT MICE,** Wai-Ping Fung-Leung*, Thomas M. Kündig‡, Karen Ngo*, Julie Panakos*, Jean De Sousa-Hitzler*, Elizabeth Wang*, Pamela S. Ohashi‡, Tak W. Mak‡ and Catherine Y. Lau*, The *R.W. Johnson Pharmaceutical Research Institute (Toronto), Don Mills, Ontario M3C 1L9, Canada; and The ‡Ontario Cancer Institute, Toronto, Ontario M4X 1K9, Canada.

CD8 is a cell surface glycoprotein on MHC class I-restricted T cells. Thymocytes and most peripheral T cells express CD8 as heterodimers of CD8α and CD8ß. The intestinal intraepithelial lymphocytes (IEL), which have been suggested to be generated extra-thymically, express CD8 predominantly as homodimers of CD8α. We have generated CD8ß gene-targeted mice. The $CD8\alpha^+$ T cell populations in the thymus and in most peripheral lymphoid organs were reduced to 20%-30% of that in wild type littermates. CD8α expression on thymocytes and peripheral T cells also decreased to 44% and 53% of the normal levels, respectively. In contrast, neither the population size nor the CD8α expression level of $CD8\alpha^+$ IEL was reduced. Cytotoxic T cell responses were studied in CD8ß -/- mice after infection with lymphocytic choriomeningitis virus (LCMV) or vesicular stomatitis virus (VSV). Efficient *in vivo* cytotoxic activity in these mice was demonstrated by an immunopathological swelling reaction after local injection of LCMV. Primary and secondary *ex vivo* cytotoxic T cell functions against LCMV or VSV were assessed with ^{51}Cr release assays. Virus specific T cells with efficient cytotoxic activity was observed in CD8ß -/- mice. The results indicate that CD8ß is important only for ontogeny of $CD8^+$ T cells in the thymus but is not essential for the cytotoxic effector function of $CD8^+$ T cells.

C2-331 **$CD8^+$ T CELLS ARE THE PREDOMINANT SOURCE OF $B220^+$ DOUBLE NEGATIVE T CELLS IN *lpr* AND *gld* MICE,** Thomas Giese and Wendy F. Davidson, Laboratory of Genetics, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892-4255

Mice homozygous for *lpr*, a defect at the *fas* locus and *gld*, a defect at the *fas* ligand locus, develop autoimmunity and progressive lymphoproliferative disease characterized by the accumulation of two unusual populations of functionally impaired $B220^+$ TCR α/β^+ T-cells, a predominant $CD4^-$, $CD8^-$ (DN) subset and a minor $CD4^{dull+}$ subset. Although $B220^+$DN T cells have been studied extensively their immediate precursors have not been identified. Recently, we reported that chronic treatment of *lpr* and *gld* mice with anti-CD8 mAb greatly reduced the development of lymphadenopathy, primarily by preventing the vast accumulation of $B220^+$ DN T-cells. There was a compensatory increase in the proportions of $CD4^+$ T-cells and $CD4^+B220^+$ T-cells. To further investigate the contributions of $CD8^+$ thymus-derived T-cells to the production of $B220^+$DN T-cells and the accumulation of $CD4^+$ T-cells we introduced disrupted $\beta_2$microglobulin genes into *lpr* and *gld* mice. In addition, we depleted *gld* mice of $CD8^+$ T-cells by a combination of anti-CD8 mAb treatment and thymectomy. Both groups of $CD8^+$ T-cell deficient mice developed massive lymphadenopathy. $CD4^+B220^+$ T-cells and $CD4^+$ T-cells were the dominant T cell subset and $B220^+$DN T cells were a minor subset. The combination of effects observed in *lpr* and *gld* depleted of $CD8^+$ T-cells by three different techniques provide strong evidence that, 1) that the majority of $B220^+$DN T-cells arise from peripheral $CD8^+$ T-cells selected on class I MHC in the thymus, 2) $CD4^+B220^+$ T-cells and possibly a minor subset of $B220^+$ DN T-cells arise from peripheral $CD4^+$ T-cells and, 3) the lack of Fas receptor expression in *lpr* does not interfere with positive selection in the thymus.

C2-332 PRODUCTION OF CELLS BY THE THYMUS AND THE ROLE OF THE THYMIC MEDULLA, Dale Godfrey and Roland Scollay, Centenary Institute of Cancer Medicine and Cell Biology, University of Sydney, NSW, 2006, Australia.

The thymic medulla is the compartment in which post-positive selection thymocytes accumulate before they are exported to the periphery. All or most of the lymphocytes in the medulla are $CD4^+8^-$ or $CD4^-8^+$ (single positive). $CD4^+8^+$ cells, typical of the thymic cortex, are rare or absent from the medulla. The medulla has been viewed as a holding compartment in which a few final maturation events (loss of HSA, acquisition of Qa-2, increase in immune function etc.) occur in the absence of significant levels of proliferation. However analysis of emigrants seems to show that the exported cells include both more and less phenotypically mature single positive cells, suggesting some cells are exported before undergoing the full medullary maturation process. Together with kinetic analysis suggestive of stochastic export, rather than first in first out, this has led to the concept that cells can leave the medulla at any stage in the final maturation process (i.e. random export from the pool of maturing cells). Recent experiments have further complicated the issue and have been interpreted to indicate that many of the cells leaving the thymus have recently divided (Tough and Sprent, JEM 179, 1127. 1994), which further complicates the picture. We are attempting to clarify the issue by looking in more detail at cell division and phenotypic changes amongst medullary thymocytes and recent thymic emigrants. Our results will be discussed in the context of models of cellular processing in the medulla.

C2-333 IMMUNOGLOBULIN VARIABLE REGION HYPERMUTATION IN B CELLS. Nancy S. Green, Jennifer Rabinowitz, Minghua Zhu, Matthew D. Scharff; Department of Cell Biology, Albert Einstein College of Medicine, Bronx, NY 10461

Immunoglobulin (Ig) gene variable region somatic hypermutation is a major molecular event in developing B cells. Rates of mutation in vivo are estimated to be 10-3 to 10-4 per base pair/cell generation. As a model for this process, Ig gene constructs containing V and C region nonsense mutations were tranfected into several murine B cell lines representing different stages of differentiation. Mutation rates were quantitated by reversion analysis using ELISA spot assay. One pre-B cell line, 18.81, mutates the Ig construct at a frequency of 10-5/bp/generation , whereas the myeloma line NSO mutates 100 times less frequently. Some hybrids between these two lines mutate at even higher rates than 18.81. This mutation dominant phenotype may reflect a synergistic effect, selective gene assortment, or simply an improved detection rate in this secretion-based assay. Hybrids between 18.81 and NSO with BCL-2 mutate at 100 fold lower rates than the comparable hybrids that do not contain BCL-2. Ig sequences from reverted cells show that reversion is by point mutation (as is seen in vivo), and supports the notion of a "hot spot" motif, independent of cellular selection. Both IgM and IgG2a constructs have been studied in these cell lines. This system can be used to identify the cis acting DNA sequences and molecular and cellular controls involved in somatic mutation.

C2-334 **PHENOTYPICAL AND FUNCTIONAL PROPERTIES OF $CD4^+$ T CELLS CHARACTERIZED BY DULL EXPRESSION OF BOTH CD45RA AND CD45RO ISOFORMS**

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$CD4^+$ T cells can, based on the expression of CD45 isoforms, be separated into $CD45RA^+$ (unprimed) and $CD45RO^+$ (primed) cells. Next to these major subsets, a smaller transitional population exists in the peripheral blood of adults that expresses both isoforms in low density (Ddull). Ddull cells were found to have an intermediate phenotype for the adhesion molecules CD11a/CD18, CD29, CD49e, CD58, CD62L and CD31 that are differentially expressed on unprimed versus primed cells. Only a very small percentage of Ddull cells expressed activation antigens (CD25, HLA class II) or was found to be in the G_2/M phase of the cell cycle. To measure cytokine production, purified cells were stimulated with CD2 and CD28 mAb in bulk cultures. While $CD45RA^+$ cells secreted IL-2, but hardly any other cytokines, Ddull cells produced IL-2, IFN-γ, IL-4 and IL-10 thus showing a cytokine secretion pattern comparable to $CD45RO^+$ cells. This could be confirmed by analyzing the cytokine production of T-cell clones that were generated from the different populations. The frequency of mutant cells (as a measurement for the amount of cell divisions that have occurred in certain populations) within the Ddull subset was comparable to that of the $CD45RO^+$ cells. $CD45RA^+$ cells had on average a 3 times lower mutation frequency. In summary, our data show that the Ddull cells found in the peripheral blood of adults share some functional characteristics with primed cells. We are now investigating whether this population is constituted of cells developing from single CD45RA to CD45RO expression or contains primed cells reexpressing the CD45RA isoform.

C2-335 NOVEL PHENOTYPIC AND FUNCTIONAL LYMPHOCYTE DEVELOPMENT IN NEONATALLY CONSTRUCTED $RAG2^{-/-}$ CHIMERAS

Fiona A. Harding, Deborah L. Jones and Manley T.F. Huang, GenPharm International. Mountain View, CA 94043

Chimeric mouse models are often used to study the development of the immune system. These models are created by transferring bone marrow cells into lethally irradiated or genetically immunodeficient adult mice. Analysis of lymphocyte regeneration in such models has yielded most of our current knowledge of the differentiation and selection processes that are necessary to give rise to mature lymphocytes. However, many discrete subpopulations of lymphocytes normally found in unmanipulated animals are not seen in chimeric mice, including $CD5^+$ peritoneal B cells and Thy-1^+ dECs. Further analysis of lymphocyte progenitors has lead to the generalized conclusion that precursors differentiate with age, losing the capacity to generate certain lymphocytes with "primordial" specificities. We have created chimeric animals by engrafting neonatal $RAG2^{-/-}$ mutant mice with allogeneic and syngeneic bone marrow cells. Upon analysis, these chimeras exhibit all the characteristics of chimeras created in adult animals in that they are engrafted with nearly normal numbers and percentages of T and B cells, and lymphocytes from both lineages are responsive to mitogens. However, we observe a discrete populaton of $CD5^+$ peritoneal B cells previously undetected in chimeras constructed with adult bone marrow. We also demonstrate that allogeneic chimeras contain antigen-specific T cells restricted to the donor MHC type which allow an IgG response to be mounted. Furthermore, contrary to results found using neonatal SCID mice, the bone marrow of these chimeras is well engrafted with precursor B lymphocytes. We conclude that neonatally constructed $RAG2^{-/-}$ chimeras may more accurately reflect lymphocyte development in mice.

C2-336 ANTIGEN-SPECIFIC SUPPRESSION OF AUTO-IMMUNE β-CELL DESTRUCTION IN THE NON-OBESE DIABETIC (NOD) MOUSE, Leonard C. Harrison and Majella Dempsey-Collier, Walter &Eliza Hall Institute of Medical Research, Royal Melbourne Hospital, Victoria, PO 3050, Australia.

The non-obese diabetic (NOD) mouse is a model of spontaneous insulin-dependent diabetes (IDD), in which destruction of insulin-producing pancreatic β-cells is mediated by both CD4 and CD8 T cells. The effect of IFN-γ and IL-12 to accelerate and of IFN-γ antibodies to retard 'insulitis' and diabetes in the NOD mouse is evidence for a pathogenic role for Th1 cells in β-cell destruction. *In situ* analysis has confirmed the presence of Th1 cytokines in intra-islet T cells.

Several autoantigens have been identified in the NOD mouse, two of which, insulin and glutamic acid decarboxylase (GAD), elicit immune responses before the onset of clinical disease, as in humans at-risk for IDD. To determine if recombinant insulin or GAD proteins are involved in driving β-cell destruction *in vivo*, these antigens or synthetic peptides corresponding to known or predicted antigenic sequences within them were given intravenously or subcutaneously (at 3-4 weeks), orally (daily from 3-4 weeks) or by aerosol (weekly from 3-4 weeks). Antigen-specific 'tolerance' was induced by all strategies, with significant reductions in the severity of insulitis and diabetes. Peptides that 'tolerized' overlapped with those that stimulated T-cells , not only from NOD mice but also from humans with pre-clinical diabetes. Tolerance was transferable; T cells from antigen-treated mice prevented the development of diabetes when co-transferred, with T cells from diabetic mice, into young, irradiated mice. These therapeutic effects may be due, at least in part, to immune re-programming towards Th2 responses. They support the view that insulin and GAD are pathogenic autoantigens that contribute to beta cell destruction, and have implications for the prevention of IDD in humans.

C2-337 TRANSFECTION OF HUMAN αβ TCR cDNA'S FROM A MYELIN BASIC PROTEIN (MBP)-SPECIFIC T CELL CLONE, A.E. Hastings, E.D. Robinson, C.K. Hurley, J.R. Richert. Depts. of Microbiology and Immunology and Neurology, Georgetown Univ. Medical Center, Washington D.C., 20007

MBP is a putative autoantigen in multiple sclerosis (MS). In order to define the molecular interaction between MBP, MHC and the human TCR, peptide binding assays and TCR transfection studies were performed. Full length α and β TCR cDNAs were isolated from a HLA DR(α,B1*1301)-restricted T cell clone isolated from an MS patient and reactive with an immunodominant epitope on MBP (peptide 152-170). These MBP specific TCR α and β cDNAs were sequenced and cloned into the pMCFR expression vectors and transfected with hCD4 into murine T cell lines lacking endogenous TCR expression. Transfectants were isolated by drug resistance and analyzed by flow cytometry for cell surface expression of mCD3, hVβ22 and hCD4. A stable transfectant line with high levels of surface expression was established by serially sorting the brightest 2-30% of hCD4 and mCD3 expressing cells. Functional assays to measure the transfectant's ability to respond to its cognate ligand are being established. Additionally, biotinylated, truncated peptides and peptide analogs were used in binding assays to define anchor residues for binding MPB 152-170 to its MHC restricting element, HLA DR(α,B1*1301), using flow cytometry. Binding assays with truncated MBP peptides have defined the region 152-155 as necessary for anchoring immunodominant MBP peptide 152-170 to DR(α,B*1301) and MBP analogs further defined residue 154 (phe) as the anchor in this region. Elucidation of molecular contact residues in the TCR-MHC-autoantigen complex will aid in the development of immunomodulatory therapies for MS aimed at blocking or altering these residues.

C2-338 **B CELLS PRIME AND DIRECT THE DEVELOPMENT OF CD4+ T CELLS IN A TCR-TRANSGENIC MOUSE MODEL**

Nancy Hosken[1], Steven Macatonia[1], Kazuko Shibuya[1], Chyi-Song Hsieh[2], Maria Wysocka[3], Giorgio Trinchieri[3], Kenneth M. Murphy[2], Anne O'Garra[1] *[1]Departments of Immunology and Molecular Biology, DNAX Research Institute, Palo Alto, CA, 94304; [2]Department of Pathology, Washington University School of Medicine, St. Louis, MO, 63110; [3]The Wistar Institute, Philadelphia, PA, 19104.*

We have used mice expressing a transgenic αβ T cell receptor specific for $OVA_{323-339}$ plus $I\text{-}A^d$ to investigate whether different types of professional antigen presenting cells are capable of priming or skewing development of CD4+ T cells. We have previously shown that dendritic cells, but not Listeria-activated macrophages, are capable of priming naive CD4+ T cells. Further, we have shown that both dendritic cells and Listeria-activated macrophages are capable of skewing CD4+ T cell development towards a Th1 phenotype by their production of an important cytokine for Th1 development, IL-12. Listeria-activated macrophages were able to direct Th1 development of both total CD4+ T cells and the "naive" $Mel14^{hi}CD4^+$ T cell subset. In contrast, dendritic cells were able to direct only the $Mel14^{hi}CD4^+$ T cell subset to develop a Th1 phenotype; development of a Th1 phenotype by total CD4+ T cells required the neutralization of endogenous IL-4. We report here on the ability of B cells to prime and direct development of both total CD4+ and the $Mel14^{hi}$ subset of CD4+ T cells. We have identified B cells which can stimulate a primary proliferative response by naive CD4+ T cells as strongly as dendritic cells. The ability of B cells to prime and direct development of naive CD4+ T cells in our system is dependent on 1) their activation by particular stimuli, 2) their expression of particular costimulatory molecules, and 3) the dose of antigen present during T cell priming.

C2-339 **LAG-3/MHC CLASS II INTERACTION MODULATES THE ANTIGENIC RESPONSE OF CD4+ T CELL CLONES**

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LAG-3 (lymphocyte activation gene 3) is closely related to CD4 at the gene and protein level. Recently, a putative role for LAG-3 in CD4+ T cell response has been suggested by the use of anti-LAG-3 mAbs. To further investigate the role of this new MHC class II ligand in immune response, we have produced a soluble form of LAG-3 by fusing extracellular domain of this membrane protein to constant region of human immunoglobulin. This soluble LAG-3 bound specifically to MHC class II molecules and this interaction of LAG-3Ig with MHC class II molecules resulted in a modulation of antigenic stimulation of CD4+ T cell clone.

C2-340 Identification and functional analysis of a thymic stromal antigen, David J. Izon, *Kenji Oritani, Maaike Hamel, Elizabeth E. Eynon, , *Paul W. Kincade and Ada M. Kruisbeek, Netherlands Cancer Institute, Plesmanlaan 121, 1066 Amsterdam, The Netherlands, and *Oklahoma Medical Research Foundation, 825 Northeast 13th Street, Oklahoma City, OK 83104

We recently described a mAb (MTS23) reactive with a membrane Ag expressed on a subset of thymic medullary stromal cells (Izon et al, J. Immunol.153:2939, 1994). MTS23 also detects an antigen constitutively expressed at high levels on peripheral B cells, macrophages, and thymic and splenic dendritic cells of C57Bl/6 mice. A number of stromal cell lines derived from the thymus and from bone marrow also stain with this mAb, but thymocytes and peripheral T cells do not express the antigen detected by MTS23. Given this expression pattern, we investigated whether MTS 23 identifies an accessory molecule in regulation of T cell activation. MTS 23 was able to block up to 75% of T cell proliferation in soluble anti-CD3 and antigen induced responses, but not under conditions where no antigen presenting cells were required. This suggests a role at the APC level, but other data point towards a role at the level of T cells as well. Although the antigen identified by MTS23 is absent from T cells and thymocytes, it can be upregulated within 24 hrs after activation through TCR cross-linking.This may in part be due to cytokines produced by T cells as a consequence of TCR signalling, since recombinant IFN-γ induced expression on peripheral T cells, and on CD4 and (to a lesser extent) CD8 thymocytes. Other cytokines, such as IL-2 and IL-4, also upregulated expression on peripheral T cells, but not on thymocytes. Experiments are in progress to study whether transmembrane signalling events can be induced by MTS23 in both hemopoietic cells and in stromal cells.

The molecule detected by MTS23 appears to be a member of the Ly-6 family of PI-anchored membrane proteins. Treatment of stromal cells with PI- PLC before staining completely abolished expression. Using transient expression of 293T cells and a cDNA library of a bone marrow stromal cell line cloned into the pEF-BOS vector, a cDNA encoding the MTS23-target antigen was isolated. Partial sequencing and restriction enzyme mapping revealed it represents the Ly-6A/E protein. MTS 23 therefore represents another mAb which detects members of the Ly-6 locus on bone marrow and thymic stromal cells. While the physiological significance of the presence of Ly-6 molecules on stromal cells is not clear, it has been known for some time that, at least in lymphocytes, cellular activation events can be induced upon Ly-6 engagement. It will therefore be of interest to test the consequences of Ly-6 crosslinking on stromal cell function directly, as well as in assays that measure the effects of stromal cells on lymphopoiesis.

C2-341 ANTI-CD28 ANTIBODY AND LIGANDS CD80 AND CD86 INDUCE DISTINCT PATTERNS OF CYTOKINE SECRETION AND MAINTAIN LONG TERM AUTOCRINE GROWTH OF CD4⁺ T CELLS, C. H. June, B. L. Levine, Y. Ueda, N. Craighead, M. L. Huang, Immune Cell Biology Program, Naval Medical Research Institute, Bethesda, MD 20889.

Recent studies have demonstrated the existence of at least two members of the B7 receptor family. Here the costimulatory signals provided by CD80 (B7-1) or CD86 (B7-2) were compared to CD28 ligation by monoclonal antibody. We demonstrate that the kinetics of induction of T cell proliferation after anti-CD3 stimulation was similar regardless of the form of costimulation. Similarly, B7-1 and B7-2 could both maintain long term expansion of CD4 cells. The costimulatory effects of both B7-1 and B7-2 were dependent on CD28 crosslinking, based on complete inhibition of proliferation by CD28 antibody Fab fragments. Costimulation with B7-1 and B7-2 induced high levels of cytokine secretion by resting T cells, and the effects of B7-1 and B7-2 could not be distinguished. This conclusion is based on analysis of the initial activation of CD28⁺ T cells, as well as T cell subpopulations consisting of CD4⁺ and CD8⁺ T cells. Furthermore, both B7-1 and B7-2 could stimulate high levels of interferon-γ and IL-4 from CD4⁺CD45RO⁺ cells, while neither B7 receptor could costimulate cytokine secretion from CD4⁺CD45RA⁺ T cells. B7-1 and B7-2 could however costimulate CD4⁺CD45RA⁺ T cells to secrete IL-2. By contrast, when previously activated T cells were tested, restimulation of CD4⁺ T cell blasts with B7-1 or B7-2 resulted in higher secretion of IL-4 and IL-5 than anti-CD28, while restimulation with anti-CD28 antibody maintained a higher level secretion of IL-2 and interferon-γ than B7-1 or B7-2. Culture of T cells in the presence of cyclosporine A also revealed differences between different forms of CD28 ligation. These observations may have important implications because they suggest that the manner of CD28 ligation can be a critical determinant in the development of cytokine secretion that corresponds to Th1 and Th2-like patterns of differentiation. Together these observations suggest that many functions of B7-1 and B7-2 are redundant in the populations of cells that we have tested.

C2-342 Vγ1Vδ6 γδ T cell Subset in MHC Class 1 Deficient Mice.

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Although great deal has been learned about the diversity and development of γδ T cells, the elucidation of their physiological role in the immune response to defined antigens has been elusive. Recently several laboratories including ours have reported evidence that γδ T cells play critical role in the control of bacterial and viral infection in mouse. However, the mechanism and recognition of the natural ligands in such responses are not yet clear. Several lines of evidence imply that γδ T cells do not require classical MHC presentation for thymic selection, development, and response to certain yet undefined antigens.

We have reported a subset of γδ T cells typically bearing a Vg1Vd6 TCR, that respond to mycobacteria-derived heat shock protein (HSP-60), and have been studying the involvement of class 1 MHC in this response. Using the β2-microglobulin knock-out mouse, we have studied the selection and functional competence of Vγ1Vδ6 cells that develop in the absence of MHC Class 1. We have generated a pool of T cell hybridomas bearing the Vγ1Vδ6 receptor from both the thymus and spleen from such mice and are now studying their responses to a stimulatory HSP-60 peptide. The relative number and frequency of Vγ1Vδ6+ cells from thymus and periphery seemed to be unaffected in β2- /- mice.

C2-343 **CYTOKINE PRODUCTION BY γδ T CELLS**

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While much is known about cytokine production by αβ T cells, very little is known about cytokine production by γδ T cells. We have therefore investigated the production of a number of cytokines upon antibody stimulation of splenic γδ T cells of IL-2, IL-3, IL-4, IL-5, IL-6, IL-10, IFNγ, GM-CSF and TNFα using ELISA and by immunofluorescent staining of the golgi apparatus. Our results indicate that IFNγ, GM-CSF and TNFα are major cytokines produced by γδ cells. Small amounts of IL-3, IL-4, IL-6, and IL-10 were also detected, but very little if any IL-2 or IL-5 were detected. Using subset-specific antibodies for stimulation we have been able to demonstrate that subsets of γδ cells (based on T cell receptor expression) are biased in their pattern of cytokine production. Vδ6⁺ cells were found to be the major producers of IL-4, while Vδ6⁺ and Vδ4⁺ cells combined can account for almost all the IL-10 produced by γδ cells. Vδ6⁺ cells produced the greatest amount of a number of cytokines on a per cell basis, although they represented only a small proportion of all γδ cells. We have therefore established an extensive cytokine production profile for splenic γδ cells.

C2-344 T CELL RECEPTOR γδ CELL DEVELOPMENT AND FUNCTION IN ζ-DEFICIENT MICE. Roli Khattri[1], Anne I. Sperling[1], Dapeng Qian[2], Paul E. Love[3], Frank W. Fitch[1], Jeffrey A. Bluestone[1]. [1]The Ben May Institute/ University of Chicago, Chicago, IL 60637. [2]Dept. of Medicine and Howard Hughes Medical Institute, UCSF, San Francisco, CA 94143. [3]NICHHD, NIH, Bethesda, MD 20892.

Activation of T cell receptor (TCR)αβ cells depends on antigen recognition by the αβ heterodimer and signalling by the CD3 invariant chains γ, δ and ε and ζ-ζ or ζ-η dimers. We have shown that unlike TCRαβ cells, TCRγδ cells express, a distinct member of the ζ family, the γ chain of FcεRI (FcεRIγ) within the TCR complex. To study the role of FcεRIγ chain in TCRγδ cells, a TCRγδ transgenic mouse (G8) has been crossed with CD3ζ chain-deficient mice. Thy-1$^+$ cells from the spleen and lymph node of these animals expressed levels of CD3/TCR that was undetectable by flow cytometric analysis. However, immunoprecipitation with anti-CD3 showed that the FcεRIγ chain is associated with the TCR complex in ζ-deficient mice. These cells proliferated in response to stimulation by TCR antibodies including anti-CD3ε, anti-pan γδ, and anti-Vγ2 mAb. These results suggested that whereas the ζ chain is required for effective TCR transport to the cell surface, the signalling through the TCR complex remained at least partially intact. However, these cells did not respond to the G8-specific antigen (T10^b), anti-Thy-1 mAb or Con A. The unresponsiveness to the antigen was not due to the reduced TCR expression because intestinal intraepithelial lymphocytes (IEL) from the ζ-deficient mice expressing high levels of TCR did not respond to antigen. The cell's inability to respond to antigen could not be overcome by providing an anti-CD28 costimulatory signal or by adding exogenous rIL-2. Thus, these data suggests that the FcεRIγ chain associates with the TCRγδ complex in absence of ζ chain, but it is not able to substitute for the ζ chain for effective transport of TCR to the cell surface. In addition, some T cell responses such as proliferation in response to antibodies remains intact, but the response to antigen is severely impaired. Future studies will examine the mechanism of this differential responsiveness and the signalling pathways involved.

C2-345 **CD8$^+$ T CELLS WITH A CLASS II MHC RESTRICTED T CELL RECEPTOR**

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Mice with a transgene encoded T cell receptor (TCR) specific for hemagglutinin peptide 111-119 presented by class II MHC molecules (I-E^d) develop CD4$^+$8$^-$ and CD4$^-$8$^+$ T cells with high levels of transgenic TCR expression in I-E^d expressing strains. Both subsets are functional as evident from proliferation and killing sensitized target cells. CD4$^+$8$^-$ and CD4$^-$8$^+$ T cells are positively selected in mice of H-2^d or H-2^{dxg2}, but not of H-2^b or H-2^{bxd} MHC haplotype. In addition, CD4$^-$8$^+$ thymocytes expressing the transgenic TCR are present in recombination and TCRα deficient H-2^d mice, excluding the possibility of selection via endogenous TCRα chains paired with transgenic TCRβ chains. Although positively selected on class II MHC molecules, CD4$^-$8$^+$ T cells bearing the transgenic TCR require class I MHC molecules for full maturation as evident in β_2-microglobulin deficient mice.

These data show that a class II MHC restricted TCR can give rise to CD4$^-$8$^+$ mature T cells, that thymic positive selection requires more than a single receptor-ligand interaction and that for positive selection there is no strict requirement for a TCR, MHC and coreceptor tri-molecular complex, at least not for full maturation of a "mismatched" receptor.

C2-346 UPREGULATION OF SURFACE MARKERS ON DYING THYMOCYTES. Hidehiro Kishimoto, Charles D. Surh and Jonathan Sprent, Department of Immunology, IMM4, The Scripps Research Institute, La Jolla, CA 92037.

Immature CD4$^+$8$^+$ thymocytes in the thymus have a rapid rate of turnover and most of these cells die in situ from a failure to undergo positive selection. Consistent with the in vivo findings, immature thymocytes die rapidly when dispersed and placed in tissue culture at 37°C; cell death is associated with DNA degradation, a cardinal sign of apoptosis, and is potentiated by corticosteroids and ionizing irradiation. Although it is known that CD4 and CD8 markers are downregulated on thymocytes undergoing negative selection-induced apoptosis, phenotypic changes on spontaneously dying thymocytes are yet to be characterized. To seek such information we have defined the surface markers expressed on CD4$^+$8$^+$ thymocytes undergoing spontaneous apoptosis in tissue culture using terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labelling (TUNEL). Some surface markers, e. g. CD4, CD8 and HSA, are down-regulated on apoptotic thymocytes. Surprisingly, however, other markers are upregulated; this applies to TCRβ/CD3, CD69 and CD25 expression. Upregulation of these markers is restricted to a discrete subset of apoptotic cells. The significance involved in up-regulation of these markers is under investigation.

C2-347 THE EFFECT OF DIFFERENT IMMUNOGLOBULIN ENHANCERS ON SOMATIC HYPERMUTATION OF LAMBDA TRANSGENES. Emily Klotz and Ursula Storb*, Comm. on Immunology and *Dept. of Molecular Genetics and Cellular Biology, Univ. Chicago, Chicago, IL 60637.

Somatic hypermutation creates diversity in the variable region of an immunoglobulin (Ig) gene by introducting single point mutations. All three Ig genes (heavy, kappa, lambda) are able to undergo mutation. While the requirements for this mutation process are only partially understood, enhancers may have an important role, as suggested by mutable kappa transgenes. In an attempt to further elucidate a role for the enhancers, we are analyzing three different lambda transgenes, each driven by a different enhancer. One lambda2 construct is driven by the lambda2-4 enhancer, another by the heavy chain intronic enhancer. The third construct, a lambda1 transgene, is driven by the 3' kappa enhancer. Each transgenic line has been bred onto a kappa knockout and lambdaSJL background to limit the Ig repetoire in an attempt to force a higher frequency of mutation. B cells from the Peyer's patches of these animals are isolated and sorted based on their B220 and PNA staining. The transgenes are amplified, cloned, and sequencing to assay for mutations.

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C2-348 **REQUIREMENT FOR NATURAL KILLER CELLS AND THEIR CD56 MOLECULES IN THE INDUCTION OF CYTOTOXIC T CELLS**, Ferdynand J. Kos and Edgar G. Engleman, Department of Pathology, Stanford University School of Medicine, Palo Alto, CA 94304

Cell-mediated immunity involves the participation of both regulatory and cytotoxic cells. The conversion of precursors to effector $CD8^+$ cytotoxic T (Tc) cells requires cell-cell collaboration in which $CD4^+$ T cells are traditionally viewed as helper cells. An in vitro system was used to demonstrate that the generation of human antigen-specific $CD8^+$ Tc cells requires the participation of $CD3^-CD16^+CD56^+$ natural killer (NK) cells but not $CD4^+$ T helper cells. Depletion of either $CD16^+$ or $CD56^+$ cells from responder peripheral blood mononuclear cells before culture abolished the induction of alloantigen-specific Tc cells. Close contact or direct physical interaction between NK and Tc cells was required for the generation of allo-specific Tc cells and studies using transwell membrane cultures showed that NK cell-derived soluble factors were not sufficient to compensate for the lack of $CD56^+$ cells among responders. Blockade of CD56 molecules by monoclonal antibodies (mAb) during 5-d allogeneic mixed lymphocyte culture abolished Tc cell generation to the background levels. Further functional and flow cytometry studies revealed that anti-CD56 mAb did not block NK cell activation per se but did prevent the accessory effect of NK cells on Tc cell induction. Our studies clearly indicate that NK cells act in an accessory manner to regulate the generation of antigen-specific, MHC class I-restricted responses of T cells and CD56 molecules on NK cells appear to play a critical role in this process. This observation represents a novel critical link between the natural and specific immune responses.

C2-349 GENETIC IMMUNIZATION MAPS IMMUNE RESPONSES AGAINST A SEQUENCE SHARED BETWEEN THE EBV PROTEIN Balf2 AND THE pJRA-RELATED HLA ALLELE DRB1*0801, Antonio La Cava, Lan Xu, Ann Montemayor, Dennis A. Carson, Salvatore Albani, Department of Pediatrics and Medicine, University of California, San Diego, CA 92093-0663, and Margherita Massa, Angelo Ravelli, Fabrizio De Benedetti, Alberto Martini, University of Pavia, Italy.

Pauciarticular juvenile rheumatoid arthritis (pJRA) is associated with HLA DRB1*1301, HLA DRB1*0801 and HLA DPB1*0201. Segments of the third hypervariable regions from each of these alleles are mimicked by Epstein-Barr virus (EBV) proteins, namely Balf1 for HLA DPB1*0201 and Balf2 for HLA DRB1*1301 and HLA DRB1*0801. In previous experiments, we have found that the shared amino acid sequences are targets of B cell responses in patients with pJRA. Here, we addressed whether exposure to intact Balf2 could elicit T cell responses aimed at the shared sequence. At weekly intervals, six BalbC mice were injected either subcutaneously or intramuscularly with 100 µg of naked recombinant DNA construct encompassing the gene for a deletion mutant of Balf2 inserted in p290, an eukaryotic expression vector. Spleen cells were harvested one week after the last injection and tested for their cytotoxic responses against P815 cells alone, incubated with Balf2 or pulsed with a synthetic peptide encompassing the sequence that Balf2 shares with HLA DRB1*0801 (PEP1). Effector to target ratios varied from 50:1 to 6:1. Percentage of lysis of Balf2-pulsed targets ranged from 11.6 ± 3.3, with a significant variation ($p<0.0004$, compared to the targets without antigen). Percentage of lysis of PEP1-pulsed cells ranged from 10 ± 3.8 to 7.8 ± 2.9 ($p<0.007$, compared to target without antigen). The sequence shared between HLA DRB1*0801 and Balf2 is therefore recognized as a T cell epitope following in vivo immunization with DNA codifying for the whole Balf2 protein. It is thus conceivable that exposure of pJRA patients to Balf2, or a related antigen, could induce self-reactive cytotoxic T cells.

C2-350 PRIMARY HUMAN THYMIC EPITHELIAL CELLS INDUCE ANTI-TCR-MEDIATED DELETION OF IMMATURE THYMOCYTES BY APOPTOSIS, Phong T. Le and Jane E. Cook. Department of Cell Biology, Neurobiology and Anatomy, Loyola University Medical Center, Maywood, IL 60153.

Deletion of self-reactive T cell clones in the thymus is a crucial step in the normal development of T cells. The specificities of TCR expressed on immature thymocytes determine whether a T cell is positively selected or negatively deleted by apoptosis. In mice, it has been well documented that thymic epithelial cell lines can mediate both positive and negative selection, and that TCR engagement is essential but not sufficient to deliver a lethal apoptotic signal in the negative selection process. In humans, whether thymic epithelial cells can mediate negative selection is not well established. We have developed an *in vitro* model using primary human thymic epithelial cells (TEC) and an allogeneic immature T cell line expressing the Vß5.1 TCR to determine whether TEC can provide costimulatory signals for anti-TCR-mediated apoptosis of the immature T cells. We demonstrate that our SUP-T13 subclone A3 (SUP-T13.A3) fails to undergo apoptosis, while the parental clone is induced to undergo apoptosis when treated with the anti-TCR Vß5.1 mAb LC4. In contrast, SUP-T13.A3 cells are induced to undergo apoptosis when cultured with anti-TCR mAb in the presence of TEC (21.0 ± 1.0). The effect of TEC is specific since human thymic fibroblasts from the same source fail to induce apoptosis (2.0 ± 4.0). Thus, we directly demonstrate that primary human TEC are capable of providing costimulatory signal for TCR-mediated apoptosis. The *in vitro* model system will allow us to identify TEC costimulatory signals, as well as to determine the role of different human TEC subsets in the negative selection process (supported by NIH grant AR34808 and a grant from the Potts Foundation).

C2-351 ANALYSIS OF CYTOKINE GENE EXPRESSION AND IL-2/IL-4 PROMOTER SPECIFIC TRANSCRIPTION FACTORS DURING CYTOKINE-INDUCED DIFFERENTIATION OF NAIVE $CD4^+$ T-CELLS, James A. Lederer, Victor L. Perez, Abul K. Abbas and Andrew H. Lichtman. Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115

Interleukin-12 (IL-12) and interleukin-4 (IL-4) can induce the differentiation of $CD4^+$ T-cells into Th1-like and Th2-like cell populations both *in vivo* and *in vitro*. The kinetics of T-helper cell subset differentiation and the molecular events that determine selective cytokine production by Th1 and Th2 cells are not well characterized. In this study, we examined the kinetics of IL-2, IFN-γ and IL-4 mRNA expression during IL-12- and IL-4-induced differentiation of naive pigeon cytochrome C (PCC)-specific T-cell receptor (TcR) transgenic $CD4^+$ T-cells. Quantitative RT-PCR analysis indicated that IL-2 mRNA is expressed 12 hours after antigen stimulation at equal levels in the presence of IL-12 or IL-4. IFN-γ transcripts were also expressed by 12 hours of stimulation, but higher levels of IFN-γ were expressed in IL-12 treated cultures. IL-4 mRNA was not detectable at 12 or 24 hours after initiation of IL-4-induced differentiation. At 48 hours, IL-2 mRNA is expressed at equal levels in cultures treated with IL-12 or IL-4, whereas IFN-γ transcript levels are 30-fold higher in IL-12-treated cultures than in untreated or IL-4-treated cultures. IL-4 transcripts were first detected at 48 hours in IL-4-treated cultures, while IL-4 mRNA was not expressed in untreated cultures or in cultures treated with IL-12. Quantitative RT-PCR analysis of cytokine transcripts in antigen restimulated differentiated T-cell populations demonstrated that IL-2 mRNA levels were significantly reduced in IL-4 differentiated T-cells as compared with IL-12 and untreated differentiated T-cell populations, IFN-γ transcipts were enhanced in IL-12 differentiated populations by 10-30-fold, and IL-4 transcripts were 30- to 100-fold higher in IL-4 differentiated cells. Thus, these findings suggest that the development of Th1- or Th2-like phenotypes result from independent regulatory pathways for each cytokine gene. To determine possible mechanisms responsible for regulation of cytokine gene transcription during T-helper cell differentiation, we assessed by electrophoretic mobility shift assays (EMSA) whether there were any detectable differences in the presence of the IL-2-promoter binding transcription factors (AP-1, NFIL-2A, NFκB, IL-2NFAT) and the IL-4-promoter binding factors (IL-4NFAT, IL-4NAF/ IL-4STAT) specific transcription factors at 48 hours after initiation of IL-12- and IL-4-induced differentiation or upon restimulation of differentiated Th1 and Th2 cell populations. At 48 hours, we observed no significant differences in AP-1, NFIL-2A, NFκB, IL-2NFAT, or IL-4NFAT nuclear binding factors in IL-12 or IL-4 treated populations, however, factors binding an IL-4NAF/ IL-4STAT binding site in the IL-4 promoter were detected only in IL-4 treated T-cells. Restimulation of differentiated Th1 and Th2 cell populations with immobilized anti-CD3 antibody revealed that IL-4NAF/IL-4STAT binding factors were detectable only in T cells differentiated in the presence of IL-4. These findings demonstrate that the presence of nuclear IL-4NAF/ IL-4STAT may be necessary for the develoment of the Th2 phenotype.

C2-352 INHIBITION OF THYMOCYTE DEVELOPMENT BY ANTI-CD45 mAb TREATMENT IN FTOC, Angela L. Lee and Jane R. Parnes, Department of Medicine, Division of Immunology and Rheumatology, Stanford University, Stanford CA 94305-5487

We have investigated the role of CD45 in thymocyte development by treating differentiating murine thymocytes in fetal thymic organ culture (FTOC) with anti-CD45 antibody, and have analyzed their maturational status by examining their expression of cell surface differentiation markers. As was shown in CD45 exon-6 deficient mice (1), CD45 may be required in the transition from $TCR^{intermediate}CD4^+CD8^+$ (double positive) cells to mature (single positive) $TCR^{high}CD4^+CD8^-$ or $TCR^{high}CD4^-CD8^+$ thymocytes, and we have data in accord with this observation. In our system the proportion of $CD4^+$ thymocytes is decreased in treated lobes compared to controls, but in contrast to the published data, the proportion of $CD8^+$ cells is not. These $CD8^+$ thymocytes, however, are low in CD3ε expression, suggesting that they are not mature $CD8^+$ thymocytes. In both single positive populations, the absolute number of cells per thymic lobe decreases with anti-CD45 mAb treatment of FTOC.

A secondary finding of ours is a dramatic increase in the proportion of $CD4^-CD8^-$ (double negative) cells and a decrease in the proportion of double positive cells. In addition, the expression level of CD3ε on most of these double negative cells is lower. In terms of absolute cell number per lobe, however, the number of double negative cells is slightly decreased while the number of double positive cells is drastically reduced compared to controls. These data suggest that anti-CD45 treatment may affect the transition of thymocytes from the $TCR^{low}CD4^-CD8^-$ to the double positive stage, and/or that double positive cells are especially sensitive to anti-CD45 mAb treatment.

C2-353 MECHANISMS REGULATING IL-13 GENE EXPRESSION BY HUMAN T-LINEAGE CELLS, David B. Lewis, Mary E. McGurn, Susan J. Bort, and Douglas S. Short, Departments of Pediatrics and Immunology, University of Washington, Seattle, WA 98195.

IL-13 is a cytokine produced by activated T cells and is encoded by a gene located close to the human IL-4 gene on chromosome 5. Although IL-13 and IL-4 are usually co-expressed in murine CD4 TH2-type clones, their expression *in vivo* by CD4 T cells has been discoordinate in some animal models of infection. To gain insight into mechanisms regulating IL-13 gene expression, we analyzed normal T-lineage cell populations for IL-13 and IL-4 mRNA expression after polyclonal activation. IL-13 mRNA was routinely detected in adult T cells, with high levels in memory/effector ($CD45RA^{lo}CD45R0^{hi}$) CD4 T cells, but was undetectable in neonatal T cells or $CD4^+CD8^-$ thymocytes. Priming *in vitro* of adult or neonatal CD4 T cells markedly upregulated IL-13 mRNA expression. This pattern of IL-13 mRNA expression was virtually identical to that found for IL-4 mRNA. We next determined if NFAT-type transcriptional activator proteins, which bind IL-4 promoter region P elements and enhance transcription, might also regulate IL-13 transcription. An oligonucleotide probe of the IL-13 5′ flank region, having sequence homology with IL-4 gene P elements, specifically formed a complex with nuclear protein extracts from activated but not unstimulated T cells. For both the IL-13 and IL-4 P element probes, the abundance of this complex was markedly higher in T cells primed *in vitro* than in freshly-isolated adult or neonatal T cells. Based on competition assays, the binding specificity of the nuclear protein for the IL-13 and IL-4 P elements appeared identical. The IL-13 and IL-4 P-element nuclear complexes also both contained NFATp (antiserum was provided by Dr. Anjana Rao, Dana-Farber C.I.). Thus, coexpression of the IL-13 and IL-4 genes by memory/effector or primed T cells could be mediated, in part, by the nuclear availability of NFATp. The mechanisms by which these two cytokines can be differentially expressed *in vivo* remain to be determined.

C2-354 THE B LYMPHOCYTE RESPONSE TO THE FOREIGN ANTIGEN PIGEON CYTOCHROME *c* INITIATES AS A HETEROCLITIC RESPONSE SPECIFIC FOR THE SELF ANTIGEN MOUSE CYTOCHROME *c*, Jeanne M. Minnerath*, Paul Wakem, Linda Comfort, Fred Sherman, and Ronald Jemmerson*, Department of Microbiology*, University of Minnesota, Minneapolis, MN 55455 and Department of Biochemistry, University of Rochester, Rochester, New York 14642.

Previously, we observed an unexpected higher frequency of antigen (Ag)-specific B lymphocytes in BALB/c mice responding early (4 days after immunization) to the self Ag mouse cytochrome c (cyt) coupled to a carrier protein than to a number of foreign cyt also coupled to the carrier. The only cyt eliciting a significant response early were those, including pigeon cyt, that cross-react with monoclonal antibodies (mAb) to mouse cyt. We questioned whether the B cells responding to these cross-reactive cyt were actually specific for mouse cyt. To address this possibility, the specificity of mAb obtained from individual B lymphocytes responding to pigeon cyt in splenic fragment cultures was examined by ELISA using variants of pigeon cyt, including other naturally-occurring cyt and mutants of pigeon cyt prepared by site-directed mutagenesis. As was observed early in the response to mouse cyt, all of the mAb obtained at 4 days in the response to pigeon cyt bound the region around residues 60 and 62. A majority (75%) of these mAb bound mouse cyt with a higher affinity than pigeon cyt indicating that they were heteroclitic for the self Ag. The remaining mAb bound identically to pigeon and mouse cyt. The frequency of the heteroclitic B cells declined as the antibody response progressed so that, for example, at 45 days, the frequency of heteroclitic B cells was less than 20% of the total pigeon cyt responding B cells. This decline could be due to: 1) somatic mutations of the V genes encoding mouse cyt-specific mAb and selection of mutated B cells having higher affinity for pigeon cyt, or 2) recruitment of B cells expressing other genes encoding pigeon cyt-specific mAb. Our results may have implications for a role of some self Ag in the maintenance of the B cell repertoire in a naive animal.

C2-355 THE INDUCTION OF A Th2 POPULATION FROM A POLARIZED *Leishmania*-SPECIFIC Th1 POPULATION BY *in vitro* CULTURE WITH IL-4.

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The infection of mice with *Leishmania major* parasite induces polarized Th1 and Th2 responses that cannot be significantly changed *in vivo* after 2-3 weeks of infection, using either cytokines or anti-cytokine antibodies. It is not clear, however, whether the T cell populations are irreversibly differentiated or whether the inability to modify the cytokine production reflects inefficiencies in the experimental treatments or complications of the infection itself. To study this further, we have cultured $CD4^+$ T cells from the draining lymph nodes of *L. major* infected mice with specific antigen, APC and IL-2, in the presence or the absence of different cytokines and/or anti-cytokine antibodies. We have demonstrated that Th1 cells cultured for 1 week in the presence of IL-4 produced very low levels of IFN-γ but, instead, high levels of IL-4 and IL-10, suggesting that IL-4 was able to induce a Th1 to Th2 conversion in a chronically stimulated Th1 population. Other Th2 specific cytokines, such as IL-10 and IL-13 were not able to cause any significant change in the Th1 population.The modifications induced by IL-4 in the Th1 population were consistent with the generation of a stable Th2 population rather than a transient induction of Th2 and inhibition of Th1 cytokines. This was demonstrated by the ability of these cells to adoptively transfer a Th2 response to *L. major*-infected C.B-17 *scid* mice. IL-4 induction of a Th2 response *in vitro* was very rapid and required the presence of IL-4 between day 1 and 4 of culture, when the proliferation of the activated T cells occurred. In contrast, the presence of IFN-γ and IL-12 during the Th2 cell stimulation enhanced IFN-γ production but was not sufficient to induce a complete conversion of a Th2 into a Th1-like population. Taken together, these data show that highly polarized murine Th populations can be modified and even converted to the opposite cytokine phenotype *in vitro* . These results suggest, moreover, possible therapeutic applications of cytokines for the modification of inappropriate Th responses.

C2-356 CYTOKINE PRODUCTION IN A GRANULOMATOUS LUNG DISEASE OF UNKNOWN ETIOLOGY: ROLE OF 65 KDA HEAT SHOCK PROTEIN (HSP) SPECIFIC T-CELLS. David R. Moller, Mark C. Liu, Jeffrey D. Forman, Brian M. Greenlee, Prachi Vyas, Hui-Qing Xiao and Christopher L. Karp. Department of Medicine, Johns Hopkins School of Medicine, Baltimore, MD. 21205

To investigate the regulation of T-cell responses involved in granulomatous inflammation of the lung, we have been studying bronchoalveolar lavage (BAL) lung and blood T-cells from individuals with sarcoidosis, a multiorgan granulomatous disease of unknown etiology that frequently affects the lungs, lymph nodes, skin and eyes. To determine whether the T-cell responses associated with granulomatous inflammation in the lung generate a Th1 pattern of cytokine production, an analysis of the expression of IFNγ, IL2, IL4 and IL5 by lung BAL cells was performed using a semiquantitative RT-PCR technique. Our results indicate that IFNγ and IL2 are strongly expressed in the lungs of individuals with sarcoidosis but not normal or allergic asthmatic individuals. In contrast, IL4 or IL5 were either barely detectable or nondetectable in sarcoid, normal and asthmatic individuals. To determine the potential influence of lung macrophage-derived cytokines on Th1 producing T-cells in the sarcoid lung, expression of IL12 and IL10 mRNA was analyzed by RT-PCR and Northern blot analysis. The results demonstrated significantly increased expression of IL12 (p40 gene) in the sarcoid compared to the normal or asthmatic lung. In contrast, similar expression of IL10 was seen in the lungs of normal, sarcoid and asthmatic individuals. To assess antigen-specific mechanisms which may contribute to Th1 cytokine production in the sarcoid lung, we analyzed lung T-cell responses to mycobacterial 65kDa hsp in sarcoidosis. Our results indicate significant T-cell responses to hsp65 as assessed by IFNγ production of antigen-stimulated cultures established at time of diagnosis. We speculate that one mechanism for the persistence of Th1 cytokine production in the sarcoid lung may be the presence of an autoimmune T-cell response cross-reactive to hsp65 which results in the sustained production of IFNγ, a process enhanced by an upregulation of macrophage-derived IL12 in the lung.

C2-357 MECHANISM OF INTERFERON-GAMMA-INDUCED INHIBITION OF TH2 PROLIFERATION. Penelope A. Morel, Timothy B. Oriss, Susan A. McCarthy, and Benoit F. Morel Departments of Medicine, Surgery and Molecular Genetics and Biochemistry, University of Pittsburgh; Department of Engineering and Public Policy, Carnegie Mellon University, Pittsburgh, PA.

$CD4^+$ T helper cells are divided into two main subsets based on the lymphokines that they secrete and on the immune responses that they mediate. Th1 cells secrete IL-2, IFN-γ and lymphotoxin and mediate the delayed type hypersensitivity response whereas Th2 cells secrete IL-4, IL-10 and IL-5 and mediate specific B cell responses. An important feature of these responses is the fact that these cells mutually regulate each other. IL-10 and IL-4, secreted by Th2 cells, inhibit the activation of Th1 cells and IFN-γ, secreted by Th1 cells, has been reported to inhibit the proliferation of Th2 cells directly. As part of a larger study of Th1 and Th2 interactions we have been studying the mechanism by which IFN-γ inhibits the proliferation of a sperm whale myoglobin-specific T cell clone, 13.26. Following antigenic stimulation 13.26 produces large amounts of IL-4 and the subsequent proliferation is dependent not only on this IL-4 but also on IL-1 secreted by the APC. IFN-γ inhibits the proliferation of this clone in a dose dependent manner, both when the clone is stimulated by antigen and APC and when the clone is stimulated with crosslinked anti-CD3 monoclonal antibody. In this last scenario IL-4-mediated proliferation of 13.26 is dependent on the addition of exogenous IL-1. IFN-γ inhibits the IL-1-induced co-stimulation and this can be reversed by addition of more IL-4. Preliminary evidence suggests that production of IL-4 is not inhibited by the addition of IFN-γ. Possibilities that are under investigation include an inhibition of IL-4 receptor expression or a mechanism involving interference with signal transduction by IFN-γ. A deeper understanding of this phenomenon is important in situations where it might be desirable to inhibit a Th2 response such as in systemic lupus erythematosus or AIDS.

C2-358 IL-4 DELAYS IN VIVO T CELL ACTIVATION AND SUPPRESSES IgE PRODUCTION IN MICE INJECTED WITH GOAT ANTI-MOUSE IgD ANTIBODY (GαMδ), S.C. Morris, W.C. Gause, C.R. Maliszewski, and F.D. Finkelman, USUHS, Bethesda, MD 20814 and Immunex Corp., Seattle WA 98101.

The injection of BALB/c mice with GαMδ stimulates increased T cell IL-2 and IL-9 gene expression in 3 days, increased IL-4 and IL-10 gene expression in 4 days, and increased IgE and IgG1 secretion in 7-8 days. Although IL-4 is required to stimulate an IgE response, injecting mice with complexes of IL-4 and anti-IL-4 mAb (IL-4C), that have long-acting IL-4 activity, prior to or along with GαMδ, suppressed serum IgE responses by >84%. In contrast, IL-4C treatment that was given 1-3 days after GαMδ enhanced IgE responses. Because IL-4 suppresses IL-2 secretion in vitro and IL-2 antagonists suppress IgE responses in GαMδ-injected mice, we examined whether IL-4C treatment inhibits the expression of IL-2 and other cytokine genes in these mice. IL-4C treatment of GαMδ-injected mice inhibited day 3 increases in IL-2 and IL-9 gene expression by 87-100% and 71-95% respectively, and day 4 increases in IL-4 and IL-10 gene expression by 100% and 80%, respectively. GαMδ-induced cytokine gene expression partially recovered from the inhibitory effects of IL-4C treatment by 5 days after GαMδ injection, when considerable increases in IL-4 and IL-9 gene expression were observed, although IL-2 and IL-10 gene expression remained low. Treatment of GαMδ-injected mice with IL-2C as well as IL-4C failed to block the inhibitory effects of IL-4C. Because IL-4 is known to inhibit monocyte production of IL-1, which can contribute to T cell activation, we examined whether exogenous IL-1 could restore responses in mice treated with GαMδ plus IL-4C. These experiments demonstrated partial restoration of GαMδ-induced responses. These observations demonstrate that high concentrations of IL-4 can inhibit the expression of Th2-associated as well as Th1-associated cytokines by differentiating T cells and suppress T cell-dependent antibody responses. In addition, they raise the possibility that the inhibitory effect of early exposure to IL-4 may result from a block in the production of stimulatory monokines, such as IL-1, rather than from a direct block of IL-2 production by T cells.

C2-359 UNRESPONSIVENESS TO A SELF PEPTIDE OF MOUSE LYSOZYME OWING TO STERIC HINDRANCE OF TCR-MHC/PEPTIDE INTERACTION, Kamal D. Moudgil, Iqbal S. Grewal, Herbert Eradat, Audrey M. Chen, Oliver J. Yun and Eli E. Sercarz, Department of Microbiology and Molecular Genetics, University of California, Los Angeles,CA 90024

A self peptide containing a.a. residues 46-61 of mouse lysozyme (ML) (p46-61; it binds to the A^k but not the E^k molecule), can induce a strong proliferative T cell response in CBA/J mice (A^k, E^k) but no response at all in B10.A(4R) mice (A^k, E^o). However, two truncated forms of p46-61, p48-61 or p46-59, are immunogenic in both B10.A(4R) and CBA/J mice. The critical residues within p46-61 reside between positions 48-56. T cells of B10.A(4R) mice primed with the truncated peptides in vivo cannot be restimulated by p46-61 in vitro. Unlike B10.A(4R) mice, [B10.A(4R)xCBA/J]F1 mice responded vigorously to p46-61. Moreover, APC from B10.A(4R) mice can efficiently present p46-61 to peptide-specific T cell lines from CBA/J mice. The proliferative unresponsiveness of B10.A(4R) mice to p46-61 is not due to non-MHC genes because B10.A mice (A^k,E^k) respond well to p46-61. Interestingly, B10.A(4R) mice can raise a good proliferative response to p46-61(A61), in which the arginine residue at position 61 (R61) of p46-61 had been substituted by an alanine residue. Thus, we provide experimental demonstration of a novel mechanism for unresponsiveness to a self peptide owing to steric hindrance: in this system it is the interaction between the available TCR and the A^k/p46-61 complex, which is hindered by epitypic residue(s) within p46-61. We argue that besides possessing T cells that are hindered by R61, CBA/J and B10.A mice have developed an additional subset of T cells which are not hinderable by R61, presumably through positive selection with peptides derived from class II E^k, or class I D^k/D^d molecules. These results have important implications in self tolerance and autoimmunity. (Supp. by grants from ACS, IM-626, and NIH, AI-11183 and AR-3683406).

C2-360 UNIQUELY DERIVED, HEL PEPTIDE 74-96-SPECIFIC CD8 T CELL CLONES REVEAL A NOVEL PARADIGM FOR ACTIVATION OF CD8 T CELLS. Navreet K. Nanda, Karo Arzoo, Nilabh Shastri*, and Eli Sercarz, Dept. of Micro. and Molec. Genetics, UCLA, Los Angeles, CA 90024 and Dept. of Molec. and Cell Biol.*, UC Berkeley, CA 94720.

$CD8^+$, $CD4^-$ T cells are usually cytotoxic and in some cases shown to exert a suppressive (immunoregulatory) function. The cytotoxic function of these cells is completely established but the basis for the immunoregulatory suppressive function of these cells is still unknown.

We recently discovered that $V_\beta{}^a$ mice of the H-2^d haplotype, lacking 10 T cell receptor (TCR) V_β gene segments, make a strong T cell response to determinant within 74-96 of hen egg-white lysozyme (HEL), whereas the $V_\beta{}^b$, H-2^d mice with a wild-type, non-truncated repertoire completely fail to respond to the same peptide. This surprising result suggested that the missing V_βs might code for immunoregulatory CD8 TCR(s). We also showed that T cell responses to peptide 74-96 of HEL in $V_\beta{}^b$ mice could be restored by in vivo depletion of CD8 T cells from normal $V_\beta{}^b$ mice. Apparently, a hole in the suppressive CD8 repertoire had been produced which permitted this normally down-regulated response.

Searching for immunoregulatory CD8 T cells in $V_\beta{}^b$, H-2^d mice, we have now derived several cloned CD8 T cell hybridomas specific for HEL peptide 74-96. The activation requirements of these CD8 T cell hybridomas are unique and unlike those for conventional CD8 T cells since these cells fail to be triggered by conventional class-I^+ targets, but instead require only specially defined, antigen-specific T cells for activation. Thus, we define a novel population of CD8 T cells which use a novel paradigm for their activation. We propose that this hitherto undefined subset of CD8 T cells is activated and induced during the normal course of an immune response. (Supported by ACS and NIH grants)

C2-361 IDENTIFICATION OF HUMAN T HELPER SUBSETS GENERATED IN VITRO BY SECRETED CYTOKINE PROFILES AND CELL SURFACE PHENOTYPE, Ellen M. Palmer and Gijs A. van Seventer, Committee on Immunology, University of Chicago, Chicago, IL 60637

The immune system employs $CD4^+$ T cells in two types of immune responses. One subset of $CD4^+$ cells, called T helper 1 (Th1), is active in cell-mediated and delayed-type hypersensitivity reactions, while another subset, T helper 2 (Th2), provides B cells with "help" necessary for a humoral response. We have used an in vitro assay to influence $CD4^+$ T cell differentiation into subsets. $CD4^+$ "naive" ($CD45RA^+$, $CD45RO^-$) T cells isolated from human peripheral blood are stimulated multiple times in the presence of immobilized α-CD3 mAb, accessory cells and exogenous cytokines and are analyzed for cell surface phenotype and cytokine production at each stimulation. Our results show that a monocytic cell line, U937, which lacks expression of B7-1 and B7-2, in the presence of exogenous IL-12, can generate a Th1 cytokine profile from naive T cells. Cytokines secreted from these cells include IFN-γ and IL-2, but not IL-5. The cytokine profile produced by T cells stimulated with a B7-1- and B7-2-expressing B cell line, JY, and exogenous IL-12 is very similar to that of the T cells stimulated with the monocytic line and IL-12. However, the addition of IL-1/6 or IL-1/6 + IL-4 to T-B cell cultures results in naive T cells producing a Th0- or Th2-like cytokine profile, respectively. The Th0 cells produce both IFN-γ and IL-5 whereas IL-5, but no IFN-γ is secreted by Th2 cells. A number of cell surface molecules are differentially expressed on T helper subsets. We find markers expressed on our subsets in much the same pattern as reported for Th1 and Th2 cells. The expression of CD60 is low on Th1 cells, while CD26 and CD27 expression is high. The expression of CD60 on Th0 and Th2 cells increases with differentiation, while CD27 staining becomes less uniformly high. CD26 is expressed in both of these populations, but is lower than in cells grown with IL-12. The data suggests that signals through costimulatory molecules as well as cytokine receptors influence the differentiation of naive $CD4^+$ T cells.

C2-362 **REGULATION OF IGE PRODUCTION BY T-CELL SUBSETS,** Patel, HR, Oshiba, A, Jeppson, JD, Gelfand, EW, National Jewish Center for Immunology and Respiratory Care, Denver, Colorado 80206

Cognate interaction between peripheral human T lymphocytes and B lymphocytes is required for the induction of IgE production. In the present study, we have examined the abilities of $CD45RA^+$ and $CD45RO^+$ human T-cell subsets to provide help for IgE production by human peripheral B-cells. Purified peripheral $CD45RA^+$ T-cells are much better inducers of IgE synthesis than are $CD45RO^+$ T-cells. Activation of $CD45RA^+$ T-cells, but not $CD45RO^+$ T-cells, via the TCR/CD3 complex for 18 h is sufficient to confer the ability to provide IgE help. The CD40 ligand, an inducible T-cell surface molecule, is expressed at higher basal and CD3-stimulated levels on $CD45RA^+$ T-cells as compared with $CD45RO^+$ T-cells. Blocking of the CD40-CD40 ligand interaction completely blocks IgE production induced by $CD45RA^+$ T-cells. Finally, the *in vitro* conversion of $CD45RA^+$ T-cells to the $CD45RO^+$ phenotype is accompanied by a loss in the ability of these cells to express the CD40 ligand in response to anti-CD3 stimulation and a concurrent loss in their ability to provide IgE help. These results indicate that the $CD45RA^+$ T-cells subset is a more potent provider of IgE help than the $CD45RO^+$ subset, and that the differential expression of the CD40 ligand on these T-cell subsets may be responsible for this effect.

C2-363 CELL ADHESION MOLECULES AND T-LYMPHOCYTE DEVELOPMENT.

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Differentiation of haemopoietic stem cell into lymphocytes is regulated through interactions with a variety of growth factors and their cognate receptors. In addition, cell-cell contact mediated by cell adhesion molecules(CAMs) is thought to participate in this differentiation pathway. During development, T-cell progenitors migrate from the fetal liver or adult bone marrow to the thymus where T-cell differentiation takes place. Availability of in vitro T-cell differentiation models provide an opportunity to define the cellular interactions which may regulate production of lymphocytes. When the FTH5 Pro-T cell line or freshly isolated day 13 fetal liver T-cell precursors are co-cultured with the thymic epithelial line, ET, in the presence of interleukin 7, interleukin 11 and F factor(supernatant from the FLS4.1 stromal cell line), the lymphoid precursors differentiate into thymocytes expressing cell surface CD4 and TCR/CD3 antigens. Using this system, we have characterised the expression and function of adhesion molecules at various stages of T-cell differentiation. We have determined the patterns of CAM expression in thymic epithelial cell lines established from different regions of the fetal thymus. Our studies show that multiple adhesion molecules are present on the thymic epithelial lines, ET and EH6, and that thymic epithelial cell lines derived from the subcapsular(EH6) or cortical(ET) regions show differences in CAM expression. Distinct patterns of CAM expression are also associated with specific stages of T-cell differentiation, namely T-cell progenitors derived from day13 day fetal liver, Pro-T(FTH5 line), Pre-T(SCID/ET/27F line) and adult thymocytes. Finally evidence will be presented showing that different CAMs play distinct and active roles during differentiation from Pro-T cells($CD4^-TCR/CD3^-$) into $CD4^+TCR/CD3^-$ and subsequently into $CD4^+TCR/CD3^+$ cells.

C2-364 IDENTIFYING THE CIS TARGETING ELEMENTS OF SOMATIC HYPERMUTATION OF IMMUNOGLOBULIN GENES. Andrew Peters* and Ursula Storb, Department of Molecular Genetics and Cell Biology, and * Department of Biochemistry and Molecular Biology, The University of Chicago, Chicago IL 60637.

One of the forces contributing to affinity maturation is somatic hypermutation. Somatic hypermutation occurs in a site specific, time dependent manner. The sequence specificity of this mechanism may suggest that a targeting sequence is located cis to the mutated gene. Transgenic constructs have been designed to test this idea by placing potential targeting sequences upstream of the constant region. The variable region and its surrounding sequences have been left intact so that the construct would have an internal positive control. Presented in this poster is one such construct, P5'C-switchV. This construct has the variable region switched with the constant region. Both regions have four kilobases, from upstream of the leader to inside the leader-variable intron from the kappa 167 gene, inserted upstream.

One line containing six-eight copies of the transgene is being analyzed by both sequencing amplified products from isolated PNAhi, B220+ staining Peyer's patch B cells, and sequencing amplified products from hybridomas made from splenic B cells. Preliminary results from Pfu amplified Peyer's Patch B cells indicate that the construct may be a target for somatic hypermutation.Supported by NIH grants GM 38649 and HD 23089.

C2-365 CIRCADIAN RHYTHMICITY IN IMMUNE FUNCTION AND REGULATION AS REFLECTED BY CHANGES IN THE IFN-γ/IL-10 RATIO. Nikolai Petrovsky, Leonard C. Harrison, Walter and Eliza Hall Institute, Parkville 3050 Australia

We have recently demonstrated circadian rhythmicity in interferon-gamma (IFN-γ) production by antigen-stimulated whole blood. The aim of this study was to determine if there is also circadian variation in the balance between TH_1 and TH_2 cytokine production.

Helper T lymphocytes (T cells) can be divided into two functional subsets. TH_1 cells produce the cytokines interleukin-2 (IL-2) and IFN-γ and mediate delayed-type hypersensitity, whereas TH_2 cells produce IL-4, -5, -6 and -10 and mediate humoral and allergic responses. TH_1 and TH_2 cell subsets have been implicated in the pathogenesis of cell-mediated autoimmunity and allergy, respectively. What determines TH_1/TH_2 balance is not known although the cytokine and hormonal environment in which a T cell is activated is clearly important.

Amongst T cells IL-10 is characteristically produced by the TH_2 subset. Consequently, in these experiments, the IFN-γ/IL-10 ratio was used as a surrogate measure of TH_1/TH_2 balance. Four subjects had blood taken hourly for 24 hours for measurement of IFN-γ and IL-10 production in response to tetanus toxoid and lipopolysaccharide. In an additional experiment subjects were administered a single dose of cortisone acetate 25mg at 9pm to determine the effects of changes in plasma cortisol on the IFN-γ/IL-10 ratio.

The IFN-γ/IL-10 ratio for each antigen demonstrated circadian rhythmicity in all subjects as validated by Cosinor analysis ($p<0.001$). The IFN-γ/IL-10 ratio was highest at night and lowest during the day. Cortisone reduced the IFN-γ/IL-10 ratio (>90%), reflecting inhibition of IFN-γ production. Circadian rhythmicity in TH_1 function is consistent with *in vivo* evidence of increased cell-mediated immune responses when immunisation is performed at night. The role of plasma cortisol in regulating TH_1/TH_2 balance could have implications for the therapy of immuno-inflammatory conditions which are subject to nocturnal exacerbations such as rheumatoid arthritis or asthma.

C2-366 PRIMING OF TH1 OR TH2 IN VIVO IS A FUNCTION OF TcR-LIGAND DENSITY. Christiane Pfeiffer[1], Alessandro Sette[2], and Kim Bottomly[3], [1]Dep. of Dermatology, Ludwig-Maximilians-University, Munich, Germany, [2]Cytel Corporation, San Diego, CA and [3]Section of Immunobiology, HHMI, Yale University School of Medicine, New Haven,CT.

Humoral(HI) and cell-mediated immunity(CMI) are often mutually exclusive. HI and CMI response are regulated by two different subsets of T helper lymphocytes,Th1 and Th2, which differ in their cytokine pattern. To study the basic mechanism governing the induction of Th1 versus Th2 in vivo we employed an animal system in which priming of MHC class II recombinant inbred mice with the same peptide [a2(675-686) of human collagenIV] induces HI or CMI depending on the MHC class II-phenotype of the mouse strain immunized. I-Ab mice mount a Th2-type response as assessed by the ability of purified CD4+ lymph node cells from draining lymph nodes in primed animals to induce class switch to IgG1 in antigen-specific B lymphocytes, and antigen-specific release of IL-5 and expression of IL-4 and IL-5, while priming of I-As mice induces antigen-specific Th1 lymphocytes,which we studied in their antigen-specific in vitro proliferation, as well as their $IFN\gamma$-release and expression.

To test our hypothesis that the density of the T cell receptor ligand I-A:peptide during priming determined the type of immune response observed, we measured the affinity of both I-A molecules for the wildtype petide.This measurements revealed that I-Ab has a 10000fold lower affinity for the peptide than I-As. To test whether this difference in affinity causes the difference in immune response observed, we generated single amino acid-substituted peptides which differ in their affinity of binding from the wildtype peptide. Using wildtype peptide-specific I-Ab and I-As restricted T cell hybrids we tested the ability of the mutated peptides to be recognised by the same TcR as the wildtype peptide in a cross-specific manner. One such mutated peptide which is bound with lower affinity than the wildtype peptide in I-As induces a Th2-like response, another one which is bound with increased affinity in I-Ab induces a Th1-like response, thereby reversing the original phenotype. Study of a whole panel of mutated peptides supports this data. Thus we conclude that the amount of peptide:MHC class II-complexes during the initial encounter with the antigen determine whether a cell-mediated or humoral immune response is induced.

C2-367 VASCULAR ASSOCIATED MOLECULES INVOLVED IN EARLY THYMOPOIESIS.

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The continual thymic atrophy occuring with age, induced by the production of sex hormones and resulting in decreased T cell immunity, is reversed by castration. Immunohistological staining of the thymus following castration revealed major upregulation of antigens secreted by the stromal components of the blood-thymus barrier. Of particular interest is the endothelial associated mAb MTS 15, which displayed a more diffuse and granular staining pattern, suggesting the presence of a secreted factor. Further investigation into the functional role of this molecule was examined by the addition of purified MTS 15 mAb to fetal liver reconstituted, 2-deoxy-guanosine treated fetal thymic organ culture (F.T.O.C.) and by injection into sublethally irradiated mice. Following irradiation the addition of purified MTS 15 mAb (600μg) at days 3, 4, 5 and 6 prevented thymic regeneration at day 7, also preventing the remaining radioresistant thymic precursors from undergoing differentiation. Addition of purified MTS 15 (200μg/mL) to 2-deoxy-guanosine treated F.T.O.C. lobes reduced cell counts at day 18 and also prevented thymocyte differentiation. These results suggest the antigen detected by the MTS 15 mAb may be a stimulatory factor involved in the reactivation of T cell differentiation in the adult, having possible applications in immune enhancement in immunodeficiency states, as well as being required for normal thymopoiesis in the embryo.

C2-368 TYPE OF IMMUNOGLOBULIN JUNCTION PREDICTS CLONAL DEVELOPMENT OF B-1 CELLS,

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Predominant use of overlap sequences in the immunoglobulin heavy chain (IgH) CDR3 junctional region have been reported in fetal derived murine B cells. IgH sequences were determined for several independent NZB B-1 (CD5+) malignant clones and it was found that the majority of these sequences recombined using junctional homology. In order to determine if the type of IgH junction might be useful to predict the development of B-1 malignant clones, NZB, DBA/2, (NZB x DBA/2)F1 and F1 backcrossed to NZB animals were studied for the proportion of total IgM message which possessed junctional homology by a PCR based assay. Upstream primers were designed to detect immunoglobulin V_H gene families that were commonly observed to be involved in homology directed recombination in fetal and neonatal derived sequences. These degenerate upstream primers either include or exclude the overlap region of the V_H-D junction. The downstream primer took advantage of a single base-pair difference in the $C\mu$-C_H1 region responsible for allotypic variation between the NZB and DBA/2 strains. In all the Ig derived from NZB and DBA/2 neonatal animal sequences, homology directed recombination was the predominant mechanism used. As the DBA/2 strain aged, the proportion of IgM message which could be amplified by primers requiring overlap regions decreased. In contrast, the NZB mice continued to demonstrate an increase in Ig message in B cells from either spleen or PWC whose CDR3 possessed junctional regions encoded by either of the recombined genes. The pathology of backcrossed animals was correlated with usage of overlap regions in their immunoglobulin message. Most of the backcrossed animals which possessed B cell malignancies had IgM message of the NZB allotype and utilized homologous recombination in the CDR3 region. Allotype heterozygous backcross animals had a very low proportion of Ig message able to be primed with the upstream primer requiring overlap at the V_H-D junction. PCR analysis of the immunoglobulin of B cells derived from NZB and its limited outbreds show that this method can be used to predict a premalignant state in the animals as well as follow the progression of the disease. (NIH AI-29740)

C2-369 TSA-1/Sca-2 Regulates The Expression of RAG-1 and Hence The Development of $CD4^+CD8^+$ Thymocytes.

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Within the thymus, TSA-1/Sca-2 is present on immature thymocytes and isolated medullary epithelial cells. To examine the functional significance of TSA-1/Sca-2 in thymopoiesis, anti-TSA-1/Sca-2 (MTS 35) was added to both E14 fetal thymic organ culture, and to 2-deoxyguanosine-treated fetal thymic lobes that were reconstituted with fetal liver cells. In both instances MTS 35 markedly reduced total cell number and inhibited mainstream thymocyte differentiation beyond the $CD3^-CD4^-CD8^-$ triple negative (TN) stage. Such inhibition was also seen when E15 fetal thymocytes were prevented from "spontaneously differentiating" into $CD4^+CD8^+$ (DP) thymocytes during overnight culture with MTS 35. Examination of TN thymocytes with respect to the markers CD44 and CD25, revealed that TSA-1/Sca-2 was essential for the self renewal, proliferation and differentiation of $CD44^+CD25^-$ cells. Furthermore, analysis of RAG-1 and TcRβ chain mRNA expression revealed that the blocking of TSA-1/Sca-2 by MTS 35 also prevented the mRNA transcription of RAG-1 and the TcRβ chain. Thus, TSA-1/Sca-2 regulates DP thymocytes development by inducing RAG-1 mRNA transcritption and hence TcRβ chain surface expression.

C2-370 GERMLINE INACTIVATION OF THE MURINE SOX-4 GENE CAUSES IMPAIRED T CELL DEVELOPMENT,

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The Sox gene family is a recently described group of DNA binding factors. The prototype gene of this family is the SRY gene, responsible for sex determination in mammals. One other member, Sox-4, has been cloned and characterized in our lab. In adult mice it is expressed in thymus and the gonads. Early B and T cell lines also express the Sox-4 gene. During embryogenesis it is expressed in various tissues, most prominently in neural tissue. Two independent mouse strains were produced with mutations in the reading frame of Sox-4. Although heterozygous mice are viable and healthy, no mice were born homozygous for the mutation. They appear to die in utero around day 12-14. Mutant embryos develop edema and have circulatory problems. Blood smears of fetal livers revealed no qualitative differences in hemopoiesis. Also small numbers of $B220^+$ cells were detected suggesting the presence of pre-B cells. To study development of T cells in the absence of Sox-4, fetal thymus organ cultures were started with day 13 thymi. Differentiation of thymocytes was inhibited, because only very low numbers of $CD4^+$ and $CD8^+$ cells were detected. TcRαß expressing cells were fewer in number as well. Independently, fetal liver cells introduced in fetal thymi of $RAG\text{-}1^{-/-}$ mice, also showed an impaired maturation, indicating that the mutation of the Sox-4 gene affects thymocytes directly.

C2-371 THE USE OF ARBITRARILY PRIMED PCR TO IDENTIFY GENES INVOLVED IN THYMOCYTE MATURATION,

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During embryogenesis, thymocytes follow an ordered developmental program which produces mature T-cells capable of recognizing foreign peptide antigens in the context of MHC molecules. We have used arbitrarily primed PCR to identify genes which contribute to this development. Two strains of mice bearing a transgenic T-cell receptor specific for cytochrome c were used for this study. Thymi of mice homozygous for $H\text{-}2^b$ (AND.B6) support complete maturation of transgenic thymocytes. On the other hand, thymocytes developing on an $H\text{-}2^{h4}$ (AND.4R) background undergo minimal development beyond the double positive ($CD4^+8^+$) stage. We have identified approximately 150 gene fragments which appear to be differentially expressed by these two mouse strains. Of 25 fragments which have been cloned and characterized, twelve represent unknown genes. One of these fragments (td21) has been selected for further analysis. A comparison of td 21 mRNA expression by thymocytes from both strains reveals substantially higher expression levels by the AND.B6 thymocytes, while AND.4R thymocytes express low but detectable levels as measured by RT-PCR. We are currently investigating what role td21 has in thymocyte development by monitoring the kinetics of its expression during embryogenesis.

C2-372 GENERATION AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES TO THE HUMAN THYROTROPIN (TSH) RECEPTOR: ANTIBODIES THAT BIND TO DISCRETE CONFORMATIONAL OR LINEAR EPITOPES CAN BLOCK TSH BINDING, Gattadahalli S. Seetharamaiah, and Bellur S. Prabhakar. Department of Microbiology and Immunology, University of Texas Medical Branch, Galveston, TX 77555-1019.

Splenocytes from female BALB/c mice immunized with a recombinant extracellular domain of the human thyrotropin receptor (ETSHR) were used to generate a panel of 23 hybridomas that produce TSHR-specific monoclonal antibodies (mAbs). All mAbs were of the IgG isotype and belonged to different subclasses including IgG1, IgG2a and IgG2b. The antibodies bound to the ETSHR with relatively high affinity and several of them blocked the binding of ^{125}I-TSH to the TSHR, with some showing better blocking than others. Competitive binding studies with a subgroup of six biotinylated mAbs showed at least 3 different binding specificities. To determine the TSHR epitopes to which these mAbs were binding, we tested them against 37 overlapping synthetic peptides which span the entire ETSHR. The mAb 47, which did not block TSH binding, bound to an epitope represented by amino acid (AA) residues 22-30. The mAb 28, which had a thyrotropin binding inhibitory index (TBII) of 20%, bound to an epitope represented by AA 32-41. However, mAbs 37 and 49, with TBII's of 39% and 43% respectively, showed no significant reactivity with any of the peptides, suggesting that they react with a conformational epitope. Together, these studies showed that mAbs, with discrete binding specificities, can interact with either linear or conformational epitopes and block TSH binding.

C2-373 INTERMEDIATES IN THYMIC POSITIVE SELECTION: $CD4^+8^{int}3^+$ CELLS GIVE RISE TO $CD4^-8^+3^+$ MATURE THYMOCYTES, Ken Shortman, William Heath, Frank Köntgen, Frank Carbone, Rebecca Kydd and Katarina Lundberg, The Walter and Eliza Hall Institute of Medical Research, Melbourne, Victoria 3050 Australia

Insight into the mechanism of positive selection can be obtained by the study of small subpopulations of thymocytes representing intermediates between $CD4^+8^+$ cortical thymocytes and $CD4^-8^+$ and $CD4^+8^-$ mature thymocytes. We have isolated thymocytes which have elevated levels of CD3 and which are $CD4^+8^+$, $CD4^+8^{int}$ or $CD4^{int}8^+$. The progeny of these cells has been determined, either after intrathymic transfer into normal congenic recipients differing at the Ly 5 locus, or after culture of cells expressing a bcl-2 transgene to preserve viability. In culture only cells which were $CD3^{hi}$ matured further and all (including $CD4^+8^{int}$) gave predominantly $CD4^-8^+3^{hi}$ progeny. On intrathymic transfer, cells which were $CD3^{int}$ also matured further; $CD4^{int}8^+$ produced only CD^-8^+ progeny whereas $CD4^+8^{int}$ produced both $CD4^+8^-$ and $CD4^-8^+$ progeny. When the $CD4^+8^{int}3^{int}$ population was isolated from mice with a class I MHC-restricted TCR transgene, or mice lacking class II MHC, the only mature progeny were $CD4^-8^+$ cells. This study delineates a new and unexpected pathway for the development of $CD4^-8^+$ lineage T cells. It also negates one of the arguments for the stochastic-selective model of positive selection.

C2-374 *Abstract Withdrawn*

C2-375 HUMAN T CELL DEVELOPMENT IN REAGGREGATE CULTURES OF DISPERSED FETAL THYMIC STROMAL CELLS, Stephen B. Sinclair and Jonathan Sprent, Department of Immunology, The Scripps Research Institute, La Jolla, CA 92037

The evidence that T cell differentiation involves positive and negative selection derives almost entirely from studies with mice. We hypothesize that human T cells undergo similar mechanisms of intrathymic repertoire selection, however direct evidence for this view is lacking. Using a reaggregate fetal thymic organ culture system modified from that described by Jenkinson et al, we have studied the development of purified $TCR^{lo}to^-$ $CD4^+8^+$ thymocytes derived from either discarded human fetal or postnatal tissues. Purification requirements of thymic epithelial cells are significantly more stringent than in the murine system due to the early (1st trimester) colonization of the thymus with hematopoietic cells in humans compared to the 3rd trimester influx in the mouse. Preliminary experiments have been performed which demonstrate progressive differentiation of $CD4^+8^+$ "double positive" (DP) thymocytes into "single positive" (SP) cells during five to eleven day cultures. CD4-SP cells appear earlier than CD8-SP cells. Differentiation is preceded by upregulation of T cell receptor (TCR) levels, and CD4-SP cells express higher levels than CD8-SP cells. Ongoing experiments seek to determine the critical changes in surface markers such as CD69 ,IL-2R, and MHC class II associated with human T cells undergoing positive and negative selection, and to determine by BrdU incorporation whether thymocyte selection involves cell division in man. This work has been supported by the Medical Research Council of Canada.

C2-376 GAD-SPECIFIC CTL IN INSULIN-DEPENDENT DIABETES MELLITUS, Francesco Sinigaglia, Peter Van Endert[1] and Paola Panina-Bordignon, Roche Milano Ricerche, via Olgettina 58, 20132 Milano, Italy and [1]INSERM U25, 161 rue de Sevres, 75743 Paris Cedex 15, France

Insulin-dependent diabetes mellitus (IDDM) is caused by pathological T-cell responses which lead to the destruction of beta cells in the pancreatic islets. Recent studies suggest that MHC class II restricted CD4+ T cells specific for peptides derived from glutamic acid decarboxylase 65K (GAD65) play a crucial role in the initial phase of IDDM in NOD mice. GAD-specific CD4+ T cells have also been observed in recent onset IDDM patients and in relatives of IDDM patients at risk to develop diabetes. In the present study we describe MHC class I (*HLA-A*0201*)-restricted cytotoxic T lymphocytes (CTL) specific for a GAD-derived peptide (GAD114-123) in subjects with recent onset IDDM and at high risk to develop IDDM, but they were not found in healthy individuals carrying the same restriction element. GAD114-123-specific CTL could recognize autologous target cells infected with a recombinant vaccinia virus expressing the GAD65 gene, indicating that GAD114-123 peptide is generated by the processing of the GAD protein, and represents a natural dominant epitope. In addition to their cytolytic potential, GAD-specific CTL produced IFN-γ in response to GAD114-123 peptide-pulsed, as well as to Vacc/hGAD infected, HLA-A*0201 expressing cells. This argues for a critical role of GAD-specific CTL in the initial events of IDDM. IFN-γ might have an important immunoregulatory role *in vivo* by promoting the development of autoreactive Th1-type cells able to induce IDDM in genetically susceptible individuals.

C2-377 EXPRESSION, PURIFICATION AND CRYSTALLISATION OF HLA B ALLELE SINGLE PEPTIDE COMPLEXES.
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Recent crystal structures of five HLA A2 single peptide complexes have allowed detailed analysis of HLA/peptide interactions and have given an insight into how a single HLA molecule interacts with multiple peptide epitopes. To analyse the structural changes which may occur when the same peptide epitope is presented by different HLA alleles, we have chosen to study the B51/B35 CREG family. In order to carry out such structural studies we have developed a system for the expression, purification and crystallisation of HLA B allele single peptide complexes. HLA B allele heavy chain constructs were expressed in E.coli using a T7 expression system and then isolated as insoluble inclusion bodies. Using a dilution method single peptide complexes were made by refolding denatured B allele heavy chain and β2m together with a single peptide. Single peptide complexes were then purified and shown to be recognised by the monoclonal antibody W6/32. To date, single HLABw53/peptide complexes have been crystallised and preliminary X-ray diffraction data has been collected. This system will allow for the first time detailed structural analysis of B allele single peptide interactions and thus facilitate comparison with the interactions seen in HLA A2 single peptide complexes. It is also a unique opportunity for analysis of the same peptide epitopes complexed with natural B allele variants. This will give information about how changes in conformation of either peptide or MHC enable T cells to distinguish between the same peptide presented in the context of different HLA alleles.

C2-378 CLONING OF MURINE *TXK*: A PROTEIN TYROSINE KINASE EXPRESSED EARLY IN FETAL THYMIC DEVELOPMENT, Connie L. Sommers, Kun Huang, Alexander Grinberg and Paul E. Love, Laboratory of Mammalian Genes and Development, National Institute of Child Health and Human Development, NIH, Bethesda, MD 20892

In an effort to clone protein tyrosine kinase genes expressed early in murine thymic development, we cloned the murine form of *txk*, a tyrosine kinase previously cloned from human cells by virtue of its homology to the B cell kinase, *btk*. *Txk* was expressed in thymocytes as early as fetal day 13.5 and its expression at the mRNA level increased throughout development. All thymocyte subsets as well as peripheral $CD4^+$ and $CD8^+$ T cells expressed *txk*. However, *txk* expression was not detected in non-hematopoietic fetal or adult tissues and was not expressed in B or *macrophage/monocyte* cell lines. Txk mRNA was down-regulated after treatment with PMA and ionomycin indicating that its expression was regulated by T cell activation. DNA sequence analysis showed high homology to human *txk* (83% at the amino acid level). In addition, *txk* was related to *tec*, *itk* and *btk* since it contained the consensus sequence R/KKPLPPL/TP in its N terminus. However, the remainder of the N terminus of *txk* (upstream of the SH3 domain) diverged from the N terminal regions of *tec*, *itk* and *btk*, possibly indicating a unique function for *txk* among these family members.

C2-379 AN IL-4 MUTANT PROTEIN INHIBITS IL-4-INDUCED GENERATION OF CD4+, CD45RA+ CORD BLOOD T CELLS INTO IL-4 AND IL-10 PRODUCING CELLS, Thierry Sornasse, Patricia V. Schneider, Jan E. de Vries, and Hans Yssel, DNAX Research Institute, Human Immunology Department, 901 California Avenue, Palo Alto, CA 94304.

It is generally accepted that IL-4 in the mouse, as well as in human, plays an important role in the differentiation of immunologically naive cells into IL-4 producing T cells belonging to the Th2 subset. Here, we show that purified CD4+, CD45RA+ cord blood T cells, repetitively activated with anti-CD3 mAb, crosslinked on CD32, CD80 and LFA-3-expressing L cells and cultured in the presence of IL-4, produced high levels of IL-4 and IL-10 and low levels of IFN-γ, following restimulation with anti-CD3 mAb, presented by the L cell transfectants. These IL-4 and IL-10-inducing effects were neutralized when the cultures were carried out in the presence of a 20 to 50-fold excess of the IL-4 mutant protein IL-4-Y124D, indicating that IL-4-Y124D acts as a powerful IL-4 receptor (R) antagonist in this culture system. IL-13 shares many of its biological activities with IL-4 and it has been demonstrated that IL-4-Y124D inhibits biological activities of both IL-4 and IL-13 which is consistent with the observation that the IL-4R and IL-13R share a common component required for signal transduction. Presently, we are determining whether IL-13, like IL-4, can induce the generation of IL-4 and IL-10 producing cells in our culture system. Whether IL-4-Y124D can antagonize these effects will be discussed.

C2-380 TCR ENGAGEMENT TRIGGERS IL-1 PRODUCTION BY TH1 AND TH2 CLONES. Risa M. Stack and Frank W. Fitch. The Committee on Developmental Biology, The Ben May Institute, and the Committee on Immunology, University of Chicago, Chicago, IL, 60637. In this study we demonstrate that resting B cells, T-depleted spleen cells, and an anti-CD3 mAb all stimulate high levels of IL-1α, and in some cases, IL-1β production by Th1 and Th2 clones. Furthermore, IL-1 production reaches its peak between 25 and 40 hours, when maximal levels of both IL-2 and IL-4 are also observed. To determine if IL-1 was involved in activation of Th clones we used a mAb that binds both type I and type II IL-1R to block IL-1 utilization. When clones were stimulated with either resting B cells or an anti-CD3 mAb, the IL-1R mAb inhibited proliferation of the Th2 clones, but not the Th1 clones. Interestingly, when we stimulated the clones with T-depleted spleen cells and blocked IL-1 utilization, proliferation of neither subtype was affected. Furthermore, CD28 was only involved in APC-mediated stimulation of Th1 clones; Th2 clones were unaffected by CTLA4Ig, even when IL-1 utilization was also blocked. Additionally, TCR engagement was required for both Th2 pathways, as neither IL-4 nor syngeneic APCs augmented proliferation to IL-4 alone. Thus, while Th1 clones produce IL-1 following TCR engagement, they do not utilize it to augment proliferation. On the other hand, Th2 clones use IL-1 in the absence of APCs, but in the presence of APCs may utilize another yet uncharacterized accessory pathway.

C2-381 SURFACE ENGAGEMENT OF TCRβ COMPLEX EXPRESSED ON THE TCRβ-TRANSGENIC *SCID* FETAL THYMOCYTES INHIBITS THE GENERATION OF CD4+8+ THYMOCYTES. Y. Takahama, K. Sugaya, S. Tsuda, Y. Hashimoto, Syntex Institute of Immunology, Niihari, Ibaraki 300-41, Japan

Transgenic expression of the β-chain of TCR is known to induce the generation of CD4+8+ thymocytes in the immunodeficient *scid* mouse, in which thymocyte development is otherwise arrested at CD4-8- cells. In the present study, we have examined how the engagement by antibody of the TCRβ complex expressed on the TCRβ-transgenic *scid* fetal thymocytes can regulate the generation of CD4+8+ thymocytes. Organ cultures of CD4-8- day 14 fetal thymocytes from the TCRβ-transgenic *scid* mice resulted in the generation of CD4-8+ and then CD4+8+ cells. The initial step from CD4-8- cells to CD4-8+ cells was enhanced by the addition of anti-TCRβ antibody, whereas the subsequent step from CD4-8+ cells to CD4+8+ cells was markedly inhibited by anti-TCRβ antibody. These results indicate that ligand engagement of the TCRβ complex can positively and negatively regulate the early thymocyte development. Moreover, the finding that the engagement of the TCRβ complex inhibits the generation of CD4+8+ cells suggests that the induction of CD4+8+ thymocytes by the TCRβ transgene is not an immediate consequence of cell surface engagement of the TCRβ complex.

C2-382 ANTIGEN LIGAND-MEDIATED GENERATION OF EFFECTOR CD4 T CELL RESPONSES, Xiang Tao and Kim Bottomly, Section of Immunobiology, Yale University School of Medicine, New Haven, CT 06510

Naive CD4 T cells can differentiate into distinct effector cell types upon antigen stimulation which are distinguished by the unique cytokines they secrete. The differentiation of naive T cells is influenced by a number of factors such as cytokine environment, type of antigen-presenting cells and structure or presentation of the antigenic peptide. To investigate how peptide antigens affect the generation of effector T cell responses, we created a panel of moth cytochrome c peptide variants and used them to prime in vitro T cells from mice transgenic for the moth cytochrome-c-specific T cell receptor (TcR). These variants contained single amino acid substitutions at positions known to be critical for binding to either TcR or MHC but not both, and therefore enabled us to differentiate the effects of a change in TcR-peptide interaction versus a change in peptide-MHC interaction on the priming of naive T cells. Peptides mutated at positions critical for TcR binding were poor stimulators of T cell proliferative responses. Nevertheless, these TcR variants were potent in priming naive CD4 T cells to become both IL-4-producing and IFNγ-producing effector T cells. In contrast, the wild-type peptide was a strong stimulator of T cell proliferative responses and always primed the T cells to become IFNγ-producing Th-1 type cells. Furthermore, one of the TcR variants with a substitution of arginine for lysine at position 99, was found to be able to serve as an antagonist in the CD4 T cell proliferative response, indicating that this variant can act as both an antagonist peptide in proliferative responses and an agonist peptide for effective priming of naive T cells to become IL-4-producing cells. The data suggest that a structural change of the ligand can alter qualitatively the signaling through TcR leading to a change of the outcome of CD4 T cell effector response. Experiments are currently being performed to study the effect of MHC variants on T cell priming as well as to discern whether the IL-4-producing cells and IFNγ-producing cells induced by TcR variants belong to a single population of T cells or a mixture of Th1 and Th2 cells.

C2-383 BYSTANDER PROLIFERATION OF CD8+ T CELLS IN VIVO, David F. Tough*, Persephone Borrow¶ and Jonathan Sprent*, *Department of Immunology and ¶Department of Neuropharmacology, Division of Virology, The Scripps Research Institute, La Jolla, CA 92037

Virus infection is known to result in the activation of large numbers of T cells. This appears to include not only virus-specific T cells but also cells having other antigenic specificities. While it has been postulated that this "bystander" proliferation may have an important immunological function (eg. with regard to the maintenance of immune memory), the phenotype of the proliferating cells and the mechanisms by which they are induced to proliferate are not well understood. To investigate these issues we examined the proliferative response of T cells after infection of normal mice with lymphocytic choriomeningitis virus (LCMV). In addition, to assess whether virally-induced cytokines may play a role in the T cell proliferation, we examined the effect of injection of α/β interferon-inducing polyinosinic-polycytidylic acid (poly-IC). Proliferating cells were detected by following bromodeoxyuridine (BrdU) incorporation in vivo. Similarities were observed between the effects of LCMV infection and poly-IC injection. Both treatments induced CD8+ T cell proliferation which was most prominent among $CD44^{hi}$ memory-phenotype cells. Also, both induced rapid upregulation of Ly-6C, a GPI-linked surface molecule. Since antibodies to Ly-6C have been shown to induce T cell proliferation in vitro, it was possible that this molecule may also be playing a role in vivo. However, the increased proliferation appeared to be largely independent of Ly-6C, since it was also observed following poly-IC injection into $Ly\text{-}6C^-$ strains. Experiments are in progress using TCR-transgenic and MHC-deficient mice to determine whether TCR ligation is necessary for "bystander" proliferation.

C2-384 INFLUENCE OF THYMIC-DERIVED STEROIDS ON POSITIVE SELECTION OF THYMOCYTES.

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Occupancy of either the T cell receptor (TCR) or the glucocorticoid receptor (GR) is a potent means of inducing programmed cell death (PCD) in thymocytes. However, glucocorticoids have been shown to antagonize induction of TCR-mediated PCD in T cell hybridomas and thymocytes *in vitro* , which has led to the proposal that exposure of thymocytes to both of these stimuli during thymocyte differentiation may be a basis for positive selection. Although high avidity TCR interactions may induce a signal too potent to be antagonized, low-moderate avidity TCR interactions may transduce a weaker signal that can be antagonzied by occupancy of the GR and allow positive selection to occur. We investigated the role of endogenously-synthesized steroids during thymocyte development. First, we have demonstrated that enzymes required for glucocorticoid synthesis are present in a subset of thymic antigen-presenting cells and that detectable levels of steroids are synthesized *in vitro*. Next, addition of the glucocorticoid synthesis inhibitor metyrapone to fetal thymic organ cultures (FTOC) resulted in a 23% decrease in double positive (DP) thymocytes that was reversible by addition of 10^{-9} M corticosterone. FTOC with H-Y-specific TCR transgenic mice revealed that absence of steroids in transgenic female thymi resulted in a 60% decrease in DP cells when the selecting MHC phenotype ($H\text{-}2^b$) was expressed. However, in transgenic female thymi expressing a nonselecting MHC phenotype, recovery of DP thymocytes was not significantly different from transgene negative littermates. These results suggest that in the absence of glucocorticoids thymocytes that would normally undergo positive selection now become negatively selected.

C2-385 HETEROGENEITY OF AUTOREACTIVE T CELL CLONES SPECIFIC FOR THE E2 COMPONENT OF THE PYRUVATE DEHYDROGENASE COMPLEX IN PRIMARY BILIARY CIRRHOSIS, Judy Van de Water, Aftab Ansari, Thomas Prindiville, Nancy Ricalton, Brian L. Kotzin, Shengjiang Liu, Thomas E. Roche, and M. Eric Gershwin, Division of Rheumatology and Allergy, University of California, Davis, CA 95616

Primary biliary cirrhosis (PBC) is an autoimmune liver disease characterized by periportal inflammation. The dominant humoral response in patients with PBC is directed against the E2 component of the mitochondrial enzyme, pyruvate dehydrogenase (PDC-E2). We examined PBMC from patients with PBC for reactivity to PDC, PDC-E1 and PDC-E2. We also studied the phenotype, lymphokine profile and Vβ usage of PDC-specific T cell clones isolated from the liver of PBC patients. We found that PBMC of 16/19 patients responded to the inner and/or outer lipoyl domains of PDC-E2 in contrast to serologic observations. The liver derived antigen-specific T cell clones responded with a higher incidence to the subunits of PDC-E2 versus the entire PDC-E2 molecule. They also produced IL-2, IL-4 and IFNγ suggesting the presence of both Th1 and Th2-like clones, and showed considerable heterogeneity in their TCR Vβ usage. Our data suggests that the response of T cells isolated from the liver is directed against at least two distinct epitopes on the PDC-E2 molecule and differs from the peripheral response.

C2-386 CHANGES IN THE THREE DIMENSIONAL ARCHITECTURE OF THYMIC MICROENVIRONMENTS DURING POSITIVE AND NEGATIVE SELECTION

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Stromal cells play a regulatory role during T cell development in the thymus. In turn, recently published evidence indicates that developing T cells themselves influence the architecture of thymic stromal cells; a phenomenon known as thymic "cross-talk". We have investigated the three dimensional architecture of thymic stromal cells under condition of positive and negative selection (HY transgenic male and female mice), using scanning electronmicroscopy.

Our data indicate that under conditions of positive selection (female HY transgenic mice) the thymic stroma develops in a spongelike network, showing multiple lymphostromal interaction "niches". In contrast, the thymic stroma in the thymus of male mice shows rounding-up of stromal cells, a loss of the spongelike reticulum structure and the generation of multiple lymphocyte "reservoirs".

C2-387 **PRE-T-1: A CO-ACTIVATION MOLECULE SPECIFIC TO MOUSE Pre-T CELLS.** **Alain Vicari, Andrew G. D. Bean, and Albert Zlotnik. DNAX Research Institute, Palo Alto, CA 94304.**

In the thymus, early T cell development is primarily characterized by the onset of T Cell Receptor (TCR) gene rearrangements. In the mouse, the earliest population where the completion of β and γ gene rearrangements is found is the pre-T cell subset, which is defined by its $CD4^-CD8^-CD44^-CD25^+$ phenotype. These cells also express very low but functional levels of CD3ε. An initial and still unclear selection process, named β selection, occurs at this stage. It preferentially allows a proportion of T cells which have completed in-frame β-TCR rearrangements to greatly expand and pursue the T cell differentiation pathway. In order to further analyze the pre-T cell subset, we searched for unknown surface molecules expressed by these cells. We reportt here a monoclonal antibody, IF-7, which reacts with an antigen PRE-T-1, exclusively expressed by pre-T cells and some components of the thymic stroma. In vitro, we found that IF-7 could co-activate the proliferation of pre-T cells together with anti-CD3ε antibody. Furthermore, addition of IF-7 to fetal thymic organ culture accelerated T cell differentiation. These results suggest that PRE-T-1 may play a unique and specific role in the expansion of the pre-T cell subset. In order to clone the gene encoding for PRE-T-1 for further analysis of its molecular nature and functions, we have recently generated a cDNA library from immature thymocytes of RAG-1 deficient mice.

C2-388 CYTOTOXIC CD8+ T LYMPHOCYTES SPECIFIC FOR TCR Vβ FAMILIES EXPRESSED ON AUTOLOGOUS CD4+ T CELLS

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We have demonstrated here the existence of CD8+ T cells among human peripheral lymphocytes that can interact directly with autologous CD4+ T cells based on CD4+ T cell receptor β variable chain usage. This was accomplished by raising lines and clones of CD8+ T cells to autologous Vβ2+/CD4+ clones and examining the specificity of their interaction with other autologous CD4+ cells. First, we showed that CD8+ T cell clones induced by autologous CD4+/Vβ2 clones are cytotoxic in a standard chromium release assay to a variety of autologous, independently isolated Vβ2+/CD4+ clones but not to autologous Vβ2-/CD4+ clones. This specificity of interaction was also detected independently by the induction of IL-2 receptor expression on CD8 clones. Antigen carryover on CD4+ targets was ruled out as a mechanism of specificity. Secondly, we showed that CD8+ lines raised against Vβ2+/CD4+ clones are cytotoxic to other autologous Vβ2+/CD4+ lines and clones but not to Vβ2-/CD4+ targets. Third, we demonstrated that a polyclonal Vβ2+ line of CD4+ cells is an effective cold-target inhibitor of the cytotoxicity of this line to the Vβ2+ clone used for its induction. Finally, we provided evidence that this interaction is unaffected by W6/32, a monoclonal antibody which binds to non-polymorphic determinants involving human HLA A/B/C molecules and β2 microglobulin. Of potential significance for the in vivo relevance of these observations, the cytotoxic interactions described have been found to be highly dependent on the state of activation of the target CD4+ cells. Recent experiments in several mammalian systems have suggested that immune regulation can be mediated in part by CD8+ cells specific for a population of CD4+ cells distinguished by TCR Vβ usage. Our cytotoxic data showing direct interaction in vitro between clones of CD8+ and CD4+ cells based on the TCR Vβ usage of the CD4+ cells provides a possible mechanism for these observations.

C2-389 IDENTIFICATION OF A BIDIRECTIONAL PROMOTER IN THE FIRST INTRON OF THE MURINE T CELL RECEPTOR Vα1, David R. Webb, Toshiro Maeda, Satoshi Fukuse, and Bruce H. Devens, Institute of Immunology, Syntex Discovery Research, Palo Alto, CA 94303

The regulation of the TCRα chain gene has taken on additional interest with the discovery that these genes do not exhibit allelic exclusion. We now report the existence of an additional regulatory element located in the introns of murine Vα. In Vα1 this promoter, which resembles a serum response element, exhibits bidirectional promoter activity that is not T-cell specific. Upstream of the promoter in the antisense strand, an open reading frame has been found that could code for a small molecular weight protein that contains a proline-rich region and a tyrosine-isoleucine motif that has homology to Ig-β(the B29 gene product). By primer extension analysis and analysis of cDNA, we have detected poly A+ RNAs of appropriate size in both the sense and anti-sense direction in both T and B-cell lines. In addition, the introns from Vα3 and VαB6.2.16 were examined and found to contain promoter elements that operate in the sense direction only. Thus the Vα1 intronic promoter is unique, regulating the expression of a putative gene in the antisense direction and a putative truncated Vα1 protein.

C2-390 CD8 β AND TCR β FORM A NOVEL DISULFIDE-LINKED COMPLEX, Christopher J. Wheeler, Angela L. Lee, and Jane R. Parnes, Dept. of Medicine, Division of Immunology & Rheumatology
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CD8β is a surface glycoprotein which is normally only found coexpressed with and disulfide-linked to CD8α on mature T cells, transfectants, and thymocytes. Anti-CD8β mAb specifically recognizes a 32kD protein (reduced) on transfectants of the T cell hybridoma HTB-157.7 which express CD8αβ or chimeric CD8β-α molecules (extracellular CD8β, transmembrane and cytoplasmic CD8α). Two CD8β (wild-type cDNA) transfectants of HTB-157.7 that do not express CD8α (4βC5 and 5βB1) are also recognized by anti-CD8β. Analysis by non-reducing/reducing two dimensional gel electrophoresis of CD8β immunoprecipitates from 4βC5 and 5βB1, but not from untransfected HTB-157.7 reveals a 75-80 kD heteromeric complex consisting of 32kD CD8β disulfide-linked to a second subunit running close to the diagonal, as well as homodimers of CD8β. Western blotting indicates that the larger subunit of the heteromer is recognized by anti-TCRβ mAb. FACS analysis with anti-CD8β also reveals a population of $CD8\alpha^{neg/low}CD8\beta^{high}$ cells in normal adult thymus, which is more pronounced in day 14 and 15 fetal thymus, prior to the onset of TCRα rearrangement. These cells coexpress CD3ε and TCRβ, and are IL-$2R^{med}$, $CD44^{-}$, and $CD4^{-}$. Thus, CD8β and TCRβ are expressed as a disulfide-linked heteromeric complex on HTB-157.7 transfectants, and are coexpressed on early thymocytes in the absence of both CD8α and TCRα.

C2-391 BINDING OF CD23 TO A $CD21^{-}$ HUMAN PRE-B ACUTE LYMPHOCYTIC LEUKAEMIA CELL LINE.

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CD23 is a 45kDa antigen expressed at the surface of many haematopoietic cells. CD23 behaves as a low affinity receptor for IgE, and recent data have illustrated that CD21 is a counter-structure for full length CD23. However, the expression of CD21 is restricted, both in terms of tissue distribution and in the context of B lymphocyte development, raising the possibility that other CD23 counter-structures may exist. Studies of the influence of recombinant cytokines upon growth of a human pre-B acute lymphocytic leukaemia cell line, SMS-SB, indicate that 25kDa soluble CD23 may enhance the growth of the cells. While cytokines such as IL-4 had pronounced effects upon cell volume and morphology, none mediated a significant growth promoting effect upon SMS-SB cells. By flow cytometry, Northern blot and PCR analyses SMS-SB cells were negative for CD23 and CD21 expression. Moreover, flow cytometric and confocal microscopic studies using liposomes containing full length CD23 indicated that SMS-SB cells possessed a counter-structure for CD23. These data are consistent with the hypothesis that SMS-SB cells possess a receptor for CD23 other than CD21. Studies are in progress to elucidate the identity of this putative CD23 counter-structure.

C2-392 IMMUNOLOGICAL ABNORMALITIES ASSOCIATED WITH DOWN SYNDROME AND TRISOMY 16 MICE:- POTENTIAL ROLE FOR THE PROTOONCOGENE ETS-2.

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Down Syndrome occurs as a result of trisomy of chromosome 21 in approximately 95% of effected individuals. Trisomy 16 mice appear to be an excellent model for Down Syndrome since these embryos spontaneously develop immunological, cardiovascular, neuronal and haematological abnormalities similar to individuals with Down Syndrome. Furthermore, a large proportion of genes on the long arm of human chromosome 21 map to mouse chromosome 16. These data suggest that the genes involved in the development of Down Syndrome are highly conserved between human chromosome 21 and mouse chromosome 16. One such gene, the protooncogene Ets-2, appears likely to contribute to aspects of the Down Syndrome and Trisomy 16 phenotype since it is overexpressed in Down Syndrome, widely expressed during embryonic development and, as a transcription factor, could potentially alter the expression of many genes independent of chromosomal location.

Therefore, we have produced transgenic mice overexpressing Ets-2 to examine both the normal biological function of this transcription factor and its potential contribution to Down Syndrome. These mice display a variety of defects in common with Down Syndrome and Trisomy 16 mice including thymic hypoplasia and altered numbers of peripheral lymphocytes, altered calvaria development and cervical spine instability. More detailed analysis of thymuses from these mice has demonstrated decreased numbers of cortical CD4+CD8+ thymocytes and altered proportions of CD4-CD8-, CD4+CD8- and CD4-CD8+ thymocyte subsets, resulting in a lower CD4:CD8 ratio similar to that observed in Trisomy 16 mice. Furthermore these mice were shown to have decreased numbers of peripheral lymphocytes and alterations in CD4:CD8 ratio similar to that observed in individuals with Down Syndrome.

Thus, Ets-2 is likely to have a role in normal thymic development and potentially is responsible for the immunological abnormalities observed in individuals with Down Syndrome.

C2-393 EVALUATION OF THE ROLE OF HYDROPHOBICITY IN THE MASS SPECTROMETRY OF MHC PEPTIDES,

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Recent advances have been made in identification of specific gene products that represent tumor antigens. These antigens are peptide fragments of cytoplasmic proteins that are expressed bound to Class I MHC. In the case of human melanoma , the peptides were identified and sequenced by mass spectrometry. Our group has been utilizing MALDI in combination with exopeptidase digests as a method for sequencing MHC Class I antigens. Some modifications of this approach have resulted in important visualization thereby allowing sequencing of the isolated peptides. In particular, we took advantage of the hydrophobicity of peptides to improve their detection. To assess the role of hydrophobicity, an equimolar solution of 4 synthetic MHC class I and II peptides (1 pmol/ul) of various hydrophobicity, as calculated by the Bull and Breese index [BBI], were used. Spectra of the mixture showed peptide #2 (TYQTRALV BBI -290) to have the highest relative intensity (RI)(100%), followed by #1 (FERFEIPKE BBI -3500) (59.2%), and #3 (SINFEKL [BBI= -3790]) (8.9%), #4 (IYSTVASSL BBI -3120) was totally suppressed. However if ammonium citrate buffer is added to the mixture, the RI of #2 (100%), #1 (96.3%) is almost identical, while #3 (23.0%) is increased and #4 is now visible (14.1%). The peptides that desorbed best were not the most hydrophobic, but rather the ones most easily protonated at pH 2.0. The ammonium citrate buffer, raises the pH to 5.0, making the solution less acidic, thus giving a better chance for peptides that are not easily protonated to desorb. Other buffers changed the desorption and suppression pattern of the mixture. The identification of antigenic sequences will allow the study of novel peptide-based tumor vaccine strategies.

C2-394 V_H12-EXPRESSING PRE-B CELLS ARE LIGAND-SELECTED BASED ON THEIR V_HCDR3 STRUCTURES.

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Most CDR3 sequences of productive (P) V_H12 rearrangements in the peritoneum and spleens of adult mice are 10 amino acids in length and have a Gly in the fourth position (designated 10/G4). Moreover, the majority of other P V_H12 rearrangements (non-10/G4) are absent from these tissues. To determine the basis for this biased CDR3 repertoire, we have examined the sequences of V_H12-D-J_H1 rearrangements in bone marrow (BM) pre-B cells (sorted as $B220^+/IgM^-$) of normal mice, and in BM pre-B cells from μ "knockout" mice (μMT) that cannot express a μ/surrogate L chain receptor. We find that the V_H12 CDR3 repertoire is diverse in pre-B cells of μMT mice, but is biased in favor of 10/G4 sequences in pre-B cells of normal mice. In addition, the ratio of P and non-P rearrangements indicates that there are ~15-fold fewer P rearrangements in normal mice than expected from the same analysis of μMT mice. Thus, there is both a loss of V_H12-expressing pre-B cells and a selection of V_HCDR3 during the transition from pre-BI to pre-BII. Since surface expression of the μ/surrogate L chain receptors is required for this transition, we tested whether non-10/G4 V_H12 μ chains can associate with surrogate light chains and be expressed on the cell surface. Expression vectors encoding either a 10/G4 or a non-10/G4 μ chain were transfected into a μ chain loss variant of the pre-B cell line Bine 4.8. We find by FACScan analysis that both 10/G4 and non-10/G4 μ chains are expressed equivalently on the surface. Immunoprecipitation experiments to verify surrogate L chain association are in progress. These data indicate that expression of a μ/surrogate L chain receptor is not by itself sufficient for survival of pre-B cells, and that ligand binding by this receptor is dependent on the structure of the V_H region.

C2-395 PRESENCE OF SHORT HOMOLOGY REPEATS AND LACK OF TDT ACTIVITY: TWO CRITICAL FACTORS CONTRIBUTE TO THE GENERATION OF INVARIANT γδ T CELL RECEPTORS IN FETAL THYMUS,

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Fetal and adult $V\gamma3^+$ and $V\gamma4^+$ T cells contain antigen receptors with very limited repertoire diversity. Majorities of these T cells express identical junctional sequences, referred to as canonical junctions. It is not clear how these γδ T cells are generated; but it seems that cellular selections are not required for shaping the repertoire during fetal thymic development. We examined the function of AT short homologies at the Vγ3 and Jγ1 junctional breakpoint in directing the TCR rearrangement. A series of transgenic mice were generated with Vγ4/Vγ3 rearrangement substrates containing mutations /substitutions at Vγ3 gene junctional breakpoint to destroy or replace the short homology repeats. A point mutation that destroys the AT direct repeat totally eliminates the appearance of transgenic Vγ3-Jγ1 canonical sequence. The addition of a new Vγ4-like ATA direct repeat at the Vγ3-Jγ1 junction generated a Vγ4-like canonical sequence. These data indicate that AT rich short homologies are both required and sufficient for directing γ gene rearrangement. The function of terminal deoxynucleotidyl transferase(TdT) during γδ T cell development was explored by generating mice carrying a TdT expressing transgene. Analysis of the Vγ3-Jγ1 junctional sequences in the TdT transgenic fetal thymocytes indicates that the junctional diversity is increased in these mice due to the frequent N-nucleotide insertion. Our results show that short sequence homologies play an critical role in the generation of γδ T cells bearing invariant repertoire. Other factors, such as the lack of TdT activity in the $V\gamma3^+$ and $V\gamma4^+$ T cells, further tighten the restriction on repertoire diversity.

Immunity to Viral Infections and Loss of Self Tolerance in Autoimmunity

C2-400 P18-SPECIFIC, HLA-A2 RESTRICTED AND H-2D^d RESTRICTED CTL SHARE A COMMON MINIMAL EPITOPE WHICH UTILIZES SIMILAR RESIDUES FOR MHC BINDING, Martha A. Alexander-Miller *, Kenneth C. Parker#, Taku Tsukui*, C. David Pendleton*, John E. Coligan# and Jay A. Berzofsky*, *Metabolism Branch, NCI, NIH, #Laboratory of Molecular Structure, NIAID, NIH, Bethesda, MD 20892

In previous studies from this lab, the P18$_{315-329}$(RIQRGPGRAFVTIGK) peptide was shown to be the immunodominant epitope from gp120 in BALB/c (H-2^d) mice. P18-specific CTL have also been observed in unstimulated PBMC from HLA-A2$^+$, HIV seropositive donors. Thus this peptide appears to be highly immunogenic in both mouse and human responses to gp120. To precisely define the presentation and recognition of this peptide in the context of the HLA-A2 molecule, P18-specific CTL were generated by in vitro stimulation of PBMC from an HLA-A2$^+$, HIV seronegative donor. The minimal epitope recognized by these CTL was identified using a panel of overlapping 10mers and 9mers from the P18 peptide. Of these, I10 (318-327) was the only peptide that sensitized target cells for lysis. The two 9mers contained within I10 were not recognized. Interestingly, in an earlier study from this lab I10 was also identified as the minimal epitope recognized by murine P18-specific, D^d-restricted CTL as well as by CTL from 3 other strains of mice, H-2^p, H-2^u, and H-2^q. Thus the same peptide binds at least five class I molecules. These results suggest that this peptide may possess structural features which confer promiscuous class I binding. P18 does not have a classic A2-binding motif. To ascertain the residues involved in HLA-A2 binding, a panel of alanine-substituted peptides was utilized for binding and functional studies. Residues 320, 324, and to a lesser extent 327 were found to be involved in binding to the HLA-A2 molecule. Residues 320, 324, and 327 are also involved in I10 binding to D^d suggesting these two molecules may bind I10 in a similar manner. The one difference found in I10 binding to these two molecules is at position 322 which is agretopic for D^d and epitopic for A2. The similarities in minimal epitope recognition and MHC binding between the mouse and human CTL lines and class I molecules, respectively, suggest that understanding the biochemical basis for the promiscuous binding of this peptide may contribute to the rational design of more effective peptide vaccines.

C2-401 ACCELERATION OF DIABETES IN THE NOD MOUSE BY IN SITU IL-2, Janette Allison, Peter McClive, Alan Baxter*, Grant Morahan and Jacques Miller, The Walter and Eliza Hall Institute of Medical Research, PO Royal Melbourne Hospital, Parkville 3050, Australia and *Centenary Institute for Cancer and Medicine and Cell Biology, University of Sydney, Australia

Transgenic mice that were engineered to express IL-2 in their islet β cells developed a massive inflammatory infiltrate localized to the islets. Mice with a single copy of the transgene did not become diabetic, those with two copies did develop diabetes and those with higher levels of β cell IL-2 failed to survive beyond 15 days of age because of inflammatory destruction of the exocrine pancreas. When the single copy transgene was introduced into a low diabetes incidence NOD strain, it dramatically accelerated diabetes onset. By doing genetic backcrosses to C57BL/6 it was found that the NOD Idd-1 and Idd-3/10 diabetes susceptibility loci were essential for the acute onset. If mice lacked CD8$^+$ T the acute effect was seriously diminished although animals did eventually progress to diabetes. Islet specific T cells could not, however, be detected in the circulation of diabetic mice that were grafted with syngeneic islet grafts not expressing IL-2. It was possible, however, that islet specific CD8$^+$ T cells were present, but they could not respond to islet grafts in the absence of a local source of IL-2. It appeared from these studies that much of the damage to the islets was mediated by the non-specific inflammatory consequence of local IL-2 production that was potentiated in the NOD genetic background. Experiments are underway to see if the accelerating effect of CD8$^+$ T cells was related to antigen specific or non-specific effects.

C2-402 **CLONAL EXPANSION OF AUTOREACTIVE Vα12$^+$ CD8$^+$ T CELLS IN RHEUMATOID ARTHRITIS.**

Samuel M. Behar, Karen Taraszka, Chris Roy, and Michael B. Brenner, Dept. of Rheum. & Immunol., Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115.

The detection of clonal expansions of T cells, biases in the T cell receptor (TCR) repertoire, and MHC associations in patients with rheumatoid arthritis are evidence that antigen specific T cells play a pivotal role in the pathogenesis of RA. We previously published that 15-20% of RA patients have clonal or oligoclonal expansions of peripheral blood CD8+ T cells expressing TCRs encoded by the Vα12 gene, and 88% of these patients were HLA-DQ2+. Since an antigen driven T cell response is the most likely explanation for the clonal expansion of these cells, our hypothesis is that these cells may be chronically stimulated by an autoantigen or persistent foreign antigen located in the synovium. We isolated Vα12+ CD8+ T cells from an RA patient by two color cell sorting using anti-TCR mAbs that react with Vα12 and Vβ5.1, followed by limiting dilution cloning in the presence of allogeneic feeders, PHA, and Il-2. We obtained Vα12+ CD8+ T cell clones and determined by CDR3 sequence analysis that they are identical to the T cells that are expanded *in vivo* in the peripheral blood of the patient. Functional analysis of the T cell clones shows them to be autoreactive since they secrete cytokines and proliferate when they are cultured with autologous but not allogeneic APCs. Furthermore, they are able to kill autologous targets in a CTL assay. Our preliminary data suggests that they may recognize a MHC class II peptide presented by a MHC class I molecule. Thus, a large population of CD8+ T cells in RA patients are clonally expanded and autoreactive. These cells define a novel immune aberration in RA and provide a tool to define the autoantigens that activate expanded T cell populations in vivo.

C2-403 MOLECULAR BASIS OF FUNCTIONAL BINDING OF THE SAME HIV PEPTIDE TO BOTH A CLASS I AND A CLASS II MHC MOLECULE, Jay A. Berzofsky, Toshiyuki Takeshita, Hidemi Takahashi, Steven Kozlowski, Jeffrey D. Ahlers, C. David Pendleton, Richard L. Moore, Yohko Nakagawa, Kozo Yokomuro, Barbara S. Fox, and David H. Margulies, Metabolism Branch, NCI, and Laboratory of Immunology, NIAID, NIH, Bethesda, MD 20892, Department of Medicine, University of Maryland Medical School, Baltimore, MD 21201, and Department of Microbiology and Immunology, Nippon Medical School, Tokyo 113, Japan

Several peptides have been found to bind to both class I and class II molecules, but the basis for this binding of the same peptide to two classes of MHC molecules has not been compared previously. We have analyzed one such peptide, P18 from the V3 loop of HIV-1 gp160, which we previously showed to be recognized by CD8$^+$ CTL with the class I molecule H-2D^d, and by CD4$^+$ helper T cells with the class II molecule I-A^d. Using truncated and substituted peptides, we found that the minimal core peptides are very similar, that the residues required for class I binding precisely fit the recently identified consensus motif for peptides binding to D^d (XGPX[R/K/H]XXX(X)[L/I/F]), and that at least three of the same residues play a role in binding to class II I-A^d. In addition, several of the same residues are involved in T-cell receptor interaction when the peptide is presented by class I and class II molecules. Modeling shows results to be consistent with the crystal structure of a peptide-class II MHC complex. Thus, the recognition of this versatile peptide by CD4$^+$ helper T cells with class II MHC molecules and by CD8$^+$ cytotoxic T cells with class I MHC molecules is remarkably concordant in both the core peptide used and the role of different residues in the ternary complex. Because we have seen that the CD4$^+$ T-cell response to this peptide can help induce a CD8$^+$ T-cell response to the same peptide, this concordance may have functional significance in determining immunodominance.

C2-404 IN VIVO ROLE OF THE CD40-CD40L, CTLA4/CD28-B7 AND CD2-CD48 INTERACTIONS IN MERCURIC CHLORIDE-INDUCED AUTOIMMUNE DISEASE. Luigi Biancone, Giuseppe Andres, Hannah Ahn, Charlotte Dai and Ivan Stamenkovic. Department of Pathology, Massachussets General Hospital and Harvard Medical School and Pathology Research Laboratory, Charlestown Navy Yard, Boston, Massachussets, 02129.

The receptor-ligand pairs CD40-CD40L, CTLA4/CD28-B7 and CD2-CD48 play a central role in lymphocyte activation and may therefore be important in the initiation and progression of autoimmune diseases. We tested the effect of a CTLA4-Ig fusion protein (CTLA4-Rg, for Receptor globulin) and of monoclonal antibodies (mAb) to CD40L and CD2 on autoimmune disease induced by HgCl2 in SJL mice. In mice, injection of HgCl2 increases the serum levels of IgG and IgE and induces the developement of circulating anti-nucleolar antibodies (ANolA), and deposition of IgG in the glomerular mesangium and in vessel wall are detectable at 4 weeks. It is proposed that the disease induced by HgCl2 is mainly mediated by Th2 cells and IL-4.

HgCl2 injected mice treated with CTLA4-Rg showed normal serum IgG and IgE levels, undetectable ANola and absence of mesangial and vascular lesions. MAb to CD40L markedly reduced serum IgE and IgG and ANolA production, and prevented the development of renal lesions. Mice treated with both CTLA4-Rg and mAb to CD40L showed normal serological parameters and absence of renal disease.

By contrast, mice treated with mAb to CD2 showed marked exacerbation of the disease with higher serum levels of IgE, IgG and ANolA and increased mesangial and vascular deposition of IgG in the kidney.

Immunohistochemical analysis of cytokines in the spleen at 4 weeks following injection showed marked staining for IL-4 in mice injected with HgCl2, and in mice injected with HgCl2 and treated with mAb to CD2. In contrast, expression of IL-4 was reduced or absent in mice treated with CTLA4-Rg, mAb to CD40L or a combination of CTLA4-Rg and mAb to CD40L.

These results suggest that several T cell-B cell costimulatory pathways participate in the development of Th2-dependent autoimmune disease. Selective abrogation of some of these pathways using mAbs and recombinant fusion proteins may provide a powerful means to prevent and/or to reverse Th2-associated autoimmune disorders.

C2-405 LETHAL EFFECT OF BACTERIAL SUPERANTIGEN IN INFLUENZA-INFECTED MICE, Marcia A. Blackman, Sally R. Sarawar, David L. Woodland, Peter C. Doherty and Wen Jie Zhang, Department of Immunology, St. Jude Children's Research Hospital, Memphis, TN 38105.

Superantigen-producing strains of Staphylococcus aureus have been associated with unexpected death resulting from influenza virus infection in man. We have examined the effect of Staphylococcal enterotoxin B (SEB) in mice that were infected intranasally with non-lethal doses of influenza virus. The results showed that doses of SEB that did not affect survival in uninfected mice, induced the rapid onset of death in influenza-infected mice. Importantly, the timepoint of addition of SEB during infection was critical. Whereas SEB injected at day 5 after influenza infection had no effect on survival, SEB injected at day 7 caused death. The death correlated with a dramatic increase in cytokine levels. Whereas SEB alone induced high serum levels of IL-2, IL-6, TNF and γIFN, the levels in SEB-injected influenza-infected mice were elevated a further ten-fold, indicating a synergistic effect. Current experiments are directed toward understanding the mechanism by which a normally non-lethal viral infection can pre-sensitive mice for SEB-mediated death. These studies have clinical implications, because secondary bacterial infections of the respiratory tract often accompany respiratory viral infections.

C2-406 CYTOKINE DYSREGULATION IN HUMAN FETAL THYMUS ORGAN CULTURE AND IN SCID-hu THY/LIV MICE FOLLOWING INFECTION WITH HIV. Mark L. Bonyhadi, Jennifer Auten, Joseph M. McCune, and Hideto Kaneshima. SyStemix, Inc., 3155 Porter Dr., Palo Alto, CA, 94304

There is a large body of evidence suggesting that one way in which human immunodeficiency virus (HIV) impairs normal immune responses, including those against HIV itself, is through the aberrant dysregulation of cytokine expression. The irregular expression of various cytokines may precede and underlie many of the observed pathophysiological abnormalities associated with HIV infection. On the other hand, aberrant cytokine expression may accompany disease progression without actually playing a causative role in HIV pathogenesis. Understanding the role of cytokine dysregulation in the immunopathogenesis of HIV infection may be essential for the development of effective therapies for AIDS. In order to address this issue, we have analyzed cytokine expression patterns following infection with HIV in both the SCID-hu Thy/Liv mouse model and in a human fetal thymus organ culture system (TOC). Both the *in vivo* and *in vitro* models demonstrate similar parameters of HIV-mediated pathology, including time-dependent viral replication, thymocyte depletion, and inversion of the CD4/CD8 ratio. In both models, depletion appears to mediated in part by the process of programmed cell death. Thymocytes from HIV-infected SCID-hu Thy/Liv mice as well as from infected TOC displayed upregulation of several cytokines, including IL-4, IL-6, IL-10 and TNF-a, while in the case of infection with certain virus isolates, IL-2 expression appeared diminished. The SCID-hu Thy/Liv mouse and TOC models for HIV-infection are amenable to protocols which are designed to interfere with HIV-mediated cytokine dysregulation, including the use of neutralizing antibodies, recombinant cytokines, and agents which interfere with cytokine secretion or expression. Recent data will be discussed. In contrast to traditional cell culture systems, both the SCID-hu Thy/Liv mouse and TOC models make use of organ systems in which tissue organization and cellular composition approximate the human setting. In this respect, both models may serve to provide clinically relevant information as to the role that cytokines play in the pathogenesis and modulation of immune responses following infection with HIV.

C2-407 A REPETITIVE STIMULATION PROTOCOL USING STAPHYLOCOCCUS AUREUS ENTEROTOXIN B (SEB) TO EXPAND SPECIFIC TCRVß AS DETECTED BY PCR AND FACS, Annemieke Boots, Astrid Wimmers-Bertens, Rob Nelissen and Antonius Rijnders. Department of immunology, NV Organon, PO box 20, 5340 BH Oss, The Netherlands.

The pathogenesis of joint destruction in rheumatoid arthritis (RA) remains ill-defined. Joint destruction is thought to be the result of tissue damage mediated by T-cells. The presence of cartilage appears responsible for sustaining the chronic inflammatory process and thereby forwards a role for cartilage-responsive T-cells in RA pathogenesis. Some studies of RA patients have suggested that the T-cells in diseased synovia are relatively oligoclonal. Moreover, Vß14-bearing T-cells have been implicated in RA pathology. In addition, it has been suggested that superantigen-activated T-cells are more likely to cross-react with self antigens. It is our goal to test this hypothesis by using superantigen-activated T-cells from rheumatoid synovia and test their reactivity to crude cartilage fractions. Since synovial samples are generally small, repeated stimulations with superantigen will be necessary to acquire workable amounts of T-cells bearing the desired Vß-specificities. Staphylococcal enterotoxins have been shown to bind to major histocompatibility complex class II molecules and stimulate T-cells in a Vß-specific manner. These Vß-specificities have been well-documented in mice and humans using the PCR method for detection. We have devised a repetitive SEB stimulation protocol for enrichment of T-cells bearing certain Vß-specificities starting from human peripheral blood lymphocytes (PBL). After four rounds of stimulation with SEB, the T-cell population showed an enrichment of certain Vß (Vß3, 12, 14, 15, 17, 20 and 24) as detected using the PCR method. In addition, the relative percentage of selected TCRVß was measured using the FACS and Vß-specific mAb.

C2-408 ISOLATION AND CHARACTERIZATION OF ANTIGEN PRESENTING DENDRITIC CELLS OF RAT CHOROID. William E. Bowers*†, V. Al Pakalnis†* and Aniruddha Choudhury*, Departments of Microbiology & Immunology* and Ophthalmology†, University of South Carolina School of Medicine, Columbia, SC 29208

Dendritic cells (DC) are potent stimulators of T cell-mediated responses. However, they exist in immunoprivileged sites and were recently discovered in the choroid of the eye. This study examines their functional properties. Fresh preparations of posterior segment cells (PSC) do not show DC functional activities. When choroidal DC are isolated from PSC by using mAb against rat DC in combination with an immunomagnetic technique, they are able to stimulate allogeneic and periodate-treated T cells and to process and present myelin basic protein and ovalbumin, as well as their peptides, to specific antigen-primed T cells. The level of these functional activities does not change during three days of culture. Exposure to IL-1β or GM-CSF augments the ability of choroidal DC to stimulate periodate-treated T cells by as much as 5- and 15-fold, respectively. The enhanced functional activity is blocked by cycloheximide. DC within the rat choroid are therefore capable of stimulating T cell responses *in situ* but may be maintained in a state of latency by neighboring cells and/or soluble factors. Given their anatomical location and the known release of IL-1β and GM-CSF in the progression of autoimmune uveitis, choroidal DC may play a critical role in this disease process.

C2-409 THE EFFECTS OF UPSTREAM GOLLI DETERMINANT EXPRESSION ON RESPONSIVENESS TO MYELIN BASIC PROTEIN. Laurent Brossay*, Anthony Campagnoni¶ and Eli Sercarz*, *Department of Microbiology & Molecular Genetics and ¶Mental Retardation Research Center, University of California, Los Angeles, CA 90024

Experimental allergic encephalomyelitis (EAE) is a T cell mediated autoimmune disease that involves recognition of myelin antigens such as myelin basic protein (MBP). However it has remained a mystery why the specific dominant determinant (acetylated 1-9 peptide) does not induce neonatal tolerance.

An important finding recently described the fetal expression of a novel upstream genetic element, Golli, which is spliced in frame into MBP exons, permitting a 105 kilobase transcription unit, termed Golli-MBP to be produced. Of greatest interest is the fact that the MBP gene is thereby shown to be part of a more complex genetic structure which may play an important role in oligodendrocyte differentiation. In this study we ask whether the existence of upstream Golli protein will act to competitively induce tolerance to Golli determinants, thereby protecting and preventing MBP determinants themselves from inducing tolerance. The in frame Golli-mbp peptide from clone BG21 which includes the sequences up through residue 56 of MBP has been cloned and expressed in the Qiagen vector. The corresponding recombinant protein has been used as an immunogen in B10.PL mice. Preliminary data suggest that there is no proliferative response to the MBP determinants. Consequently, the existence of the Golli exon may act to preemptively and competitively induce tolerance to Golli determinants. To examine this hypothesis, peptides covering the entire Golli-MBP sequence (190 amino-acids of the Golli MBP) have been synthesized and used to study MHC binding of each of these peptides, as well as the ability to activate a T cell response. The rationale is that those peptides which bind to the MHC molecule but do not induce a proliferative response are considered dominant determinants which must have induced tolerance but nevertheless compete effectively with MBP determinants for MBP binding. Supported by NMSS and Medical Research Postdoctoral Fellowship from Canada to L.B.

C2-410 INDUCTION OF BOTH CONTROL AND CYTOKINE GENES IN RELATIVE RT-PCR ANALYSIS OF ACTIVATED LYMPHOCYTES FROM MULTIPLE SCLEROSIS PATIENTS, Calabresi, PA, McFarland, HF, Cowan EP, NINDS,NIH & FDA Bethesda MD 20892.

We are studying cytokine gene expression in peripheral blood lymphocytes (PBL) and cerebrospinal fluid (CSF) cells of patients with multiple sclerosis (MS). We have used relative RT-PCR to determine whether type I cytokine gene expression correlates with disease activity. This method is dependent on having a stable control gene to account for variability in the amount of starting RNA and error introduced during PCR amplification. In preliminary studies we have tried three different control amplimer sets; *B*-Actin, Glyceraldehyde 3-phosphate dehydrogenase (G3PDH), and transferrin receptor. We found that PBL's and T-cell lines, that are activated by mitogen or antigen respectively, express more control gene mRNA than resting cells. This effect can partially be accounted for by quantifying the total RNA extracted from the cells, but even after controlling for the amount of starting RNA, there is an apparent induction of control gene mRNA by both mitogen and antigen. This effect may significantly impair the ability to measure increases in cytokine mRNA from activated cells. The extent of this effect and its relevance to cytokine analysis of in vivo activated lymphocytes from MS patients will be discussed.

C2-411 T CELL RECEPTOR USAGE BY SIVmac GAG-SPECIFIC CYTOTOXIC T LYMPHOCYTES, Zheng Wei Chen, Ling Shen, Julie Regan, Zhongchen Kou and Norman L. Letvin. Harvard Medical School, Beth Israel Hospital, Boston, MA 02215

Cytotoxic T lymphocytes (CTL) may play an important role in limiting the spread of AIDS viruses. Elucidating the molecular interactions of CTL and the virus is therefore of central importance for exploring the immune control of an AIDS virus infection. A previous study has shawn that TCR of SIVmac Gag-specific CTL from a single animal can exhibit a restricted use of the Vß23 gene family. To extend these studies, thirty five CTL clones were generated from three SIVmac-infected monkeys expressing the MHC class I Mamu-A01 gene product and assessed for TCR gene usage. All CTL clones were shawn to recognize a single SIVmac Gag peptide in association with Mamu-A01. Sixty six percent of CTL clones generated from two infected monkeys employed TCR-ß chains from Vß13 gene family; 60% from the other infected animal expressed TCR employing ß chains from the Vß6 family. In addition, the Vα1 gene famiy was used in the TCR expressed by 45% 0f CTL clones from all monkeys. Nevertherless, this CTL response was clearly polyclonal. CTL clones ex-pressing TCR that employed genes from other Vß or Vα were also readily demonstrated, including Vß1,2,5,12,21,22, and 23, as well as Vα2,7,8,20,21, and 23. Futhermore, TCR employed by these CTL clones exhibited diverse sequences in their CDR3 regions. These results indicate that SIVmac-infected monkeys sharing the same restricting MHC class I molecule have SIVmac Gag-specific CTL in their PBL whose TCR exhibit different patterns of Vß usage. These studies, therefore, suggest that the TCR repertoire of SIVmac Gag-specific CTL can be diverse in virus-infectd, Mamu-A01+ rhesus monkeys. Such a broad CTL TCR repertoire may provide the host an advantage in containing an AIDS virus infection.

C2-412 IMMUNOMODULATION OF EXPERIMENTAL AUTOIMMUNE MYASTHENIA GRAVIS WITH INTERFERON-α. P. Christadoss, S. Baron, M. Shenoy, E. Goluszko and Bo Wu+. Department of Microbiology and Immunology, University of Texas Medical Branch, Galveston, TX 77555-1019.

Interferon-α (IFN-α) has been shown to enhance MHC class I expression, block the induction of MHC class II expression, activate suppressor T cells, down-regulate TH1 responses, and up-regulate acetylcholine receptor (AChR) expression. Because of these properties of IFN-α, we evaluated the immunomodulatory aspect of IFN-α on experimental autoimmune myasthenia gravis (EAMG). IFN-α (10^5 IU/thrice weekly for 5-6 weeks) or placebo treatment was started one week after the second immunization with AChR in CFA, when autoimmunity to AChR is well established. In this blinded study all the mice were monitored over a period of 8-9 weeks after the start of IFN-α treatment and mice were graded daily for clinical manifestations of EAMG. In the first experiment 11/19 (58%) PBS treated and 5/19 (25%) IFN-α treated mice developed clinical EAMG. In the second experiment 16/19 (84%) PBS treated and 7/17 (41%) IFN-α treated mice developed clinical EAMG. Thus in two separate experiments IFN-α treatment reduced the incidence of disease by more than 50% ($p=0.04$ and 0.008) compared to PBS treated control. The MHC class II expression on peripheral blood lymphocytes (PBL) increased after immunization with AChR in CFA (naive - 42%; after boosting with AChR - 57%). IFN-α treatment after established autoimmunity to AChR reduced the MHC class II expression on PBL (PBS-57%; IFN-α-48%). These findings indicate that IFNα's protective effect against EAMG may be caused in part by down-regulation of MHC class II expression. IFN-α is FDA approved, and has minimal side effects, and therefore, could be used as a potential therapeutic agent in the treatment of MG.

+ J.W. McLaughlin Postdoctoral Fellow

C2-413 EVIDENCE FOR FUNCTIONAL HETEROGENEITY IN SPLENIC MACROPHAGE SUBSETS DURING PRIMING OF CLASS I RESTRICTED ANTI-VIRAL CTL, Richard P. Ciavarra, Francis J. Liuzzi, Thomas Bass and Bruce Tedeschi, Department of Microbiology and Immunology, Department of Pediatrics and the Department of Anatomy and Neurobiology, Eastern Virginia Medical School, Norfolk, VA 23501

A number of cell types have been shown to function as efficient antigen presenting cells (APC) for protein or peptide antigens. However, few *in vivo* studies have been conducted on complex, macromolecular antigens such as viruses. Because extensive degradation may be required to present these antigens, macrophages (mΦ) may play an important role as APC for anti-viral immune responses. We have therefore utilized a mΦ "suicide" technique which utilizes liposome-mediated intracellular delivery of dichloromethylene diphosphate (Cl2MDP) to deplete mΦ *in vivo*. Analysis of tissue sections by enzyme histochemistry demonstrated that virtually all splenic mΦ subpopulations were depleted following Cl2MDP treatment. Priming to vesicular stomatitis virus (VSV) was dramatically inhibited in mΦ-depleted mice; thus, secondary anti-VSV CTL and proliferative responses *in vitro* were barely detectable in mΦ-depleted mice. In contrast, primary alloreactive CTL and proliferative responses remained unaffected. Because splenic mΦ subsets are highly compartmentalized and may possess specialized functions, we tested the possibility that priming was mediated by a specific mΦ subpopulation. For this purpose, mΦ-depleted mice were primed with VSV 7 days after Cl2MDP-treatment. At this time, all mΦ subpopulations had repopulated the spleen to normal numbers with the exception of marginal metallophilic mΦ (MMM) and marginal zone mΦ (MZM). However, normal secondary anti-VSV CTL responses were generated in mice depleted of MMM and MZM indicating that these two mΦ subpopulations were not required for successful priming of VSV precursors. Present studies are directed to isolate purified mΦ subpopulations to directly assess their functional capabilities as APC.

C2-414 EVIDENCE FOR IMMUNE PROTECTION TO HIV. Mario Clerici, Ligia Pinto* and Gene M. Shearer*, Cattedra di Immunologia. Universita' degli Studi di Milano. 20133 Milano, and
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We hypothesized that progression of HIV-infected individuals to AIDS is controlled by two functional subsets of T helper lymphocytes: Th1-like, mainly enhancing cell mediated immunity (CMI), and Th2-like mainly augmenting antibody (Ab) production. During progression of HIV+ individuals to AIDS, we described a decline in the production of type-1 cytokines interleukin 2 (IL 2) and interferon γ (IFN γ), and an increase in the production of type-2 cytokines interleukin 4 (IL4) and interleukin 10 (IL10) by HIV+ PBMC. Additionally, in vitro stimulation of HIV+PBMC in the presence of neutralizing antibodies to IL-4 and/or IL-10 could circumvent defective antigen-stimulated IL2 production and proliferation. Furthermore, a predominant type-2 cytokine profile can be moved to predominant type-1 by incubation of PBMC with IL12, a type 1 cytokine which is defective in HIV infection. The importance of the type-1 to type-2 switch is indicated by the observation that such switch is predictive for: 1) reduction in CD4 counts; 2) time to AIDS diagnosis; and 3) time to death. We also suggested that a strong CMI can reduce susceptibility to HIV infection. Thus, we analyzed HIV-specific immune responses of HIV-seronegative individuals exposed to HIV including: gay men, IV drug users, exposed health care workers and newborn of HIV+ mothers. PBMC from 30-70% of these individuals, but from only 3-5% of HIV presumably unexposed individuals, showed HIV peptides-stimulated proliferation and IL-2 production. The finding of HIV-specific TH lymphocytes in these individuals strongly suggests the presence of immunologic memory, secondary to in vivo lymphocyte priming with HIV. Additionally, HIV-specific CD8+ MHC class I restricted CTL were detected in 35% of HIV seronegative health care workers exposed to HIV. Because peptide presentation in association with MHC class-I is secundary to active viral replication inside the host cells, the detection of HIV-specific MHC class-I restricted CTL suggests the presence of a silent infection in these individuals, which presumably has been kept under control by CMI. These findings indicate that CMI may be a correlate of immune protection in HIV infection

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C2-415 **BREAKING OF THE AUTOTOLERANCE TOWARDS THE HIGHLY CONSERVED PROTEIN UBIQUITIN.** Dalum, I., Jensen, M. R., Hindersson, P., Elsner, H., Mouritsen, S., M & E, Lersø Parkallé 40, 2100 Copenhagen Ø, Denmark.

In order to be recognized by a T_H cell antigens (self or non-self) must be processed in a professional APC. Selected fragments are subsequently exposed on the APC surface in complex with MHC class II. Normally, selected self-peptides do not stimulate T cells implying that the discrimination between self- and non-self antigens is conferred to the T_H cell population. Using APCs as a kind of tutor T cells were taught in early life to tolerate MHC associated peptides derived from self proteins.

Ubiquitin (UBI) is a highly conserved protein which is present in all eukaryotic cells and therefore is expected to be non-immunogenic. In this study we examined the antigen processing and presentation of genetically modified UBI molecules. UBI sequences were substituted with one of the known T cell epitopes HEL(50-61), which binds specifically to the murine A^k MHC class II molecule, or OVA(325-336), which binds to A^d. The substitutions were placed in a molecular context expected to disturb the UBI structure as little as possible. The recombinant proteins (UBI-HEL and UBI-OVA) as well as native UBI were expressed in *E. coli*, and the influence of their substitutions on the immunogenicity in mice as well as on the processing and presentation was studied.

Already within two weeks UBI-OVA and UBI-HEL elicited strong autoantibody responses in Balb/c ($H\text{-}2^d$) mice against native UBI whereas native recombinant UBI was non-immunogenic. UBI-OVA also elicited autoantibodies in BALB/k ($H\text{-}2^k$) whereas both UBI-HEL and UBI were non-immunogenic. Serological studies showed that the antibodies reacted with soluble native UBI epitopes although reactivities towards the inserted peptides were also present. These results support the notion that autoreactive B cells are not clonally deleted in the individual.

Clearly the induced immune responses did not follow the known MHC restriction of the inserted T cell epitopes. The T cell regulatory mechanism of this breaking of the B cell tolerance was studied further by competitive MHC class II binding experiments using overlapping peptides representing the entire sequence of UBI-OVA as well as of UBI.

C2-416 **EVIDENCE FOR AGE-RELATED ABNORMALITIES IN THE DELETION OF ACTIVATED PERIPHERAL T CELLS IN *lpr* AND *gld* MICE,** Wendy F. Davidson and Thomas Giese, Laboratory of Genetics, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892-4255

Mice homozygous for *lpr or gld* develop lymphoproliferative disease characterized by the progressive accumulation of B220$^+$ DN T cells and primed CD4$^+$ and CD8$^+$ T cells. The mechanisms leading to the accumulation of these T cell subsets are poorly understood but clearly dependent on defective expression of Fas in *lpr* mice and FasL in *gld* mice. Recently, a variety of experimental approaches revealed that the majority of B220$^+$ DN T cells are derived from thymus-selected peripheral CD8$^+$ T cells. Here we used the potent mitogen, Staphylococcus enterotoxin B (SEB), to detect abnormalities in the deletion of Vβ8$^+$ T cell subsets in C3H-*lpr* and -*gld* mice and also to determine if SEB-activated Vβ8$^+$CD8$^+$ T cells could differentiate into B220$^+$ DN T cells. Mice were injected with a single dose or with multiple doses of SEB. these studies showed that Vβ8$^+$CD4$^+$ T cells from 6-8 wk old *lpr* and *gld* mice were deleted normally but that Vβ8$^+$CD8$^+$ T cells from young and diseased mice and Vβ8$^+$CD4$^+$ T cells from diseased animals were deleted inefficiently. Activation of +/+, *lpr* and *gld* Vβ8$^+$CD8$^+$ T cells resulted in transient expression of B220. B220 levels were maximal 2 days after SEB treatment and were undetectable 5 days later. There was no evidence for conversion of B220$^+$ or B220$^-$ Vβ8$^+$CD8$^+$ T cells into B220$^+$ DN T cells and the preexisting B220$^+$ DN T cells neither proliferated nor were deleted in response to SEB. These data imply that deletion pathways independent of Fas/FasL expression function in young *lpr* and *gld* mice and delete CD4$^+$ T cells more efficiently than CD8$^+$ T cells. As the mice age these alternative pathways become less effective and this may explain the progressive accumulation of memory T cells. Single or multiple exposures of CD8$^+$ T cells to a strong mitogenic stimulus that induces B220 expression is insufficient for conversion to B220$^+$ DN T cells.

C2-417 DNA IMMUNIZATION TO HEPATITIS B SURFACE ANTIGEN (HBsAg) OVERCOMES H-2 RESTRICTED NON-RESPONSE TO HBsAg AND BREAKS TOLERANCE TO HBsAg IN TRANSGENIC MICE.

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We have developed a murine model of DNA-mediated immunization to hepatitis B virus (HBV) by intramuscular injection of plasmid DNA encoding HBV envelope proteins. A single DNA injection induces rapid, strong and sustained humoral and cell mediated responses. HBV envelope is encoded by an open reading frame which is divided by 3 in-frame ATG start codons into 3 domains (pre-S1, pre-S2 and S), each of which contain B- and T-helper cell epitopes. Three envelope proteins are expressed: small (S), middle (pre-S2+S) and large (pre-S1+pre-S2+S). Some strains of mice do not respond to the primary antigenic determinant of S protein (HBV surface antigen = HBsAg) unless pre-S1 and/or pre-S2 epitopes are also included and this nonresponse to HBsAg is H-2 haplotype restricted. Up to 10% of humans fail to respond to vaccintation with HBsAg and this is HLA-restricted in 25% of these cases. Since the immune response with DNA is superior to that with injected recombinant protein, it was desirable to determine the effect of DNA immunization on haplotype-restricted non-response to HBsAg in congenic mice differing in H-2 haplotype. Good and poor responding strains of mice were immunized with HBsAg by injection of DNA encoding S protein or by injection of the protein itself. In poor responder strains, anti-HBsAg antibodies were induced by a single DNA injection but required two protein injections. In good responders, antibodies appeared earlier with DNA than protein. DNA immunization induces very high CTL activity. For example, expression of the HBV envelope proteins from DNA gives spleen cells capable of specific lysis of 80-90% at effector:target ratios as low as 2.5:1 after specific restimulation with antigen-presenting cells. After non-specific stimulation in culture with IL2, it is possible to obtain nearly 40% specific lysis at effector:target ratios of 200:1. The high efficiency of this response may be due to the combined effects of *in situ* synthesis of the protein antigens, which leads to processing of protein and presentation of peptide epitopes via class I MHC, as well as the unique ability of HBsAg to induce CTL activity even if delivered as a pure recombinant protein. Immunization with DNA encoding HBsAg into transgenic mice tolerant to circulating HBsAg produced in the liver induces anti-HBsAg antibodies and down-regulate HBV gene expression in the liver. This effect may be mediated by CTL activity. DNA may prove useful for vaccination of humans who fail to respond to recombinant HBsAg or for treatment of HBV chronic carriers.

C2-418 IMMUNITY TO DNA AND AN AUTOLOGOUS DNA-BINDING PEPTIDE INDUCED BY COMPLEXES OF DNA AND PEPTIDE, Dharmesh D. Desai and Tony N. Marion, Department of Microbiology and Immunology, University of Tennessee, Memphis, Memphis ,TN 38163.

We have previously demonstrated that DNA-peptide complexes consisting of native, duplex, mammalian, B-form DNA bound to Fus1 (DNA-Fus1) can induce antibody in normal, non autoimmune-predisposed mice that is structurally and serologically similar to autoimmune anti-DNA antibody in (NZB x NZW)F_1 mice. The synthetic 27 amino acid peptide, Fus1, is derived from a 52 amino acid ubiquitin-carboxyl extension protein found in *Trypanosoma cruzi*. Fus1 is highly basic, binds to DNA, is highly conserved among all eukaryotes including mice and humans, and is immunogenic.

The mouse homologue of the Fus1 peptide, MFus1, shares 67% identity and 74% similarity with Fus1. Like Fus1, MFus1 is also highly basic and has the ability to bind DNA. Despite the similarity between MFus1 and Fus1, anti-Fus1 antibody in three different strains of mice immunized with DNA-Fus1 or Fus1 alone binds weakly to MFus1. More recently, mice immunized with MFus1 produced IgG anti-MFus1 antibody. These results suggest that tolerance to the self peptide MFus1 may not be complete. Prior immunization or co-immunization with the foreign peptide Fus1 was not required for induction of antibody with specificity for the self peptide MFus1. Given that an immune response to the self peptide MFus can occur, DNA-MFus1 complexes may be able to generate anti-DNA antibodies similar to those produced in DNA-Fus1 immune mice. If so, this may provide a completely autologous model system for the induction of anti-DNA antibodies and would prove useful in understanding the origin of autoimmune anti-DNA antibodies that occur in (NZB x NZW)F_1 mice where the autoantigen remains unknown.

C2-419 **HUMAN CYTOMEGALOVIRUS IN MONOCYTES ENHANCES HIV-1 REPLICATION IN Vβ12 T CELLS: EVIDENCE FOR A HERPES VIRUS SUPERANTIGEN.** Dana Dobrescu, Bogdan Ursea, Adam Asch and David N. Posnett, Dept. of Medicine, Cornell Univ. Medical College, New York, NY 10021.

HIV-1 replicates more efficiently in IL-2 dependent T cell lines expressing Vβ12 TCR rather than other TCRs. In HIV-1 infected patients a viral reservoir is frequently established in Vβ12 CD4 cells.

Six to eight days after *in vitro* infection of fresh T cells from HIV-1 negative donors, HIV-1 replicates primarily in Vβ12 cells, as compared to 11 other Vβ subsets and assessed by a quantitative PCR with gag primers. Non-T cells are required in these cultures. In particular, CD14+ monocytes from CMV seropositive donors promoted HIV-1 replication in Vβ12 cells, whereas CD19+ B cells and CD14-CD19- dendritic cells did not. The monocytic cell line, U937, bears MHC class II and is permissive for CMV infection. When infected with CMV (3 different isolates), U937 cells promoted HIV-1 replication in Vβ12 T cells. This was observed with 3 unrelated HIV-1 isolates (TIIIB, JR-FL, BAL). Vβ12 depleted cells showed significantly decreased HIV-1 viral replication as compared to Vβ17 depleted cells, indicating that Vβ12 represents the predominant subset infected. The CMV effect was abrogated by prior UV radiation of CMV stock. Preferential replication of HIV-1 in Vβ12 cells was not due to a cytokine because (a) CMV infected U937 cells did not stimulate HIV-1 replication in a transwell system and (b) exogenous TNFα resulted in HIV-1 replication in all Vβ subsets tested.

Because HIV-1 replication is targeted to Vβ subsets after culture of infected T cells with exogenous superantigens (SAG), the current data suggest that CMV directs production of a Vβ12 specific SAG which plays a role *in vivo* by targeting HIV-1 replication to this subset to form a viral reservoir.

C2-420 THE ROLE OF CYTOKINES IN A MURINE MODEL OF ACQUIRED IMMUNOLOGIC UNRESPONSIVENESS

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Infection of C57BL/6 mice with a mixture of murine leukemia viruses designated LP-BM5 MuLV leads to an AIDS-like disease characterised by progressive immunodeficiency and lymphoproliferation, known as murine acquired immunodeficiency syndrome (MAIDS). The development of MAIDS is associated with increased B cell lymphoblast proliferation, but there is reason to believe that T cell function, and particularly, T cell-derived cytokines, may also play a role. It has been suggested that the immunodeficiency associated with MAIDS is caused by a conversion of immune responses normally characterised by Th1 development towards a Th2-dominated response. We have directly examined this hypothesis by inducing strongly polarised Th1 and Th2 responses by injecting mice co-infected with LP-BM5 MuLV with *Leishmania major* or TNP-KLH respectively, and assaying immune responses as the disease progresses. Our results clearly show that polarised Th1 responses are not converted to Th2 responses, but that both Th1 and Th2 responses are inhibited by MAIDs with similar kinetics. However, we have found that a strongly polarised Th1 response can modulate the effect of MAIDS on T cells, leading to the prolonged survival of antigen-specific CD4+ T cells.

Further, we show that treatment of mice with neutralising antibodies to cytokines affects the development of MAIDS. Infected mice treated with antibodies to interleukin-4 and interleukin-10 exhibited a delayed development of MAIDS-related pathology and maintained T cell responsiveness compared to mice treated with control antibody. Conversely, treatment with anti-interferon-γ led to a significant increase in MAIDS pathology and exacerbated loss of T cell function. These data suggest that while immunodeficiency is not due to conversion of specific Th responses, production of Th2-associated cytokines may promote MAIDS pathology, while Th1-associated cytokines may help control the infection.

C2-421 THE NZB CONTRIBUTION TO LUPUS-LIKE RENAL DISEASE: MULTIPLE GENES THAT OPERATE IN A THRESHOLD MANNER,

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F_1 progeny of New Zealand Black (NZB) and New Zealand White (NZW) mice spontaneously develop an autoimmune process remarkably similar to the human disease, systemic lupus erythematosus (SLE). Genes from each parent are required for disease. To further elucidate the NZB contribution to disease, NZB x SM/J (NXSM) recombinant inbred (RI) strains were crossed with NZW mice and F_1 progeny analyzed for the presence of progressive lupus-like renal disease. The incidence of lupus nephritis varied greatly among the F_1 cohorts, with some RI strains generating a high incidence of disease, similar to (NZB x NZW)F_1 mice, and a few cohorts demonstrating very little disease, similar to (SM/J x NZW)F_1 mice. Additionally, a number of cohorts developed renal disease at intermediate levels, suggesting a multigenic process. Based on previous genotyping of the RI strains, the results of this analysis suggested the chromosomal positions of multiple potential contributory loci. In order to verify these provisional mapping data, (NZB x SM/J)F_1 x NZW backcross mice were analyzed for the development of severe renal disease and comprehensively genotyped. Significant associations between the presence of the NZB genotype and disease were noted on chromosomes 1, 4, 7, 10, 13 and 19. Of these, only the chromosome 1 locus was predicted by the original RI analysis. Overall, both the backcross data and a subsequent reanalysis of the RI genotypes supported the conclusion that no single NZB gene is required for the development of lupus-like autoimmune disease in this model. Instead, heterogeneous combinations of contributing NZB genes appear to operate in a threshold manner to generate the disease phenotype.

C2-422 NEUROPEPTIDES MODULATE ANTIGEN PRESENTATION IN THE EYE.

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Injection of antigen into the anterior chamber (AC) of the eye results in induction of antibody and CTL responses, but suppression of DTH. We have found that neuropeptides are primarily responsible for the outcome of immune reactions initiated in the eye, and that the level of two neuropeptides, vasoactive intestinal peptide (VIP) and substnace P (SP), are the most critical. Concentrations of these peptides can be regulated by neurologic stimulation of the eye by light. For example, mice reared under standard diurnal conditions (12 hr light/12 hr dark) have VIP associated with neurons of the iris and ciliary body in the AC (500-700 pg/mg), but not free in aqueous humor. Injection of antigen into the AC results in the release of VIP and the induction of suppressed DTH. A VIP antagonist reverses the induction of inhibited DTH. SP is found in very low levels in the normal eye, but following a period of dark-adaptation, the levels increase in the sympathetic neurons of the iris and ciliary body (to 100-200 pg/mg), as well as in the aqueous humor (to 200-300 pg/ml). SP is a potent inducer of inflammation and under conditions of increased SP, injection of antigen into the eye leads to induction of DTH. A SP receptor (NK-1) antagonist restores suppression in this instance. The effect of SP always dominates over the effect of VIP, since injection of antigen and SP into normal mice results in activation of immunity. Thus, the outcome of antigen presentation in the eye is determined by the level of neuropeptides with opposing effects. These studies have important implications to our understanding of neuropeptide regulation of organ specific immune responses.

C2-423 $V_H4.21$ EXPRESSION IN THE NORMAL HUMAN B CELL REPERTOIRE

by David F. Friedman, MD, Piotr Kraj, DVM, and Leslie E. Silberstein, MD, Depts. of Pediatrics and Pathology and Laboratory. Medicine, Univ. of Pennsylvania School of Medicine.

During fetal development, in B cell neoplasms, and in autoreactive B cell clones, expression of the germline Ig heavy chain variable (V_H) genes in the genome is non-random. The patterns of normal V_H gene expression are critical to evaluating V_H gene usage in disease. We prepared unbiased phage libraries of V_H genes rearranged (DNA based) and expressed (cDNA based) in normal newborns (NB) and adults (AD) for repertoire analysis. We focused on the conserved $V_H4.21$ gene because it is associated with B cell neoplasms and autoimmunity. The proportions of the 6 V_H gene families in libraries from NB and AD were similar, with V_H4 comprising 30% of rearrangements. Sequencing of V_H4 clones showed that $V_H4.21$ alone accounted for > 10% of the expressed C_μ repertoire, and that 5-7 V_H4 genes accounted for > 90% of V_H4-C_μ and V_H4-C_γ rearrangements. All V_H sequences were 95-100% homologous to germline genes. Flow cytometry showed $V_H4.21$ expression, by the 9G4 idiotype, on 2.5-16.5% of B cells. These findings suggest that human V_H gene expression may normally be highly skewed, and that this pattern persists from birth to adulthood. The high frequency of genes such as $V_H4.21$ in neoplastic and autoimmune disease may thus reflect the normal expressed repertoire, which may be skewed by preferential V_H gene rearrangement or selection by antigen.

C2-424 **Analysis of the diversity and peptide specificity of N region-deficient T cell receptors.** Marc A. Gavin and Michael J. Bevan, Department of Immunology and the Howard Hughes Medical Institute, University of Washington, Seattle, WA 98195.

For both B and T lymphocyte antigen receptors, the random insertion of nucleotides between receptor gene segments (N regions) contributes significantly to the great diversity of antigen binding domains. In mice, the gene responsible for N region addition, terminal deoxynucleotidyltransferase (TdT), is not expressed in the thymus until post natal day 5-7. Thus the newborn repertoire of αβ T cells is far less diverse than that of an adult. What is the role of this early repertoire? Perhaps an N region-deficient αβ TCR is capable of reacting with a larger array of foreign antigens, allowing for the small number of T cells in the neonate to protect against diverse pathogens. Mice deficient for TdT expression have recently been generated (Gilfillan, S. et al. *Science 261:* 1175), and their antigen receptors are devoid of N regions. To observe the functional effects of N region diversity on the T cell repertoire, we have begun screening a random peptide library containing 1.8×10^6 H-2D^b-restricted peptides (Gavin, M. A. et al. *Eur. J. Immunol. 24*:2124) with cytotoxic T lymphocytes (CTL) raised from wild-type and TdT° mice against the D^b-restricted influenza A/PR/8/34 nucleoprotein 366-374 peptide. Consistent with the predicted decrease in repertoire diversity, polyclonal CTL lines from different TdT° mice are far more similar to each other than lines from different wild-type mice in terms of their "fingerprints" of cross-reactivity to the library. We have also found that N region-deficient CTL clones recognize more library peptides than wild-type clones, and that the degree of peptide promiscuity tends to correlate with CDR3 loop length and amino acid usage. If the TCR's affinity threshold for antigen recognition is constant, this reduced specificity for peptide may coincide with an increased affinity for the α-helices of the MHC, indicating a higher propensity for autoreactive T cells in the newborn repertoire.

C2-425 ADMINISTRATION OF IL12 TO DBA/1 MICE IMMUNIZED WITH TYPE II COLLAGEN CAUSES SEVERE ARTHRITIS, Tieno Germann, Jacek Szeliga, Henry Hess, Edgar Schmitt, Frank J. Podlaski*, Maurice K. Gately* and Erwin Rüde,
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DBA/1 mice immunized with type II collagen emulsified with Mycobacterium tuberculosis in oil develop arthritis associated with a strong anti-collagen immune response of the Th1 type. Several microorganismn including mycobacteria activate macrophages to produce IL12. This cytokine is implicated in the development of Th1 cells. Therefore, we tested whether IL12 could replace mycobacteria and cause arthritis of DBA/1 mice immunized with type II collagen in oil. Immunization of DBA/1 mice with type II collagen alone resulted in a weak immune response and only a few animals developed arthritis (10-30%). Administration of IL12 simultaneously to each immunization strongly enhanced the anti-collagen immune response as shown by an about 10-fold increase of collagen-specific IFNγ synthesis by ex vivo activated spleen cells and a 10 to 100-fold upregulation of collagen-specific IgG2a and IgG2b antibody production. The incidence of arthritis was very high (80-100%) in the group immunized with collagen plus IL12. The developing arthritis was severe, involving about 50% of all limbs, and was associated with swelling of whole footpads in many cases. Histological examination revealed massive cellular infiltration, synovial hyperplasia and cartilage as well as bone destruction. A neutralizing anti-IFNγ mAb given to IL12 treated collagen immunized mice prevented the development of arthritis and blocked most of the enhanced IgG2a production. Thus, endogenous IFNγ is involved in the enhancement of anti-collagen immunity induced by IL12. Administration of IL12 and collagen promoted an autoimmune response even in DBA/1 mice that were immunized before in a way not resulting in arthritis (collagen in oil, collagen in oil plus IL12 plus anti-IFNγ mAb). In conclusion, our data show that in vivo administered IL12 can profoundly upregulate an immune response directed to a potential autoantigen resulting in arthritis of DBA/1 mice.

C2-426 A POTENTIAL MECHANISM FOR GP120-MEDIATED INHIBITION OF TCR SIGNALING, Frederick Goldman, John Crabtree and Gary Koretzky, Department of Pediatrics and Internal Medicine, University of Iowa Hospitals and Clinics, Iowa City, IA 52242

HIV infection is characterized by qualitative defects in T cell function which can be mimicked in vitro by ligation of CD4 with envelope glycoprotein gp120. The mechanisms involved in the "desensitizing" effects of gp120 appear to involve the uncoupling of T cell antigen receptor (TCR) from its signal transduction cascade. We previously demonstrated that gp120 inhibits the earliest biochemical event required for TCR stimulation; specifically protein tyrosine kinase activation. In our current studies we have examined the kinetics of gp120-mediated desensitization in the Jurkat T cell line. Cells treated with gp120 + anti-gp120 for 1 or 2 hrs were unresponsive to TCR challenge as assessed by anti-phosphotyrosine Western blot analysis of stimulated whole cell lysates. Following shorter or longer incubations with gp120, TCR-mediated responses were minimally inhibited. A number of recent observations suggest that the inhibitory effects of gp120 may be mediated by p56lck, an important component of the TCR signaling cascade. To determine if CD4 ligation results in the sequestration of p56lck, cells were treated with gp120 + anti-gp120 over a 24 hr time course and then subjected to NP-40 detergent lysis. p56lck was immunoprecipitated with either anti-p56lck or anti-CD4 antibodies from the soluble or insoluble detergent fractions and subjected to Western blot analysis. During the time period of TCR desensitization (1-2 hr incubation with CD4 ligands), significantly less p56lck could be immunoprecipitated from the soluble fraction with anti-p56lck, while treatment with gp120 had no effect on the amount of p56lck that could be immunoprecipitated with anti-CD4. To determine if p56lck was being targeted to the cytoskeleton, the NP-40 detergent insoluble fractions were solubilized in an SDS-containing buffer and immunoprecipitation was carried out with anti-p56lck antibodies. Concurrent with the decrease of p56lck in the soluble fraction, p56lck could be recovered from the cytoskeletal fraction. These data suggest that inhibition of TCR signals by gp120 may be temporally regulated. Furthermore, gp120 may inhibit TCR signals by translocating p56lck to the cytoskeleton.

C2-427 CONTRIBUTION OF CD28/CTLA4/B7 AND gp39/CD40 COSTIMULATION PATHWAYS IN CLONAL EXPANSION AND FUNCTIONAL ACQUISITION OF SELF REACTIVE T CELLS. Nathan Griggs, Sally Agersborg, Randolf Noelle, Jeffrey Ledbetter, Peter Linsley, Kenneth Tung, Dept of Pathol., Univ. Virginia, Charlottesville, VA 22908; Department of Microbiol., Dartmouth Medical Coll., Hanover, NH 03756; Dept. Cell Immunol., Bristol-Myers Squibb Pharmaceut. Res. Inst. Seattle., WA 98121

The zona pellucida (ZP), an ovarian extracellular structure, contains three major glycoproteins: ZP1, ZP2 and ZP3. A peptide from murine ZP3, ZP3(330-342), has a T cell epitope that induces autoimmune oophoritis and a B-cell epitope that induces antibody to the ZP. We have shown that blockage of gp39/CD40 interaction with anti-gp39 antibody results in failure of ZP3(330-342) to induce both autoimmune oophoritis and ZP antibody production. By limiting dilution analysis, the frequencies of ZP3 specific T cells in gp39 antibody-treated mice and in mice given control hamster IgG are comparable. T cells from gp39-antibody treated mice, upon in vitro activation, transfer severe oophoritis to normal mice. Inhibition of ligand binding to the B7 receptor with the fusion protein, CTLA4-Ig, results in failure to generate antibody to the ZP, but has no effect on autoimmune disease progression following ZP3(330-342) peptide immunization. When anti-gp39 antibody and CTLA4-Ig are given together, the effect is additive, leading to variable blockage of ZP3-specific T cell activation, as determined by T cell proliferation and ZP3 specific T cell frequency analysis. This is the first parallel analysis of the two costimulation pathways in a single experimental autoimmune model. The results indicate that autoimmune disease and autoantibody production are inhibitable by costimulation blockade. In the case of gp39/CD40 blockade, gp39 antibody treatment has no apparent effect on clonal expansion of antigen specific T cells, but it inhibits the acquisition of effector T cell functions.

C2-428 IMMUNIZATION WITH INFLUENZA NUCLEOPROTEIN USING mRNA EXPRESSION VECTORS, Stanislaw H. Gromkowski, Michelle Yankauckas, Michelle Nolasco, Marston Manthorpe, Michael Sawdey, VICAL INCORPORATED, San Diego, CA 92121

Recent studies have indicated that immunological memory may depend on the persistence of low doses of antigen, rather than on the existence of long-lived memory cells. In genetic immunization, direct intramuscular injection of a single dose of plasmid DNA coding for a foreign antigen leads to the generation of persistent humoral and cellular immune responses against the gene product. We have shown that intramuscular injection of reporter genes results in permanent, low-level expression of the reporter proteins. However, injection of mRNA vectors results in only short-term expression of the same reporter proteins. In order to test directly the hypothesis that long-term, low-level antigen expression is required to produce persistent humoral and cellular immune responses, we immunized mice using mRNA that codes for an immunogenic protein. We designed and synthesized in vitro a mRNA expression vector that contains a (5' to 3') Cap-Independent Translational Enhancer (CITE), influenza nucleoprotein (NP) gene, ß-globin, and poly(A)23 sequences. This NP mRNA was injected intramuscularly into mice and the development of humoral and cellular immune responses was monitored. Anti-NP specific Ab were detected in sera of NP mRNA injected mice. Splenocytes from immunized mice specifically lysed NP peptide-pulsed syngeneic tumor cells after in vitro restimulation. Thus, NP-specific Ab and CTL responses can be generated in mice by intramuscular injection of transiently expressing NP mRNA. This mRNA transfection approach is being used to gain a better understanding of the phenomenon of immunological memory.

C2-429 ANALYSIS OF T15-IDIOTYPE NEGATIVE ANTI-PHOSPHOCHOLINE ANTIBODIES FROM X-LINKED IMMUNE DEFICIENT, XID, MICE, Wei-Xing Guo#, Angelica Burger*, Randy T. Fischer#, Dan L. Longo#, and James J. Kenny*, #Biological Response Modifiers Program & *PRI/DynCorp., NCI-FCRDC, Frederick, MD 21702.

X-linked immune deficient, Xid, mice are highly susceptible to infection with Streptococcus pneumoniae because they fail to produce immunodominant anti-phosphocholine (PC) antibodies or antibodies to capsular polysaccharides. Anti-PC-specific B cells bearing the dominant T15-idiotype (id) (V_H1:κ22), as well as those expressing the M603-id (V_H1:κ8) or M167-id (V_H1:κ24) appear to be either clonally deleted via an antigen-specific, receptor-mediated process or they are not positively selected from the bone marrow into the peripheral lymphoid tissues of Xid mice. However, Xid mice can produce anti-PC antibodies following multiple immunization with a new thymus dependent PC-antigen, 6-(O-phosphocholine) hydroxyhexanoate, conjugated to KLH (EPC-KLH). Eleven anti-PC hybridomas from 5 separate fusions of EPC-KLH immune C.CBA/N spleen cells have been analyzed for their: 1) ability to bind to and passively protect against virulent S. pneumoniae; 2) ability to capture PC-carbohydrate antigens; and 3) functional heavy (H) and light (L) chain gene sequences. Seven of 11 hybridomas express a V_H1 gene sequence having a 95H Asp → Gly replacement. All of these V_H1-Gly variants utilize either J_H2 or J_H4 rather than the usual J_H1 gene segment, and this variant H-chain is associated with a Vκ1c L-chain, which in 6 of 7 hybridomas, has rearranged to Jκ1 rather than the generally utilized Jκ5 gene segment. These V_H1-Gly:κ1c anti-PC antibodies provide low or no protection against S. pneumoniae although most of them bind to the virulent, encapsulated bacteria and can capture EPC-dextran. Three Xid anti-PC hybridomas express the germ-line Asp 95H, and one of these (27-7C3) utilizes a κ22 L-chain and thus appears to be a somatic variant of T15. A 2nd Asp 95H variant associates with κ1c, while the L-chain of the 3rd variant has yet to be determined. The final anti-PC hybridoma antibody (31-23-1) appears to be a M603-like 95H Asp → Asn variant which expresses the expected κ8 L-chain, and exhibits strong binding to and protection against virulent S. pneumoniae.

C2-430 *TIME* COURSE ADAPTATION OF HIV-1 NEP-SPECIFIC CTL TO EPITOPE VARIATIONS

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Cytotoxic T cell (CTL) responses specific for HIV proteins probably play a major role in controlling HIV infection during the clinically asymptomatic stages preceding the onset of AIDS.

A cohort of 50 asymptomatic patients ($CD4^+$ counts: 150-800/mm^3) was studied to follow over three years the development of the CTL responses directed against HIV-1 proteins with special interest to the recognition of the Nef protein. Nef-specific CTL could be detected in the peripheral blood from 50 % of the patients, while CTL specific for structural proteins (Env,Gag, Pol) were detectable in 80% of patients at all stages of the infection.

We characterized 3 new CTL epitopes containing HLA-A2 anchor motifs in the following regions (a.a. according to the HIV-1 LAI sequence): Nef 136-145, Nef 180-189 and Nef 190-198 as well as two epitopes in association with HLA B7 at Nef 68-77 and Nef 128-137. Distinct patterns of epitope variation and CTL evolution were obtained:

First we observed constant CTL recognition of NEF 180-189 and 190-198 despite high levels of viral mutations observed in these regions. Indeed, CTL already present in 1992 recognized the major viral variant that disappeared two years later. A second phenomenon was the occurence of dominant viral variants in the HLA-B7 restricted epitope Nef 128-137, which were only weakly recognized by CTL. However, these CTL seemed to be amplified in vivo within 2 years in parallel to the relative decrease of the initial dominant corresponding variant . Third, we found a constant CTL recognition of the HLA-A2-restricted epitope Nef 136-145, which displayed little variation but persisted over time, indicating an insufficient control of viral replication in vivo.

Interestingly, we were not able to detect important mutations at amino acid positions containing HLA A2 or B7 anchor motifs that would have been able to abolish CTL recognition.

Altogether these data strongly suggest that the CTL repertoire is capable of adapting to viral epitope variation and control the rate of viral replication in the asymptomatic phase of disease even in patients with less than 200 $CD4^+/mm^3$ and that viral persistance can be independent of a CTL escape phenomenon. The amplification of such CTL recognizing and controlling viral variants might be useful for application in immunotherapy.

C2-431 POSITION 71 OF HLA-DR4 MOLECULES INDUCES CHANGES OF BINDING SPECIFICITY THAT CORRELATE WITH *RHEUMATOID* ARTHRITIS ASSOCIATION.

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Susceptibility to rheumatoid arthritis (RA) is specifically associated with the class II MHC alleles *DRB1*0401*, *DRB1*0404*, and *DRB1*0101*. Interestingly, the DRß chains encoded by these genes possess a "shared epitope" formed by a short strech of amino acids (at positions 67 to 74) that is highly conserved among RA-associated molecules. Since DRB1*0402, a closely related molecule not associated with RA, differs from the RA-linked DRB1*0401 and DRB1*0404 molecules only in the shared epitope region, this part of the molecule is likely to be critical for disease association.

In an attempt to gain insight into the mechanism of RA association, we have studied the influence of the shared epitope region on the peptide binding specificity of the RA associated and non-associated DR molecules using designer peptide libraries. The effect of single key residues was tested with site directed mutants of DRB1*0401. The results have demonstrated striking differences between RA-linked and non-linked DR allotypes in selecting the portion of peptides that interacts with the shared epitope area. Most differences were associated with a single amino acid exchange at position 71 of the DR ß-chain, and affected the charge of residues contacting the shared epitope. The observed binding patterns permitted an accurate prediction of natural protein-derived peptide sequences that bind selectively to RA associated DR molecules. Thus, the shared epitope, in particular position 71, induces changes of binding specificity that correlate with the genetic linkage of RA susceptibility. These findings should facilitate the identification of autoantigenic peptides involved in the pathogenesis of RA.

C2-432 **A RHEUMATOID FACTOR TRANSGENIC MOUSE MODEL OF AUTOIMMUNITY**

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During systemic autoimmune diseases, only certain autoantibodies are produced; for example, anti-DNA occurs in lupus but not Rheumatoid Arthritis. Although recent and classic work has shown that certain self-reactive B cells can be anergized or even eliminated, a breakdown of these tolerance mechanisms is unlikely to explain why the autoantibody response focuses on certain autoantigens while tolerance is apparently maintained toward other autoantigens. An understanding of this feature of systemic autoimmunity should be central to any models of autoimmune pathogenesis. To address this issue, we have developed a transgenic mouse model in which nearly all of the B cells express, as surface IgM, the RF specificity of AM14, an autoantibody derived from an autoimmune MRL/lpr mouse. A key feature of the RF system, in contrast to other disease-related autoantibodies, is the ability to manipulate both the antigen and antibody. This can be done because some anti-IgG2a RFs from autoimmune mice--such as AM14-- display allotype specificity. Thus, unlike other disease-related autoantibodies, the RF B cells can be studied in the presence and absence of the autoantigen by crossing the transgenes onto allotype congenic strains. We have recently demonstrated (Shlomchik, et al., Int. Immunol. 5:1329, 1993) that autoreactive RF B cells are not deleted in AM14 transgenic mice. We will present new data showing that autoreactive RF B cells which develop in a normal (non-autoimmune) mouse can also participate in a primary in vivo immune response and in memory responses, suggesting that they are not anergized. Indeed, there is evidence for selective spontaneous activation of autoreactive RF B cells in normal mice. This would represent a new phenotype for an autoreactive B cell and already suggests a mechanism by which systemic autoimmunity focuses on certain targets. Namely, it selects B cells which are not tolerized but which normally are not stimulated, perhaps due to lack of T cell help or antigen.

C2-433 TWO FUNCTIONAL ANTIGEN SPECIFIC RECEPTORS ON A SINGLE T CELL.

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According to the clonal selection theory lymphocytes should carry receptors with only one specificity. However, there is much evidence that some T cells, at least, bear two receptors. We have studied mice transgenic for the rearranged genes encoding the α and β chains of an autoreactive T cell receptor (TCR) specific for myelin basic protein (MBP) and the MHC class II molecule I-A^u. This system has allowed us to to examine the specificity of T cells bearing two distinct functional TCR on a single cell. We find that T cells developing in mice that do not express the MHC molecule recognized as self by the transgene encoded TCR express both this TCR and a second TCR that is specific for the MHC molecules of the strain in which it arose. Thus, these T cells have two TCR, each specific for a distinct antigen bound to a distinct MHC molecule. By contrast, when raised in mice bearing the MHC molecule for which the receptor is specific, T cells develop expressing the transgene encoded TCR almost exclusively. Such mice are highy susceptible to autoimmune disease. Our data suggest that on most T cells bearing two TCR, only one is specific for peptides bound to self MHC molecules, and thus that expression of two TCR does not ususally confer reactivity to two unrelated antigens.

C2-434 REGULATION OF PATHOGENIC ANTI-NEUTROPHIL CYTOPLASM ANTIBODY IN MRL-*LPR/LPR* MICE. Janice M. Harper, Césai Milstein* and Anne Cooke, Department of Pathology, University of Cambridge, and Laboratories of Molecular Biology*, Cambridge, UK.

Microscopic polyarteritis is thought to be mediated by anti-neutrophil cytoplasm antibody (ANCA) binding enzymes expressed by activated neutrophils, triggering degranulation and blood vessel wall destruction.
To address the origin of pathogenic autoantibody, the MRL-*lpr/lpr* mouse was used as a model for microscopic polyarteritis. We have derived several monoclonal IgG ANCA from these mice which are multireactive, binding myeloperoxidase (MPO), lactoferrin and additionally dsDNA, characteristics of 'natural autoantibodies'. One of them induced vasculitis in both MRL-*lpr/lpr* and MRL+/+ mice primed with TNFα. Primary nucleotide sequences of a larger panel of monoclonal anti-DNA antibodies were determined. Those which showed ANCA activity utilized the V_H7183 gene family while monospecific anti-DNA antibody used V_HJ558. Somatic mutations in the CDR3 led to loss of affinity of multi-reactive antibody for MPO and DNA. Late anti-CD4 therapy inhibited ANCA but not anti-dsDNA antibody production in MRL-*lpr/lpr* mice.
The results provide answers to questions concerning the pathogenesis of microscopic polyarteritis. A differential role for $CD4^-CD8^-$ and $CD4^+$ T cells is implicated in the regulation of autoantibody production in this model.

C2-435 THE *IN VITRO* SPECIFICITY AND *IN VIVO* BIOLOGICAL ACTIVITY OF CD4 TH1 AND TH2 CELL LINES PROPAGATED FROM THE SPLEEN OF DIABETIC NOD MICE. Don Healey, Sue Arden*, John Hutton* and Anne Cooke. Dept of Pathology and Clinical Biochemistry*, Cambridge University, Cambridge, UK.

CD4+ T cell lines were generated from the spleens of diabetic NOD mice against crude membrane preparations derived from an *in vivo* passaged rat insulinoma. Adoptive transfer studies into neonatal mice with these lines confirmed that overt diabetes is induced by γ-IFN secreting Th1 cells, whereas transfer of IL-4 secreting Th2 cells, resulted in a non-destructive peri-islet insulitis. Analysis of the antigens recognised by individual T cell clones from the Th1 line included reactivity against an insulinoma membrane fraction enriched in proteins of ~38kD. Immune responses to the same antigen preparation have been associated with T cell clones derived from human IDDM. The specificity of Th2 cells include reactivity with a fraction enriched in proteins of 30kD. The data suggest that the balance between β-cell destruction associated with intra-islet infiltration, versus non-destructive (potential protective) peri-islet insultis, may depend on both the antigens recognised, and the prevailing cytokine environment.

C2-436 **OPTIMAL DOSE FOR CTL INDUCTION BY SYNTHETIC LIPOPEPTIDES,** Catarina Hioe, Zuning Bian, Howard Qiu, Pei-de Chen, Peter Kuebler, Andrew Wu, Julie Richardson, Paul McGee, Derek O'Hagan, Wayne Koff, Chang Yi Wang, and Douglas F. Nixon., United Biomedical, Inc., Hauppauge, NY 11788.

Objective: An *in vivo* dose-response study was conducted to determine the optimal conditions for CTL induction by synthetic lipopeptides.
Methods: H-2K^d-restricted CTL epitopes from *Plasmodium berghei* circumsporozoite (PbCS) and influenza virus nucleoprotein (FNP) conjugated to a tripalmitoyl-S-glycerylcysteinyl lipid adjuvant (P_3C) were used to immunize BALB/c mice subcutaneously. After 1 week, the spleens were collected, cultured for 5-10 days with appropriate peptide and rat Con A supernatant, and then tested in the standard ^{51}Cr release assay.
Results: When the FNP lipopeptide containing a 12-mer epitope was titrated, the CTL response increased with the injection of 0.01 μg to 300 μg. The CTL were specific for the FNP epitope and were able to recognize virus-infected target cells. For the PbCS lipopeptide with a 9-mer epitope, the optimal dose was reached at 5 μg to 10 μg, but surprisingly the CTL response declined with 100 μg and 300 μg. We also were able to induce CTL using the PbCS lipopeptide with a 20 mer epitope, and for this longer peptide, a higher dose enhanced the CTL response.
Conclusion: These results showed that lipopeptides containing CTL epitopes of different lengths effectively elicited CTL responses. It was noted, however, that the optimal dose was distinct for each lipopeptide. Overall, this study has demonstrated a potential use of lipopeptides as synthetic vaccines which can confer CTL-mediated immunity.

C2-437 IFN-γ AND TNF-α ARE NOT REQUIRED FOR RECOVERY FROM ACUTE HSV INFECTION IN C57BL/6 MICE, Ai-Xuan L. Holterman, David M. Koelle, Judith A. Westall, Larry Corey, Tim Stewart and Christopher B. Wilson, Departments of Pediatrics, Immunology and Laboratory Medicine, University of Washington, Seattle, WA 981956 and Genentech, South San Francisco, CA 98040

The direct antiviral properties and broad immunoregulatory effects of IFN-γ and TNF-α have implicated these cytokines as crucial effectors in the control of viral infections. Their role in mediating host resistance against acute HSV infection were examined in C57BL/6 mice with targeted disruption of the IFN-γ gene (GKO) and their normal littermates (WT), as well as GKO and WT mice which have been treated with soluble TNF-α receptor antagonist p80:Fc. Animals were inoculated in the footpads and studied for 14 days following infection. GKO and WT mice had similar survival outcome (WT 69%; GKO 71% at day 14, n=16). Preliminary viral clearance results however suggested a more rapid clearance from spinal cords in WT mice after the first week of infection.

	GKO (pfu/mg)	WT (pfu/mg)	
day 8	113,000; 5250; 8500	0; 20; 390	n=3
day 10	850; 530	0; 80	n=2

Preliminary cytokine data also showed 6-fold more iNOS mRNA in nodes from infected WT mice than GKO mice. These findings parallel mortality data showing that GKO mice suffered earlier deaths than WT mice. Survival rates were not different between GKO or WT mice treated with p80:Fc (WT-IgG 62% survival, WT-p80:Fc 50%; GKO-IgG 75%; and GKO-p80Fc 50%; n=8). The GKO mice or P80:Fc treated mice all experienced accelerated deaths relative to the WT animals. In particular, the day 7 and day 8 mortality rates in the p80:Fc treated GKO mice were significantly higher than in the WT mice (WT 0%; GKO-p80:Fc 28%; p=0.05). These preliminary results suggest that IFN-γ and/or TNF-α are not required for recovery from acute HSV infection nor for acquisition of effective immunity against HSV. The trend toward earlier deaths and delayed viral clearance in cytokine depleted animals suggests that IFN-γ and TNF-α mediate protection early in the immune response.

C2-438 A TRANSGENIC MODEL FOR VIRAL INDUCED AUTOIMMUNE-MEDIATED DEMYELINATION. Marc S. Horwitz, Claire E. Evans, Robert A. Lazzarini, and Michael B. A. Oldstone. Dept. of Neuropharmocology, The Scripps Research Institute, La Jolla, CA 92037 and (RAL) Brookdale Center of Molecular Biology, Mount Sinai School of Medicine, New York, NY 10029

A transgenic mouse model has been established to examine the mechanism by which autoimmune-mediated CNS demyelination develops. One hypothesis for the onset of autoimmune diseases like multiple sclerosis is that infection by a virus which shares antigenic epitopes in common with gene products of oligodendrocytes(molecular mimicry) ellicits an immune response which controls the infection and also recognizes the self epitopes on oligodendrocytes. The viral gene products of lymphocytic choriomeningitis virus (LCMV) nucleoprotein (NP) or glycoprotein (GP) were expressed exclusively within oligodendrocytes using the oligodendrocyte specific myelin basic protein (MBP) promoter. Only following infection with LCMV did adult transgenic mice show signs of CNS autoimmune disease. Immune infiltration, primarily by CD8+ staining lymphocytes was observed in the brains of 77% (30/39) of the infected transgenic mice 3 weeks postinfection and was found within white matter, but not limited to any particular portion of the CNS. The infiltrating cells remain present within the brain as long as 11 months postinfection. These mice were given a second viral infection using a vaccinia virus recombinant also encoding the transgene in order to study the effects of stimulating the memory response to the transgene product. Following the second infection, 92% (11/12) of the mice had both infiltrating CD4+ and CD8 + lymphocytes and these cells were localized specifically along myelin tracts. Areas of microgliosis and necrosis were found to colocalize with the infiltrating lymphocytes.

C2-439 PHOTODYNAMIC THERAPY (PDT) UTILIZING BENZOPORPHYRIN DERIVATIVE (BPD, verteporfin) MODIFIES THE DEVELOPMENT OF MURINE AUTOIMMUNE DISEASE. David W.C. Hunt, Simon Leong, Martin Renke, Hui-jun Jiang, Julia G. Levy and Agnes H. Chan. Quadra Logic Technologies, Inc. 520 West 6th Avenue, Vancouver, B.C. CANADA V5Z 4H5.

Destruction of malignant tumors can been achieved by the systemic administration of certain photosensitizing compounds, usually non-metallic porphyrins, that strongly absorb visible wavelengths of light and generate toxic oxygen products in tissues exposed to sufficient quanta of light. To determine whether PDT may also be useful in non-oncologic situations, we have examined whether the photosensitizer BPD and whole-body light (690 nm) irradiation (15 Joules/cm^2) could modify the induction of collagen-induced arthritis (CIA) in DBA/1 mice or the development of adoptively transferred experimental autoimmune encephalolmyelitis (EAE) in PL mice. PDT applied 5 and 12 days after injection of arthritogenic type II collagen caused a significant (P<0.05) reduction in the frequency of arthritis from 25/53 (47%) in placebo-injected controls to 6/27 (22%) in mice given BPD and light. If PDT was applied 24 and 28 days after type II collagen was first given, the incidence of arthritis was significantly (P<0.05) reduced to 2 of 28 (8%) mice in contrast to that observed (8/28, 29%) in the control animals. PDT also significantly delayed the onset of EAE by 20 ± 3 days if it was applied within 24 hours of the receipt of pathogenic myelin basic protein-sensitized spleen cells. However, if PDT was given later than 24 hours after cell administration, it had no effect on the progression to severe EAE.

To account for the protective effects of PDT on the development of experimental autoimmune disease, *in vitro* studies of purified, resting and activated murine splenic T and B cells were performed. Flow cytometric studies indicated that anti-CD3-activated T cells and LPS-activated B cells accumulate greater amounts of BPD than resting lymphocytes. In addition, sub-lethal photodynamic damage coupled with a cellular activation signal (anti-CD3 or LPS) rendered T and B lymphocytes more vulnerable to cell death mediated by apoptosis indicating that PDT may destabilize lymphoid cell function. PDT constitutes a novel approach for the treatment of autoimmune disease.

C2-440 DIRECT INJECTION OF A RECOMBINANT RETROVIRAL VECTOR AS AN IMMUNOTHERAPEUTIC FOR HIV INFECTION, Michael J. Irwin, Sunil Chada, Elizabeth Song, Nancy Sajjadi, Gloria Peters and John F. Warner, Department of Immunobiology, Viagene, Inc., San Diego, CA 92121.

Immunization involving the direct in vivo administration of retroviral vector (retrovector) particles, represents an efficient means of introducing and expressing genes in mammalian cells. A non-replicating recombinant retrovector, encoding the human immunodeficiency virus type I, IIIB envelope and rev proteins, has been developed and examined for gene expression and subsequent stimulation of immune responses in animal models. Transfer of vector encoded genes has been observed at the intramuscular injection site and the draining lymph node. Protein expression has been detected at the injection site both enzymatically and by homing of antigen specific cytotoxic T lymphocytes (CTL).

Foreign proteins produced endogenously in host cells provide peptide fragments to class I major histocompatibility complex (MHC) molecules leading to CTL activation. Vector-immunized animals generated CTL responses that were HIV-1 protein-specific. HIV-seropositive, asyptomatic human subjects treated with the recombinant retrovector have demonstrated augmented CTL responses suggesting that retrovector-immunization may provide an effective means of inducing or augmenting CTL responses in HIV-infected individuals.

C2-441 LONG-LASTING IMMUNE RESPONSE GENERATED BY A SINGLE IMMUNISATION WITH A RECOMBINANT ADENOVIRUS VECTOR V.Juillard, P. Villefroy, D. Godfrin, A. Pavirani, A. Venet and J.G. Guillet. INSERM U152, Institut Cochin de Génétique Moléculaire, Paris and Transgène, Strasbourg, France.

Recombinant Adenoviruses (Ad-rec) are effective vectors for *in vivo* delivery and expression of foreign genes. They have several important features relevant to the development of vaccines: i) proteins are synthesized endogenously and undergo host-specific post-translational modifications such as glycosylation which may be important for antibody recognition; ii) vectors may be used to induce immune response in the mucosal tissue, which is a natural port of entry of many human pathogens.

We have examined the parameters of the immune response induced by a replication-defective adenovirus vector (lacking the E1a and E1b regions) using the response to the β-galactosidase protein in mice immunized with a single injection of recombinant adenovirus expressing the lac-Z gene (Ad-β-gal). Strong, long-lasting cytotoxic T cell response specific for β-galactosidase were obtained. In addition, a β-galactosidase specific T lymphocyte helper proliferative response was observed which secreted IL-2 but not IL-4. This response is consistent with a T1-like profile. Finally, a humoral response to the β-galactosidase was observed 15 days after the single immunization which remained elevated over 6 months without additional priming. Interestingly, the principle immunoglobulin was an IgG2a subtype.

In addition, we have investigated the effects of repeated immunizations on this immune response. Mice immunized with 4 injections did not develop enhanced immune responses to those detailed above. However, a strong neutralizing anti-adenoviral antibody response was observed as early as the second immunization which may be limiting this enhancement. Importantly, and in direct contrast, mice given a single injection of the Ad-β-gal vector did not develop neutralizing antibodies.

These results demonstrate that a single immunization with a replication defective adenovirus recombinant vector induce strong, long-lasting humoral and cellular immune responses. The details of this Ad-β-gal-induced response are currently being studied.

C2-442 SUPPRESSION AND REVERSAL OF *gld* DISEASE BY PARABIOSIS WITH NORMAL MICE, Vellalore N. Kakkanaiah, Glen C. MacDonald, Philip L. Cohen and Robert A. Eisenberg, Departments of Medicine and Microbiology / Immunology, University of North Carolina, Chapel Hill, NC 27599-7280.

The disruption of the Fas receptor or Fas ligand by the *lpr* or *gld* mutations, respectively, results in severe autoimmune and lymphoproliferative disease due to the failure of Fas-mediated deletion of self-reactive lymphocytes. Recently, we have shown in mixed chimeras that *gld*-induced autoimmunity could be corrected by normal bone marrow, in particular by normal T cells. In contrast, *lpr*-mediated autoimmunity could not be influenced by normal bone marrow-derived cells. In the present report, we have studied the role of normal lymphocytes in suppressing or reversing *gld*-induced autoimmunity through parabiosis with normal mice. Our results show a suppression of lymphadenopathy, fewer $CD4^-CD8^-$ T cells, and lower levels of autoantibody production in *gld* mice parabiosed with normal mice at 4-6 weeks of age. *Gld* mice parabiosed with normal mice at four months of age also exhibited a substantial reduction of both total and $CD4^-CD8^-$ T cells in the periphery two months after surgery. However, they showed only partial reduction of autoantibodies compared to *gld* mice parabiosed with *gld* mice. In contrast, older *lpr* mice did not exhibit any reduction in lymphadenopathy or autoantibody production after parabiosis with normal mice. The prevention or reversal of lymphadenopathy in parabiosed *gld* mice suggests that ongoing Fas-mediated deletion in the periphery may play an important role in maintaining self-tolerance. The relative irreversibility of autoantibody synthesis in older parabiosed *gld* mice suggests that autoantibody producing B cells or their committed precursors are long lived and do not express functional Fas receptor.

C2-443 ADAPTION OF THE NK REPERTOIRE TO SELF MHC CLASS I Klas Kärre, Charles Sentman, Petter Höglund, Maria Johansson, Mats Olsson, Margareta Waldenström, Jonas Sundbäck Microbiology and Tumor Biology Center, Karolinska Institute, Stockholm.

The ability of NK cells to detect "missing self" is determined by the MHC class I phenotype of the host. This is illustrated by the altered NK repertoire of MHC class I ($H\text{-}2D^d$) transgenic B6 mice and β_2 microglobulin deficient mice. The effector inhibition model for missing self recognition postulates MHC class I specific NK receptors that turn off the NK lytic program, and there is now strong evidence that Ly 49a molecules act as such receptors, capable of mediating inhibitory signals after recognition of $H\text{-}2D^d$ (Yokoyama, Karlhofer, Seaman et al). The observations on Ly 49a, a C type lectin like receptor, nevertheless impose a paradox in relation to NK cells and "missing self" recognition: Ly 49a molecules cells are downregulated on NK cells in $H\text{-}2D^d$ expressing mice, where they ought to be be useful, but expressed at high levels in mice lacking $H\text{-}2D^d$, where they would be expected to be harmful. We propose a model based on "receptor calibration by avidity" to account for how NK cells adapt to self MHC. The key elements of this model are 1) Each NK cell can express several MHC class I receptors that transmit negative signals to inhibit killing 2) Host expression of the MHC class I ligand for an NK receptor leads to the development or selection of NK cells with reduced (but not deleted) expression of that receptor. This makes the receptor "useful", since NK cells carrying the receptor are more difficult to inhibit with low levels of ligand, and hence become more sensitive to detect "missing self". This is in contrast to the T-cell system, where high receptor expression is thought to correlate with higher sensitivity to detect the aberrant cells. 3) NK receptors for nonself MHC class I molecules may be functional and expressed at high levels; these are "useless" but not "harmful" as long as each NK cell carries at least one receptor for a self MHC class I molecule. The model may explain why allogeneic MHC molecules are sometimes more protective than syngeneic when NK subsets or clones are tested, while the autologous MHC combination would always be optimal for protection against the whole population of NK cells. Instructive versus selective models for determination of the NK repertoire are discussed.

C2-444 T CELL RECEPTOR Vß REPERTOIRE IN ACUTE INFECTION OF RHESUS MONKEYS WITH SIMIAN IMMUNODEFICIENCY VIRUS OR SIMIAN HUMAN IMMUNODEFICIENCY VIRUS, Zhong Chen Kou, Christine Lekutis, DejiangZhou, Matilda Halloran, Ling Shen, David Lee-Parritz, Norman Letvin and Zheng Wei Chen, Beth Israel Hospital, Harvard Medical School, Boston, MA 02215.

To investigate an alteration in T cell receptor (TCR) Vß repertoire and its correlate to the virologic events in acute infection of individuals with an AIDS virus, we have employed an animal model of AIDS, the simian immunodeficiency virus (SIV)-infected macaque monkeys. A cohort of ten genetically-defined rhesus monkeys were experimentally infected with SIV_{mac} or a chimera of SIV and HIV (SHIV), and prospectively assessed for TCR Vß gene expression in PBL and lymph nodes. The acutely infected monkeys exhibited an expansion of the selected Vß-expressing T lymphocyte subpopulations in PBL and lymph nodes, which could be detected in PBL obtained from a monkey as early as three days post-infection. Moreover, the selected Vß-expressing lymphocytes were predominantly expanded in CD8+, but not CD4+ cell population. Sequence analyses of PCR-generated TCR-ß cDNA clones derived from the PBL, which exhibits Vß expansion, showed that the virus-driven Vß expansion was oligoclonal, and the restricted CDR3 lengths predominated. Interestingly, five of six infected monkeys sharing a single MHC class I allele exhibited the same expansion of Vß14-expressing lymphocyte subpopulations in PBL, suggesting that the virus-driven Vß response is MHC class I-associated. Finally, the expansion of the selected Vß-expressing lymphocytes in PBL coincided with the emergence and clearance of SIVp27 protein in plasma of the infected monkeys. These results suggest that acute infection of macaque monkeys with SIV_{mac} or SHIV resulted in an expansion of CD8+ lymphocyte subpopulations expressing the selected Vß gene families. The selectively expanded T lymphocytes may contribute to the active antiviral immunity in the acute SIV_{mac} or SHIV infection.

C2-445 The CNS environment controls effector CD4+ T cell cytokine profile in EAE. Michelle Krakowski and Trevor Owens, Montréal Neurological Institute, McGill University, Montréal, Canada H3A 2B4

EAE is a CD4+ T cell-dependent disease characterized by CNS inflammation and infiltration. We have observed a Th1 cytokine biased T cell response in the CNS of antigen-induced EAE. This significant cytokine switch is likely to influence the development and outcome of the immune response. To understand the mechanism involved, we generated short term, SJL/J CD4+ T cell lines which produced both IFN-γ and IL-4 and were specific for the encephalitogenic MBP peptides p89-101 and p91-103. These were labelled with PKH2 fluorescent dye and transferred to naive mice. As soon as EAE was evident, mononuclear cells were FACS sorted from both the inflamed CNS and LN. Cytokine mRNA production was assayed by reverse transcriptase-PCR. There was a strong Th1 biased cytokine profile within the inflamed CNS, whereas both Th1 (IFN-γ) and Th2 (IL-4) mRNAs were found within the LN. Microglia (Mac-1+, $CD45^{lo}$) taken from naive mice supported the production of IFN-γ and IL-4 by peptide-specific CD4+ T cell lines in response to both exogenously and endogenously presented peptides *in vitro*. The Th1 cytokine bias is therefore effected by other APCs, or components of the inflamed CNS environment. One potential component would be the production of IL-12 by CNS APCs and we are currently analyzing its correlation to disease.

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C2-446 DO MEASLES VIRUS-INFECTED AND NON-INFECTES CELLS COMMUNICATE VIA VIRAL HAEMAGGLUTININ?, Slavica Krantic, Cyrille Gimenez, Chantal Rabordin-Combe, Lab. Biol. Mol. Cell., Lyon, France

Recently, it has been shown that measles virus (MV) downregulate virus receptor (CD46) from the cell surface: this phenomenon is associated to expression of virus haemagglutinin (H) protein on the cell surface of the same cell. To assess whether CD46 downregulation occurs also after CD46-H interaction when two molecules are expressed on two different cells, we used human T cell line Jurkat and murine fibroblast L-line stably expressing MVH protein. Our FACS analysis show that a contact of one hour at 37°C is sufficient to trigger a shift in immunofluorescent labelling of CD46 present on Jurkat cell surface similar to those observed after MV infection. This shift reflects a downregulation rather than simple masking of the CD46 epitope by interaction with H because it was detected not only whith monoclonal antibody (MAb) 20.6 directed against CD46 epitope involved in CD46-H interaction but also with two other MAbs (GB24 and J4-48) that do not interfere with H binding to CD46. CD46-H interaction-dependent CD46 downregulation is specific: other cell surface markers (CD3, CD14, CD63) are not afected. Finally, cellular background is irrelevant for CD46 downregulation: it occurs also in murine fibroblasts expressing CD46 (L.CD46, 3T3.CD46) after their exposure to murine lymphocytes (M12) engineered for high expression of MVH protein (M12.CD46.Hallé). The possible relevance of cell-cell interaction through MVH protein in MV associated lymphopöenia and immunosupression will be discussed.

C2-447 STRATEGIES FOR THE INHIBITION OF T CELL RESPONSES INVOLVED IN MURINE EXPERIMENTAL MYASTHENIA GRAVIS USING Vβ-SPECIFIC IMMUNOTOXINS AND AN ANTIBODY SPECIFIC FOR CD40 LIGAND (ANTI-gp39). K. A. Krolick, P. A. Thompson, A. J. Infante, T. E. Zoda, and K. M. Brandon. Department of Microbiology, University of Texas Health Science Center at San Antonio, San Antonio, TX 78284.

Immunization of C57BL/6 mice with purified acetylcholine receptor (AChR) is known to induce a T cell-dependent antibody response that results in Experimental Autoimmune Myasthenia Gravis (EAMG). Two immunosuppressive strategies were evaluated in an attempt to "short-circuit" the regulatory requirements of this autoantibody response. Strategy 1 is based on our past observations linking $V\beta6^+$ T cells with a prominent AChR epitope reactivity. Thus, a Vβ6-specific immunotoxin was tested *in vitro* for its ability to selectively kill monoclonal and polyclonal T cells that demonstrate reactivity against AChR; results clearly demonstrated the ability to selectively kill AChR-reactive T cells based on their expression of a particular Vβ-associated antigen receptor. Strategy 2 is based on observations by other investigators demonstrating that antibody responsiveness to a number of different antigens requires regulatory signals mediated by CD40/CD40 ligand (gp39) interactions associated with B cells and T cells, respectively. Thus, the monoclonal antibody MR1 (anti-gp39) was tested *in vivo* for its ability to interfere with the communication between AChR-reactive helper T cells and AChR-reactive B cells. Results described demonstrate the ability to inhibit anti-AChR antibody production by MR1 treatment during early stages of T cell activation. These two strategies are discussed with regard to the advantages of T cell inhibition with narrow vs. broad selectivity.

C2-448 **DIFFERENTIAL ROLES OF THE B7-1 AND B7-2 CO-STIMULATORY MOLECULES IN THE PATHOGENESIS OF AUTOIMMUNE DIABETES.** Deborah J. Lenschow*, Lesley Rhee+, Nasrin Nabavi#, Gary Gray$, Kevan C. Herold*, and Jeffrey A. Bluestone+. *The Ben May Institute and the Committee of Immunology, + the Department of Medicine and the Committee of Immunology, The University of Chicago, Chicago, IL 60637. # Roche Research Center, Nutley, NY 07110. $ Repligen Corporation, Cambridge, MA 02139.

Autoreactive T cells play a major role in the development and progression of autoimmune diabetes in the NOD mouse. To determine potential signals required for the activation of these T cells, we examined the role of the CD28/B7 co-stimulation pathway in disease pathogenesis. Female NOD mice, treated at the onset of insulitis (2 to 4 weeks of age) with CTLA4Ig (a soluble CD28 antagonist) or a mAb specific for B7-2 (a CD28 ligand), did not develop diabetes. However, neither of these treatments altered the disease process when administered late, at greater than 10 weeks of age. Histological examination of islets from the various treatment groups showed that these reagents had little effect on the development or severity of insulitis. Together these results suggest that blockade of co-stimulatory signals by CTLA4Ig or anti-B7-2 after insulitis but prior to the onset of frank diabetes. NOD mice were also treated with mAbs to another CD28 ligand, B7-1. In contrast to the previous results, the anti-B7-1 treatment significantly accelerated the development of disease in female mice, and most strikingly, induced diabetes in normally resistent male mice. Furthermore, treatment with anti-B7-1 mAbs resulted in a more rapid and severe infiltrate. T cells isolated from the pancreases of these anti-B7-1-treated animals exhibited a more activated phenotype than T cells isolated from any of the other treatment groups. Finally, a combination of anti-B7-1 and anti-B7-2 mAbs also resulted in an accelerated onset of diabetes, similar to that observed with anti-B7-1 mAb treatment alone, suggesting that the anti-B7-1 mAb's effect was dominant. These studies demonstrate that co-stimulatory signals play an important role in the autoimmune process and that different members of the B7 family have distinct regulatory functions during the development of autoimmune diabetes. Current studies are underway to examine the mechanisms by which CTLA4Ig and anti-B7-2 mAb treatment inhibits diabetes and anti-B7-1 mAb treatment exacerbates disease progression.

C2-449 EVALUATION OF VIRAL MUTATIONAL ESCAPE FROM ADOPTIVELY TRANSFERRED CD8+ HIV GAG-SPECIFIC CTL IN HIV SEROPOSITIVE INDIVIDUALS, Deborah Lewinsohn, Philip Greenberg, Faith Yoshimura, Larry Corey and Stan Riddell, Fred Hutchinson Cancer Research Center, Seattle, WA 98104

The development of CD8+ class I MHC-restricted HIV-specific CTL responses after acute HIV infection correlates with clearance of viremia but these responses fail to completely control infection and decline with progression to AIDS. In HIV infected individuals, CTL responses are elicited against several immunodominant epitopes. Mutations in these epitopes that alter peptide binding to MHC or alter T-cell receptor recognition of the MHC-peptide complex have been proposed as mechanisms by which HIV escapes host T-cell control. In an attempt to augment HIV-specific immunity in HIV seropositive individuals, our laboratory is evaluating the safety and anti-viral effects of the adoptive transfer of autologous *in vitro*-expanded CD8+ gag-specific CTL clones. These CTL clones are generated against a vaccinia recombinant virus that contains a full-length gag construct. The clones are genetically modified to contain a marker/suicide gene prior to adoptive transfer. This study provides a unique opportunity to evaluate if augmenting responses against selected epitopes results in the emergence *in vivo* of HIV isolates that escape recognition by the infused CTL clones due to mutation(s) in the sequences encoding the epitopes. The viral peptides recognized by the CTL clones were mapped to individual core protein components of gag by sensitizing autologous target cells using infection with vaccinia recombinant viruses encoding individual core proteins (provided by Dennis Panicali, Therion). Subsequently, the specific epitope was mapped using target cells incubated with synthetic overlapping peptides. The epitopes recognized by seven CTL clones administered to five individuals in the study have been mapped and include peptides derived from each of p15, p17, and p24, including three previously undescribed epitopes. Sequencing of gag clones obtained by PCR from the plasma of these individuals before and after adoptive immunotherapy is currently underway. If mutations are identified in regions encoding the epitopes, synthetic peptides corresponding to identified mutations will be evaluated for recognition by CD8+ CTL These data should provide insight into the ability of transferred gag-specific CTL clones to mediate an anti-viral effect and the probability that HIV will mutate specific gag epitopes to avoid recognition by CTL.

C2-450 REACTIVATION OF QUIESCENT β-CELL AUTOIMMUNITY IN OLD NON-DIABETIC BB RATS. Peter MacKay, Diabetes Immunology Department, Novo Nordisk, Copenhagen, DENMARK.

4% of diabetes-prone (DP) BB/Mol rats escape overt diabetes which occurs spontaneously in other DP rats between age 56-130 days. Histologic examination of pancreata from such old non-diabetic (OND) rats revealed lymphocytic infiltration (insulitis) levels intermediate between those seen in acutely diabetic (AD, $p<0.001$) and in diabetes resistant (DR, $p<0.01$) BB rats. Also, pancreatic insulin content in OND rats was intermediate between that of AD ($p<0.001$) and DR rats ($p<0.05$) and thus OND rats showed evidence of prior cell-mediated β-cell destruction. Treatment with the IFN-inducer poly I:C rendered 4/4 OND rats diabetic after 2, 5, 7 and 8 days, while diabetes was induced in 4/4 young DP rats at 11, 12, 17 and 24 days ($p<0.05$). In adoptive transfer (AT) experiments, pooled PMA/ionomycin-activated OND donor spleen cells transferred IDDM to 8/9 DP recipients, while similar numbers of AD spleen cells transferred IDDM to 10/10 DP rats with mean onset times ±SEM of 13±0 and 15±1 days, respectively (NS). As recipients, 5/5 OND rats became diabetic after AT with AD spleen cells, while 2/2 became diabetic after AT from other OND rats, and all cell recipients, but no controls, had end-stage insulitis lesions. Transfusion of non-activated OND spleen cells did not protect young DP rats from spontaneous IDDM (4/4 diabetic), while cells from DR rats did (0/5 diabetic, $p<0.02$). Spleen cells from OND-cell recipients were 1.4±0.6% $RT6.1^+$, while cells from DR-cell recipients were 21.8±2.7% positive for the diabetes-suppressive RT6.1 T cell subset ($p<0.02$). Conclusion: Autoaggressive effector cells have become quiescent in OND BB rats but can be reactivated by non-antigen specific agents both in vitro and in vivo. Normoglyceamia is not due to cell-mediated suppression.

C2-451 LUPUS AUTOIMMUNITY ORIGINATING FROM SINGLE SELF PEPTIDES. Mark J. Mamula and Linda K. Bockenstedt. Department of Internal Medicine. Yale University School of Medicine, New Haven, CT 06520.

Autoantibodies specific for the U1 and Sm small nuclear ribonucleoprotein particles (snRNPs) are a serologic hallmark of human SLE as well as murine models of this disease. It is clear that fulminant lupus autoimmunity is directed at a number of peptides of the snRNP including the 70kDa, A, B and D protein components . These IgG class autoantibody specificities are frequently found in a single patient (or lupus-prone mouse) suggesting that their genesis is T cell-mediated and driven by the snRNP autoantigen. We examined the B and T cell tolerance and generation of autoimmunologic diversity in a murine model of SLE. Overlapping peptides of the D protein of murine snRNPs were used as immunogens in mice. We found two cryptic self peptides, sequences 26-40 and 56-70, to which strong T cell responses could be generated. These T cells initially did not respond to stimulation with native mouse snRNPs. A third isoaspartyl form of self peptide (residues 65-79), linking the Asp73 residue to Ser74 via the beta carboxyl group, also elicited autoreactive T cell responses. The latter isoaspartyl form of self peptide can occur naturally in cells and it was our intent to investigate tolerance (or lack thereof) to these "aberrant" self peptides. Induction of autoimmunity with all three peptides resulted in an expanded autoantibody response as measured by fluorescent anti-nuclear antibodies on murine cell substrates and by the ability to blot multiple proteins of the murine snRNP, responses that resemble spontaneous lupus autoimmunity. These studies illustrate that T cells to self peptides exist in the normal repertoire and are capable of driving autoantibody production by the appropriate (or inappropriate) expression of self peptides. Once immunity is generated to a single self peptide, the response can diversify to other sites on the lupus autoantigens.

C2-452 SUPPRESSION OF HIV-SPECIFIC CTL RESPONSE BY LYMPHOCYTES FROM HELMINTH-INFECTED MICE, Margaret A. Marshall, Jeffrey K Actor, Alan Sher, and Jay A Berzofsky, Metabolism Branch, NCI, and Laboratory of Parasitic Disease, NIAID, NIH, Bethesda, MD 20892.

We have previously shown that the cytokine response of spleen cells from mice infected with *Schistosoma mansoni* to mitogens or to an unrelated antigen is shifted from a predominantly TH1 response to a predominantly TH2 response, and that the in vitro CTL response to unrelated antigens is diminished. The purpose of this study is to explore the mechanism by which infection with *S. mansoni* inhibits the CTL response to an unrelated antigen, HIV gp160 peptide P18IIIB. BALB/c mice were infected with 50 *S. mansoni* cercariae, and spleen cells were harvested 4 to15 weeks later and used as suppressor cells. Responder spleen cells were obtained from uninfected mice which had been inoculated i.p. with a recombinant vaccinia which expresses HIV gp160, and restimulated in vitro with P18IIIB, the predominant CTL epitope of gp160. Spleen cells from mice infected with *S. mansoni* not only make a markedly diminished in vitro CTL response to P18IIIB, but they also suppress the CTL response of spleen cells from noninfected mice when the two cell populations are mixed together during the in vitro restimulation culture. Time course experiments show that the suppression begins at about the time of egg deposition and increases through chronic infection. The suppressor cell expresses T cell markers. Experiments in which suppressor cells (from *S. mansoni* infected mice) are separated from responder cells (from noninfected mice) by a 0.4 micron membrane show that this novel suppression of CTL response may be mediated by a soluble factor. In conclusion, spleens of mice infected with *Schistosoma mansoni* contain T cells that can suppress the induction of a CTL response to an HIV antigen. This result may have a bearing on the progression of HIV disease in areas where helminth infection is endemic.

C2-453 IMMUNOGENICITY OF SOLUBLE BDC 2.5 TCR IN SYNGENEIC NOD MICE. Una McKeever, Sanjay Khandekar, John Newcomb, Pamela Brauer, Jerome Naylor, Paul Gregory, Michael Jesson, Amy Alderson, Julian Banerji, *Kathryn Haskins and Barry Jones. Procept Inc., Cambridge MA & *Barbara Davis Center for Childhood Diabetes, Denver, CO.

The BDC 2.5 (Vβ-4/Vα-1) and 6.9 (Vβ-4/Vα-13.1) CD4+ T cell clones from the NOD mouse are pancreatic islet antigen-specific and I-A^{g7} restricted. They accelerate diabetes in young NOD mice. Utilizing the baculovirus expression system we have produced soluble TCR either as chimeric proteins in which the α- and β-chains of the TCR are linked to the Fc portion of mouse IgG1 to form a disulfide-bonded dimeric molecule, or as truncated TCR molecules which lack their transmembrane and cytoplasmic segments. Using a panel of IgG1 chimeric and non-chimeric soluble TCR proteins derived from BDC 2.5, BDC 6.9, B10, & D10, T cell clones, we have analyzed the specificity of the T cell and antibody responses of BDC 2.5 TCR-IgG1 immunized syngeneic mice. *In vitro* T cell proliferative responses appeared to be directed towards constant region epitopes of both the α- and β-chains of the TCR. Results of indirect immunofluorescence showed that approximately 56% of 32 mice produced antibodies that bound to the BDC 2.5 T cell surface. Neither BDC 6.9 nor NOD splenic T cells were stained, thereby indicating the production of clonotype-specific antibodies. *In vitro*, these antisera blocked the I-A^{g7}-restricted, islet-antigen stimulated proliferative response of the BDC 2.5 T cell clone in a clonotype-specific fashion because they had virtually no effect on the BDC 6.9 response to the same antigen. Antisera raised against the soluble D10 TCR did not block either response. In ELISA with soluble TCRs adsorbed to plastic, all the antiserum samples from the same 32 mice were found to contain antibodies specific for constant region epitopes of both the α- and β-chains of the TCR.

The data demonstrate that a TCR can be immunogenic in mice of the strain in which it originated. Tolerance may fail to occur within the thymus because developing T cells are not exposed to MHC class II complexes containing TCR peptides. The antibody response appears to contain a component that is directed towards clonotypic epitopes of the TCR. The *in vitro* TCR-blocking activity of clonotype-specific antibodies suggests that immunization with soluble TCR might effect T cell clone-specific regulation *in vivo*, and thereby suppress the progression of diabetes.

C2-454 **EFFECTS OF PERIPHERAL TOLERANCE AND ANTI-B7 ANTIBODIES ON DISEASE RELAPSES AND EPITOPE SPREADING IN PLP-INDUCED MURINE EAE.** Stephen D. Miller, Carol J. Vanderlugt, Bradford L. McRae, Debra J. Lenschow, and Jeffrey A. Bluestone. Dept. of Micro-Immunology, Northwestern Univ. Med. School and Dept. of Pathology, Univ. of Chicago, Chicago, IL.

The efficacy of peripheral tolerance and antibodies directed against costimulatory molecules on treatment of ongoing relapsing-remitting EAE (R-EAE) in SJL/J mice was examined. Using peripherally induced immunologic tolerance induced by the i.v. injection of ECDI-fixed APCs as a probe to analyze the neuropathologic T cell repertoire, we determined that the majority of the immunopathologic reactivity during the acute phase of R-EAE induced by active immunization with the intact proteolipid (PLP) molecule is directed at the immunodominant $PLP_{139-151}$ epitope. However, following the initiation of acute tissue damage by $PLP_{139-151}$-specific T cells, endogenous host T cells specific for a secondary, non-crossreactive PLP epitope ($PLP_{178-191}$) are demonstrable (*i.e.*, epitope spreading). The recruited $PLP_{178-191}$-specific cells have encephalitogenic potential as they induce R-EAE upon adoptive secondary transfer to naive recipients. The $PLP_{178-191}$-specific response is activated as a result of and correlates with the degree of acute tissue damage since it does not develop in mice tolerized to the $PLP_{139-151}$ initiating epitope prior to expression of acute disease and can be regulated by induction of $PLP_{178-191}$-specific tolerance during acute disease. *In vivo* injection of either CTLA4-Ig or anti-B7-2 during the clinical remission has little effect on ameliorating subsequent relapses. In contrast, injection of anti-B7-1 results in a significant exacerbation in the incidence and severity of clinical relapses. However, treatment with the F(ab) fragment of anti-B7-1 during remission effectively prevents clinical relapses and the accompanying $PLP_{178-191}$-specific responses. These results strongly support a contributory role of T cell responses to epitopes released as a result of acute tissue damage to the immunopathogenesis of relapsing clinical episodes, indicate the importance of B7-mediated costimulatory molecules in this process, and have important implications for the design of specific and co-stimulatory directed immunotherapies for the treatment of chronic autoimmune disorders in humans.

C2-455 THE B CELL RESPONSE TO AUTOLOGOUS TYPE II COLLAGEN: BIASED V GENE REPERTOIRE WITH V GENE SHARING AND EPITOPE SHIFT. John A. Mo, Robert Karlsson and Rikard Holmdahl, Department of Medical Inflammation Research, Box 94, Lund University, S-221 00 Lund, Sweden and Biosensor, Pharmacia AB, Uppsala, Sweden.

Collagen induced arthritis (CIA) is an autoimmune model disease induced in the DBA/1 mouse immunized with type II collagen (CII). B cells are thought to play an important role in the disease both as producers of pathogenic anti-CII antibodies, and maybe also as antigen presenting cells for activation of autoreactive T cells. After a single immunization with CII, we have isolated high frequencies of V gene selected, CII specific and IgG positive B cell hybridomas from the draining lymph nodes. In the present study we analyze the V gene usage, epitope specificity and affinity of CII reactive B cell hybridomas, randomly isolated from the primary and secondary response in mice immunized with rat CII. We make the following conclusions: 1) There are major epitopes in the native CII molecule to which the B cells preferentially respond. 2) B cells specific for the same epitope show a preferential pairing of certain V_H/V_K genes, or a biased usage of individual V_H (V_H J558, V_H X24) or V_K genes (V_K 21). 3) The V genes are germline encoded in the primary response and somatically mutated in the secondary response. Somatic mutations give the antibodies crossreactivity between CII-epitopes and epitope shift, i.e another epitope within the CII molecule is recognized. 4) There is a sharing of certain V genes in B cell clones specific for different epitopes, indicating structural similarities of the different CII epitopes.

C2-456 THE ALTERED ABILITY OF HIV INFECTED MACROPHAGES TO ATTRACT AND ACTIVATE AUTOLOGOUS LYMPHOCYTES. Marilyn Moore, Donald Innes and Andrew Leigh-Brown. Centre for HIV Research, ICAPB, University of Edinburgh,Scotland.

Using primary human macrophage cultures, we set out to analyse the ability of these cells to act as antigen presenting cells (APCs) while infected with appropriate HIV variants. The macrophage tropic strain Ba-L was used to infect the cultures which were then followed for a period of one month. Cells were stained for viral antigens and assayed for APC function and compared to uninfected cells. We found that the infected macrophages transiently displayed enhanced ability to stimulate autologous CD4+ T cells to respond to recall antigens such as tetanus toxoid. This occured at days 5-7 post infection and was followed by a period of depressed APC function. We also observed a longer term ability of infected macrophages to stimulate autologous T cells in the absence of exogenous antigen. This correlated with an increased ability to attract lymphocytes to the surface of the infected APCs. The incoming lymphocytes are rapidly activated and we are currently looking at the profiles of cytokines and adhesion molecules involved and whether this activation permits infection of the T cells.

C2-457 RELATIONSHIPS OF CYTOKINE EXPRESSION AND CELL SIGNALING TO PATHOGENESIS OF MAIDS, A RETROVIRUS INDUCED IMMUNODEFICIENCY SYNDROME OF MICE. Renate Morawetz, Natasha Giese, Herbert C. Morse III, Laboratory of Immunopathology, NIAID, NIH, Bethesda, MD 20892

Development of MAIDS in susceptible B6 mice is associated with enhanced expression of transcripts for the Type 2 cytokines, IL-4 and IL-10, as well as increased expression of the Type 1 cytokines, IL-12, TNF-α and IFN-γ. In contrast, transcripts for IL-2 decrease with time after infection and progression of MAIDS. We first studied mice deficient in expression in IL-4, IL-10, IL-4 and IL-10 or IFN-γ as a result of gene knockouts. Mice deficient in IL-4, IL-10 or both cytokines showed normal induction of MAIDS, suggesting that these cytokines are not required for disease. In contrast, mice deficient in IFN-γ expression had a prolonged time course of disease. Further studies showed that IFN-γ effects on disease are dose-dependent as mice expressing high levels of IFN-γ resulting from in vivo treatment with IL-12 were suppressed for MAIDS. The results of these and other studies lead us to suggest a model for AIDS in which the cytokine pattern of MAIDS-sensitive mice reflects induction of anergy in $CD4^+$ T cells in association with activation and expansion of NK cells and activation of macrophages. Cellular interactions that could contribute to induction of T cell anergy while driving B cell activation will be discussed.

C2-458 GENETIC ANALYSIS OF SYSTEMIC LUPUS ERYTHEMATOSUS SUSCEPTIBILITY IN THE NZM MOUSE. L. Morel[1], U.H. Rudofsky[2], and E. K. Wakeland[1]. [1]Center for Mammalian Genetics and Department of Pathology, University of Florida, Gainesville, Fl, and [2]Wadsworth Center for Laboratories and Research, New York State Health Department, Albany, NY.

The inbred mouse strain NZM/Aeg2410, derived from an intercross between NZB and NZW, spontaneously develops highly penetrant (80%) SLE in both sexes by 6 months of age. We have used NZM/Aeg2410 to identify the number and positions of genomic intervals affecting SLE-susceptibility. We produced 158 (C57BL/6 X NZM2410)F1 X NZM2410 backcross progeny (BC1), with C57BL/6 used as a resistant strain. The BC1 progeny was monitored monthly from weaning to 1 year of age for proteinuria and autoantibody production. Animals were sacrificed at one year of age, or when found severely proteinuric, and their kidneys were histologically scored for glomerulonephritis (GN). Phenotypic analysis of the F1 and BC1 progeny showed that most of the GN-susceptibility alleles are recessive. However, dominant alleles play a major role in autoantibody production. We have initiated an analysis of an F2 cross to identify dominant alleles affecting GN and autoantibody production.

The genome of each BC1 progeny was typed with 75 polymorphic microsatellite markers and the locations of intervals containing GN-susceptibility loci were identified using MAPMAKER.QTL and by standard Chi-square analysis. Three intervals containing recessive NZM2410 GN-susceptibility alleles were identified: *Sle-1*, on chromosome 1 (LOD = 10.1); *Sle-2* , on chromosome 4 (LOD = 6.5); and *Sle-3*, on chromosome 7 (LOD = 4.0). In addition, the $H2^{b/z}$ heterozygous genotype at the MHC complex was also linked to GN-susceptibility. None of these loci is necessary or sufficient for GN expression. Instead, our results showed that GN is inherited as a threshold trait whose expression can be achieved by the combination of any 3 of these 4 susceptibility loci.

C2-459 TOLERANCE OF $CD8^+$ T CELLS SPECIFIC FOR THE INFLUENZA VIRUS HAEMAGGLUTININ EXPRESSED ON PANCREATIC ISLET BETA CELLS, **David J. Morgan*, Roland Liblau#, Hugh O. McDevitt# David Lo* and Linda A. Sherman*, *Dept. of Immunology, Scripps Research Institute, La Jolla, CA 92037. #Dept. of Microbiology and Immunology, Stanford University School of Medicine, Stanford, CA 94305.**

To study the mechanism for the induction of tolerance to peripheral tissue specific antigens, we generated transgenic mice that express the influenza virus A/PR/8 hemagglutinin (HA) on the pancreatic islet beta (b) cells (Ins-HA). After challenging with influenza A/PR/8 these mice did not develop autoimmune diabetes and were functionally tolerant to the b cell-specific HA. In addition, HA-specific CTL could not be demonstrated following *in vitro* stimulation with A/PR/8-infected cells. In contrast, when stimulated *in vitro* with HA peptide-pulsed cells HA peptide-specific CTL were generated. We suggest that these CTL maybe of lower affinity for the K^d/HA complex than those obtained from control animals. To determine the fate of the HA specific CTLs in Ins-HA mice we generated TCR-transgenic mice which express a TCR obtained from a CTL clone (clone-4) specific for an HA peptide restricted by H-$2K^d$ class I, and is recognised by F23.1 antibody (clone-4 TCR H-2^d). Initial characterization of the clone-4 TCR mice revealed that the majority of the thymocytes expressed high levels of the TCR, and in the peripheral lymphoid organs there were far more $CD8^+$ cells than $CD4^+$ cells. However, when H-2^b was present, there was a decrease in the percentage of TCR^{hi} in the thymus, whereas in the periphery the number of functional K^d HA-specific T cells was unaffected by the gene. We also generated double transgenic (clone 4-TCR x Ins-HA)F_1 mice that were homozygous for H-2^d. Initial characterization of these mice showed that the expression of the TCR in both the thymus and periphery at 2 day was similar to that seen in the adult clone-4 TCR (H-2^d) mice with the majority of cells expressing high levels of the TCR. These double transgenic mice developed autoimmune diabetes and were dead at day 7. In contrast, chimeric Ins-HA mice given TCR (H-2^d) bone marrow had a slight decrease in the level of clone-4 TCR^{hi} in the thymus and did not develop diabetes unless immunized with A/PR/8. Most interesting was the combined effect of both the HA and the H-2^b genes upon expression of the clone-4 TCR. In the thymus of double transgenic mice that were H-2^{bxd} the majority of the cells, although double positive for CD8 and CD4, were TCR negative. In the periphery there were no mature K^d HA-specific T cells. Consequently these H-2^{bxd} double transgenic mice did not develop diabetes, and so we concluded that the presence of H-2^b protects these mice from diabetes.

C2-460 MODULATION OF INFILTRATE SPECIALIZATION IN AUTOIMMUNE DIABETES BY REGULATORY CYTOKINES, Regula Mueller, Lise Wogensen, Myung-Shik Lee, and Nora Sarvetnick, The Scripps Research Institute, La Jolla, CA 92037

Cytokines are potent mediators of the immune system and have recently been implicated in the pathogenesis of autoimmune diseases such as insulin-dependent diabetes mellitus (IDDM). The development of autoimmunity is generally attributed to the type 1 subset of T helper cells (Th1) and the cytokines these cells secrete upon activation (e.g. interferon-gamma (IFN-g)), whereas Th2 cells and their cytokines (e.g. interleukin (IL)-4, IL-10) are regarded as protective. We have generated several lines of transgenic non-obese diabetic (NOD) mice, a murine model for IDDM, which express individual regulatory cytokines within the insulin-producing beta cells in the Langerhans' islets of the pancreas. They are excellent model systems to study how cytokines modulate the balance of Th1 and Th2 cells in the pancreatic infiltrates and how this modulation influences the pathogenicity of autoreactive T cells and the disease outcome. Characterization of the inflammatory foci in the pancreas of transgenic IL-10 mice by immunocytochemistry techniques revealed the predominant presence of Th2 cells as determined by intensive staining for IL-4. In IFN-g transgenic mice, only a few inflammatory cells stained positive for IL-4. A divergent disease development could be observed in IL-10 and IFN-g transgenic mice. Interestingly, the transgenic expression of IL-10 resulted in a acceleration of the onset and prevalence of diabetes as compared to nontransgenic littermates. Preliminary data on transgenic mice expressing IFN-g show no differences in blood glucose levels up to 10 weeks of age as compared to nontransgenic littermates. *In vitro* and *in vivo* experiments will now be carried out to characterize the differences in the functional specialization of the inflammatory cells in these mice in more detail.

C2-461 EXPERIMENTAL AUTOIMMUNE THYROIDITIS (EAT) INDUCED BY THYROXINE (T4) PEPTIDE. H. Braley-Mullen, H. Tang and G. Sharp, Dept. of Medicine, University of Missouri, Columbia, MO 65212.

Mouse thyroglobulin (MTg) primed spleen cells transfer classical lymphocytic (L) EAT after in vitro activation by MTg; MTg primed cells activated by MTg and anti-IL2R mAb M7/20 induce severe granulomatous EAT (G-EAT) differing qualitatively from L-EAT. CD4+ T cells are effector cells for both L and G-EAT; i.v. injection of dMTg induces tolerance of CD4+ effector cells resulting in suppression of anti-MTg autoantibody and an inability of cells to transfer L or G EAT after in vitro activation with MTg ± M7/20. Hutchings et al. (JEM 175:869, 1992) showed that a 12 amino acid peptide (2549-2559 of HTg) containing T4 at position 2553 induced EAT after immunization with T4 or activation of MTg-primed cells in vitro. To determine if T4 could induce G-EAT, mice were immunized with T4 or MTg and adjuvant (LPS) and cells were activated with T4 or MTg ± M7/20. Cells from MTg/LPS primed donors activated with T4 or MTg ± M7/20 transferred G-EAT; cells activated by T4 induced more severe G-EAT than cells activated by MTg. Immunization with T4/LPS and activation of cells by T4 ± M7/20 also induced G-EAT but lesions were less severe than those induced by T4-activated MTg primed cells. Activation by T4 but no M7/20 induced less severe EAT. Immunization with T4/LPS induced minimal autoantibody (MTg, HTg or T4-specific). T4-activated MTg-primed cells produced anti-MTg derived from primed donor B cells. T4 is apparently a dominant EAT-inducing peptide since i.v. injection of T4 prior to immunization with MTg/LPS was as effective as dMTg for inducing tolerance of CD4+ EAT effector cells, i.e. cells from T4 pretreated donors induced little or no EAT after activation by MTg. Tolerance induced by T4 resulted in minimal suppression of donor anti-MTg autoantibody indicating that tolerance of EAT effector cells can be dissociated from suppression of anti-MTg autoantibody. Studies are in progress to determine if T4 also suppresses effector cells for G-EAT. Supported in part by NIH DK-35527.

C2-462 **Generation of microbe- and tumor-specific CD4-independent CTLs by in vivo peptide immunization**, Janko Nikolić-Žugić, Ljiljana Vasović, Alberto Molano and Rubendra Dyall, Immunology Program, Sloan-Kettering Institute, New York, NY 10021

Cytotoxic T lymphocytes (CTL) combat intracellular pathogens by killing infected cells. The molecular targets of their attack are foreign peptides bound to self major histocompatibility complex (MHC) encoded class I molecules. Immunization of mice with peptides containing CTL determinants was shown to elicit CD4-dependent CTLs. Here, we have achieved in vivo CTL priming with naturally processed 8-10 amino acid long class I-restricted peptides emulsified in an adjuvant. A potent, reproducible and physiologically relevant response was obtained using peptides from an intracellular bacterium and five viruses (including the HIV) in two murine MHC haplotypes. This method is suitable for multiple vaccination, since a "cocktail" of peptides derived from three pathogens elicited effector CTLs against each pathogen. This method of CD8 stimulation was also effective in preventing development of solid tumors. Importantly, in all cases peptide-induced $CD8^+4^-$ CTLs were $CD4^+$-independent. These results have implications for *HIV* immunization and vaccine design.

C2-463 ANTI-IL12 ANTIBODY CAN PREVENT AUTOIMMUNE DIABETES IN AN ADOPTIVE TRANSFER MURINE MODEL, Lynn C. Ogata, Christina Reilly, Lori Anne M. Marconi, and David Lo, Department of Immunology, The Scripps Research Institute, La Jolla, CA 92037

Previous work from our laboratory has suggested that one mechanism of tolerance to peripheral antigen may be "clonal diversion" of autoreactive CD4+ cells from a TH1 to a TH2 phenotype. Since IL12 has been shown to promote IFNγ secretion, which in turn promotes differentiation to TH1 CD4+ cells, we investigated the effects of an anti-IL12 on autoimmune diabetes. InsHA.scid mice express influenza hemagglutinin (HA) specifically on pancreatic islet β cells. When HA specific, class II restricted TcR transgenic lymphocytes are adoptively transferred into InsHA.scid mice, autoimmune diabetes develops within 2 weeks. In contrast, animals that had received anti-IL12 Ab beginning one day prior to adoptive transfer did not became diabetic. Anti-IL12 also prevented diabetes even when the donor cells were from double transgenic TcRHNTxInsHA mice in which spontaneous autoimmunity had already developed in vivo. These data are novel because they suggest a role for IL12 in auto-immune responses. Interestingly, anti-IL12 protected mice still had islet infiltrates and spleen cells from these mice proliferated in vitro to peptide, demonstrating that antigen reactive cells were present. Although ELISA and RT PCR data show that restimulated spleen cells have IFNγ mRNA and are secreting IFNγ, preliminary data suggest that anti-IL12 treated mice have a higher IL4/IFNγ ratio than diabetic controls. These data are consistent with a clonal diversion model in which autoreactive CD4+ cells can be shifted to a nonpathogenic TH2 phenotype.

C2-464 DIHYDROTESTOSTERONE (DHT) IN AUTOIMMUNITY: DIFFERENTIAL ALTERATION OF STIMULATED IL-2 AND IL-4 LEVELS IN VITRO AND SUPPRESSION IN VIVO OF A SEXUALLY DIMORPHIC, DIABETES-LINKED PHENOTYPE, **Richard B. Pearce, Kirean Healey, Bent Formby, and Charles M. Peterson, Sansum Medical Research Foundation.**

The penetrance of autoimmune diabetes in NOD/Smrf mice is greater among females than males (66% *vs.* 5%). A role for androgens is suggested in that diabetes can be prevented by dihydrotestosterone (DHT) implants. Since diabetes is T-cell mediated, we examined whether or not DHT could affect T-cells directly. We found that cytokines released by Con A activated NOD splenocytes were differentially altered in vitro by physiological levels of DHT. Following a 60-minute exposure to 10(-7) M DHT and 24-hr culture in the presence of 4 μg/ml Con A, the levels of immunoreactive and bioactive IL-2 rose 22% while the level of immunoreactive IL-4 fell 39% (P<0.0001). DHT, over a wide rage of concentrations, had no effect on the level of high-affinity IL-2R. Another unusual feature of the NOD strain is an elevation of the percentage of CD4+ PBL. Mean %CD4+ for NOD is 48.6 ± 7.7 compared to 26.8 ± 3.2 for C57BL/6 and 30.3 ± 9.3 for the non-obese, non-diabetic (NON) strain. Within the NOD strain, female levels are higher than males (50.2 ± 5.2 vs. 44.0 ± 6.6; P = 0.008). Amongst [NOD X (NON X NOD)F1]BC1 segregants we observed that only females showing high levels of CD4+ T cells at an early age (4 wks) went on to develop diabetes later in life (>12 wks). Thus this T-cell phenotype, like diabetes, is sexually dimorphic and links with diabetic gene(s) and/or is necessary for the diabetic process. In vivo, DHT lowered the %CD4+ PBL to a male-like level (control, 46.8 ± 3.4 vs. DHT, 41.6 ± 2.9; P < 0.01). We conclude (1) that elevated CD4+ percentages are associated with the development of diabetes in female NOD mice and can be reduced by a regimen of DHT that also prevents diabetes, and (2) that DHT may exert some of its effects on the immune system by modulating Th1 and Th2 pathways. Androgen levels reportedly correlate inversely with disease progression in lupus and in AIDS. Our findings in a mouse model for autoimmune diabetes suggest a possible role for androgens as modulators of such Th1/Th2 activity.

C2-465 PEPTIDE AFFINITY FOR MHC CLASS II CORRELATES WITH EFFICACY OF PERIPHERAL ACTIVATION IN MBP-SPECIFIC TCR TRANSGENIC MICE. Cecelia I. Pearson, Roland S. Liblau, and Hugh O. McDevitt, Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, CA 94305.

Experimental autoimmune encephalomyelitis (EAE) is an autoimmune disorder in mice that has been extensively studied as a model of multiple sclerosis. Mice that express the class II molecule I-A^u are susceptible to EAE when induced with the N-terminal peptide of myelin basic protein (MBP), Ac1-11. EAE can be treated prior to induction of disease or at the time of disease onset with Ac1-11 and its analogs Ac1-11[4A] and Ac1-11[4Y], in which the native lysine at position four has been replaced with alanine or tyrosine, respectively. Currently, the mechanism for this treatment of EAE is not clear. We have established two lines of transgenic mice that express a Vβ8.2+, Vα4+ T cell receptor (TCR) that is specific for Ac1-11 and restricted to I-A^u in order to examine the fate of encephalitogenic T cells after immunotherapy with these peptides. In one line, about 80% of lymphocytes in the periphery express CD4 and Vβ8.2 in males, while about 60% express CD4 and Vβ8.2 in females, due to integration of the transgenes on the X chromosome. Intravenous injection of 1.2 mg of each of the three peptides revealed that Ac1-11, which binds relatively poorly to I-A^u, only induces low to moderate deletion of CD4+CD8+ double positive (DP) thymocytes, while Ac1-11[4A] and Ac1-11[4Y] induce moderate and high levels of deletion, respectively. In the periphery, the level of activation induced by these peptides also correlates with the affinity of the peptides for the MHC, as determined by the level of CD69, IL-2R, and TCR expression and the proliferative response. Higher doses of each of the three peptides more efficiently deleted DP thymocytes. Furthermore, higher doses induced an initial hyperresponsiveness followed by unresponsiveness. Finally, the induction of unresponsiveness may depend on the number of T cells present in the periphery, as 2.4 mg of peptide administered to females induces unresponsiveness, while the same dose induces only activation in males. These results suggest that peptide immunotherapy may induce an initial activation phase, which is followed by a state of unresponsiveness, preventing EAE.

C2-466 PATHOGEN ANTIGEN- AND SUPERANTIGEN-REACTIVE SYNOVIAL FLUID T CELLS IN REACTIVE ARTHRITIS. Gary Peltz, Carol Soderberg, Andrea Allsup, Reijo Luukkainen*, Takehiko Uchiyama#, and Riitta Lahesmaa. Department of Leukocyte Biology, Syntex Research, Palo Alto, CA 94303; *Satalinna Hospital, SF-29200 Harjavalta, Finland; #Department of Microbiology and Immunology, Tokyo Women's Medical College, Tokyo, Japan.

We have analyzed pathogen-reactive T cell clones ($CD3^+4^+8^-TCR\alpha\beta^+$), isolated from synovial fluid of two $HLA\text{-}B27^+$ patients with *Yersinia enterocolitica*-triggered reactive arthritis. Pathogenic strains of *Y. enterocolitica* harbor a virulence plasmid. This virulence plasmid encodes for a set of proteins, that are released when the bacteria are cultured in Ca^{2+}-deficient media. We demonstrate that these secreted proteins, including a protein with tyrosine phosphatase activity (YopH), are potent immunogens stimulating $CD4^+$ T cells within the inflamed joint. Furthermore, the pathogen-reactive T cell clones present at an early stage of active synovitis preferentially utilized a limited set of TCR variable region β-gene segments. Finally, a purified *Yersinia* superantigen triggers an *in vitro* proliferative response in the *Yersinia* antigen-reactive T cell clones expressing preferentially selected TCR Vβ elements. These results suggest that a superantigen of *Yersinia* influences the cellular immune responses to its conventional antigens, which may play a role in the pathogenesis of reactive arthritis.

C2-467 snRNP TRANSGENES IN MRL MICE. Stanford L. Peng, Jian Ma, Saeed Fatenejad and Joe Craft, Department of Biology and Section of Rheumatology, Department of Internal Medicine, Yale University School of Medicine, New Haven, CT 06510.

MRL/Mp-*lpr/lpr* and MRL/Mp-+/+ mice spontaneously develop a disease that resembles the human disease systemic lupus erythematosus (SLE), including the production of affinity-matured IgG autoantibodies to chromatin and small nuclear ribonucleoprotein particles (snRNPs). Previous studies on the immune system in SLE have provided evidence suggesting the necessity of self-antigen as well as T-cell help in the generation of these autoantibodies. In order to further characterize the immunologic disorder in the murine SLE model, we have begun analyzing T- and B-cell responses in MRL and normal (B10.BR) mice. Our data demonstrate that snRNP-specific autoreactive T-cells exist in both the MRL and normal immune repertoire, and that these cells can provide help for the synthesis of anti-snRNP antibodies. Further, the production of these antibodies requires the presence of intact snRNP particles, with certain proteins within the particles behaving as immunodominant components and providing a focus upon which the antibody response diversifies. These findings suggest that autoimmunity in the MRL strain results from a subtle aberrancy in immune tolerance, not conspicuously revealed by this and previous work. As a result, we have decided to analyze T- and B-cell tolerance in these and normal animals in the presence of a snRNP transgene. Other workers have previously demonstrated the predictable tolerogenicity of such constructs for otherwise foreign antigens; such an approach for snRNPs should render tolerant to such antigens both the normal and autoimmune immune repertoires. Specifically, this model will help address questions concerning the capabilities of B- and/or T-cell tolerance in this autoimmune environment, and it will also address the possible role of immunodominant autoantigen proteins in the diversification of autoantibodies.

C2-468 **HIV-1 LOAD SKEWED TO THE Vβ12 SUBSET: EVIDENCE FOR A SUPERANTIGEN.** David N. Posnett, Dana Dobrescu, Adam Asch and Andrew S. Hodtsev, Dep. of Medicine, Cornell University Medical School, New York, NY 10021.

HIV-1 replicates 10-100 fold more efficiently in T cell lines expressing Vβ12 T cell receptors (TCR) than in Vβ6.7a cell lines, suggestive of the effects of a superantigen (SAG). To assess whether Vβ12 cells represent an *in vivo* viral reservoir in HIV-1 *infected patients*, viral load was measured by quantitative PCR with *gag* primers and with an infectivity assay for competent virus. In 16/23 patients (70%), the Vβ12 subset (2.1+/-1.2 % of CD4 cells) had a 2-360 fold greater HIV-1 load than the control Vβ6.7a subset (3.5+/-1.7 % of CD4 cells). Only 2/23 patients (9%) had more viral copies/1000 cells in the Vβ6.7a subset than in the Vβ12 subset and in 5/23 patients HIV-1 was distributed equally among both subsets. HIV-1 skewing to Vβ12 cells did not correlate with the stage of the disease and was often prominent in asymptomatic patients. However, their was a significant correlation with serum antibodies to CMV.

Selective HIV-1 replication in Vβ12 cells was also observed 6-8 days following *in vitro* infection of T cells from HIV-1 neg. donors. In a similar manner HIV-1 replication could be targeted to specific Vβ subsets by adding exogenous SAG: the Vβ17 subset for MAM (Mycoplasma Arthritidis SAG), the Vβ12 subset for DAP fibroblasts transfected with *mtv*-7 and the Vβ6.7a subset for DAP transfected with *mtv*-9. Therefore, HIV-1 replication targeted to Vβ12 cells may be due to a yet unidentified SAG, which serves to promote a biologically relevant viral reservoir.

C2-469 REGULATION OF PATHOGENIC TH1 RESPONSES AGAINST IMMUNOLOGICAL SELF ANTIGENS BY CD45RB^{LOW} CD4^{+} T CELLS : ROLE OF IL-4, IL-10 & TGF-β. Fiona Powrie, Joseph A. Carlino† and Robert L. Coffman, DNAX Research Institute of Molecular and Cellular Biology Inc., 901 California Ave, Palo Alto, CA 94304. † Celltrix Pharmaceuticals Inc. 3055 Patrick Henry Dr., Santa Clara, CA 95054.

Transfer of CD45RB^{high} CD4^{+} T cells from normal BALB/c mice to C.B-17 *scid* mice leads to the development of a progressive wasting disease and severe colitis. Th1 responses are involved in the pathogenesis of this disease as administration of anti-IFN-γ or anti-TNF mAbs prevented disease induction. Importantly, animals restored with the reciprocal CD45RB^{low} CD4^{+} subset did not develop wasting or colitis and this population, when co-transferred with the CD45RB^{high} population completely prevented wasting disease and colitis. These data indicate that CD4^{+} T cells from normal mice have the capacity to induce a lethal inflammatory response in the colon but that under normal circumstances this response is inhibited by a phenotypically distinct subpopulation of CD4^{+} T cells. CD45RB^{low} cells were capable of preventing colitis when transferred up to 3 weeks after CD45RB^{high} cells, suggesting the inhibitory effect was not due solely to effects on homing of the CD45RB^{high} population. Pathological changes are clearly present in the colon at this time, suggesting that the CD45RB^{low} population can reverse this process Studies with anti-cytokine mAbs suggest that TGF-β but not IL-4 or IL-10 is involved in the inhibition of colitis by the CD45RB^{low} population. However, systemic administration of rIL-10 was able to prevent disease induction. Further analysis of the role of IL-4 and IL-10 in the regulation of colitis are being pursued using IL-4 or IL-10 deficient mice as donors of the CD45RB^{low} popuiation and these results will be discussed.

C2-470 THYROTROPIN RECEPTOR SPECIFIC ANTIBODIES IN BALB/cJ MICE WITH EXPERIMENTAL HYPERTHYROXINEMIA SHOW A RESTRICTED BINDING SPECIFICITY AND BELONG TO THE IgG1 SUBCLASS, Bellur S. Prabhakar, John S. Dallas, John C. Morris and Neelam M. Wagle, Departments of Microbiology/Immunology and Pediatrics, University of Texas Medical Branch, Galveston, TX 77555 and Division of Endocrinology, Mayo Clinic, Rochester, MN.

Following immunization with the extracellular domain of the thyrotropin receptor (ETSHR) BALB/cJ mice developed hyperthyroxinemia, whereas, C57BL/cJ, SJL/J and B10.BR mice did not. Earlier, human studies had shown that thyroid stimulating antibodies are predominantly of the IgG1 subclass with a narrow specificity to TSHR and antibodies that block thyroid function could be of any subclass with a broader specificity. To see whether similar differences in antibody responses could be found in susceptible (BALB/cJ) and resistant (SJL/J) mice, we characterized their antibody responses against the TSHR. There were no significant differences in the titers, relative affinities and isotypes of antibodies against the TSHR between these two strains of mice. When sera were tested against 26 overlapping peptides from ETSHR, BALB/cJ mice showed reactivity with only two peptides. In contrast, SJL/J mice showed reactivity with seven peptides. The ability of sera from BALB/cJ and SJL/J mice, to block TSH binding to TSHR was reversed by one and six of the reactive peptides respectively. BALB/cJ mice showed predominantly an IgG1 response against the ETSHR and peptides, whereas, SJL/J mice showed varying levels of all subclasses of IgG. Although, SJL/J sera reacted with peptides to which blocking antibodies bind, these mice did not show hypothyroidism. These data showed that antibodies in SJL/J mice were heterogeneous and likely contained a mixture of blocking and stimulating antibodies which negated the effects of each other. In contrast, the TSHR specific antibodies in BALB/cJ were relatively homogeneous and most likely represented stimulating antibodies directed against conformational epitopes.

C2-471 **ASPARTATE AT POSITION 57 OF NOD I-Ag^{7} BETA CHAIN REDUCES THE SPONTANEOUS INCIDENCE OF DIABETES.** **Ruby Quartey-Papafio, T Lund,* Phil Chandler,§ J Picard,§ P Ozegbe, S Day, P R Hutchings, L O'Reilly, D Kioussis,# E Simpson§ and A Cooke. Department of Pathology, University of Cambridge, *Department of Immunology, University college London Medical School, London. §MRC Clinical Sciences Centre, Hammersmith, London. #National Institute for Medical Reasearch, Mill Hill, London.**

The non-obese diabetic (NOD) mouse spontaneously develops IDDM which results in the destruction of the β cells of the islets of langerhans. One of the disease susceptibility genes has been mapped to the MHC class II region. In order to examine the role of the unusual NOD Aβ chain in the development of IDDM, we have generated transgenic NOD mice (NOD-A-Asp) which express an aspartate at position 57 of the Aβ chain rather than the serine found in this position in the NOD mouse. These transgenic mice have a reduced incidence of disease though periislet infiltration is still present. T cell clones have been established which can distinguish between peptide presented by the I-Ag^{7} and I-Ag^{7}Asp molecules. Our data suggest that the disease causing T cells have not been wholly deleted from the NOD-A-Asp mice but that the diabetogenic peptide is either inefficiently presented to the T cells or that presentation results in an anergic signal.

C2-472 *IN VITRO* IMMUNOMODULATING EFFECTS OF INTERFERON-β, Martin H.G. Rep[1], Rogier Q. Hintzen[1,2], Chris H. Polman[2] and René A.W. van Lier[1], [1]*Central Laboratory of the Netherlands Red Cross Blood Transfusion Service and Laboratory of Experimental and Clinical Immunology of the University of Amsterdam; and The* [2]*Department of Neurology, Free University Hospital, Amsterdam*

Recently, results of clinical trials have shown that recombinant interferon-β (rIFN-β) is a very promising drug for the treatment of Multiple Sclerosis (MS) patients. In this study we show that the addition of increasing amounts of rIFN-β results in a dose- and also donor-dependent decrease in *in vitro* T cell proliferation, which never exceeds 50 % with the highest concentration used (10.000 U/ml). Similarly, the production of the Th1-like cytokines IFN-γ and TNF-α, measured after stimulation with a triplet of CD2 mAb in combination with CD28 mAb is dose-dependently inhibited by interferon β. However, production of IL-4 is not affected by rIFN-β, while IL-10 production shows a marked increase, up to about 350 % of the initial value. Also a higher concentration of IL-2 can be measured, which coincided with a lower expression of IL-2 receptor (IL2R) on $CD4^+$ T lymphocytes. Markedly, *in vitro* B cell proliferation and differentiation, measured after stimulation with IgM and CD40 mAb, in the absence or presence of IL-2 and IL-4, is already affected at much lower concentrations of interferon β. At the highest concentration used (10.000 U/ml) B cell proliferation and IgG production is completely blocked. Phenotypical analysis of these cells showed that, analogous to T cells, expression of IL-2R is reduced upon addition of rIFN-β. In conclusion, compared to T cells, B cells seem to be much more sensitive to the effects of interferon β. Interindividual variation in the responsiveness of T cells to interferon β may prove to be useful for pretrial assessment of MS patients.

C2-473 IG-SPECIFIC T CELLS REGULATE THE FATE OF THE B CELLS DURING THE IMMUNE RESPONSE IN A TCR TRANSGENIC MOUSE MODEL, Paola Ricciardi-Castagnoli, Giulia Marconi, Maria Rescigno, Maria Foti, Benedetta Cavanna and Francesca Granucci , CNR, Center of Cytopharmacology, 20129 Milan Italy.

To study T and B cells interactions a TCR transgenic mouse model was produced. The TCR is I-A^d restricted and is specific for an epitope in the $IgG2a^b$ CH3 domain. Two different transgenic mice were made on the following genetic backgrounds: Balb/c (*Igh*-1^a : Tg+b-) and Balb/c x CB17 (*Igh*-$1^{a/b}$: Tg+b+). The Tg+b+ mice transgenic T cells were not deleted in the thymus and their presence in the periphery, caused the disappearance of $IgG2a^b$ secreting cells. Consistently, $IgG2a^b$ were almost undetectable in the serum. Moreover, Tg+b+ lymphnode T cells, did not proliferate in vitro, in response to the specific peptide. Transgenic mice were immunized with Salmonella Typhimurium. Three weeks after this challenge, $IgG2a^b$ serum level raised to 10 μg/ml, though keeping 50 times lower than in control mice. Interestingly, a 5-fold increase of IgE serum level was observed when compared to the non transgenic mice. Further, transgenic T cells from immunized mice could proliferate in vitro by stimulating with the specific peptide, and produce IFNγ. These results suggest that anti IgG2a T cells might exist in normal animals and play a regulatory role in the control of IgG2a to IgE class switch.

C2-474 **INHIBITION OF EAE INDUCTION AND PROGRESSION WITH ANTI-INTEGRIN AND ANTI-SELECTIN ANTIBODIES**, Yacov Ron, Ethel Gordon, Kathleen J. Myers, and Joseph P. Dougherty, Department of Molecular Genetics and Microbiology, University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School, 675 Hoes Lane, Piscataway, NJ 08854; and the Graduate Program in Microbiology and Molecular Genetics, Rutgers University, Piscataway, NJ 08855

Experimental autoimmune encephalomyelitis (EAE) is a demyelinating disease of the central nervous system (CNS) induced in rodents by myelin-specific $CD4^+$ T cells. The disease is characterized by breach of the blood brain barrier, perivascular infiltration of leukocytes into the CNS, local inflammation and demyelination manifested in the form of plaques. The clinical and histological signs of EAE are very similar to those of multiple sclerosis in humans. In this study, we evaluated the ability of antibodies against two members of the β2 integrin sub-family of adhesion molecules (CD11a and CD11b), and against P- and E-selectin, to inhibit the onset and progression of EAE. Anti-CD11a antibodies could completely block the induction of EAE whereas anti-CD11b antibodies significantly delayed the onset and diminished disease severity even when injections were initiated at the first appearance of clinical signs. Anti P- and E-selectin antibodies had only a marginal effect, slightly delaying onset and severity of EAE. P- and E-selectin knock out mice are now being tested. We suggest that these effects are mainly due to blocking the entry of T cells into the CNS. However, in the case of CD11a inhibition may also be due to partial blocking of APC-lymphocyte interactions. We have not observed any side effects in treated animals and the effects of these treatments on general immune responses were transient.

C2-475 THE SELECTIVE BLOCKADE OF CLASS I PROCESSING/PRESENTATION OF AN IMMUNOGENIC VIRAL TRANSCRIPTION FACTOR INVOLVES THE PROTEIN KINASE FUNCTION OF A CMV MATRIX PROTEIN. Jennifer Rosser, Mark Gilbert, Stan Riddell, and Philip Greenberg, Fred Hutchinson Cancer Research Center and Univ. of Washington, Seattle, WA 98104

IE1, an abundantly expressed CMV transcription factor, is essential for viral replication and could potentially serve as a major target antigen for cytotoxic T lymphocytes (Tc). However, IE1-specific Tc which can efficiently lyse targets expressing IE1 alone, do not recognize IE1 expressing targets in the presence of the CMV matrix protein pp65. This effect of pp65 is selective for IE1, occurs prior to the down-regulation of Class I in CMV infected cells, and prevents the presentation of IE1 epitopes restricted by more than six HLA alleles, and taken together suggests a novel mechanism other than peptide competition. To determine which domains of pp65 are required for this effect, deletion mutants of pp65 were constructed and tested for their ability to block IE1 presentation by coinfection of target cells with vaccinia recombinants encoding these proteins. Coexpression of one pp65 mutant, Δ65, from which the serine/threonine kinase domain was deleted resulted in the efficient presentation of IE1 suggesting that this catalytic function is required for the effect. Although pp65 could phosphorylate any one of a number of cellular or viral proteins, IE1 is a phosphoprotein itself that is a substrate of a protein kinase. Thus, deletion mutants of IE1 were generated to determine what regions might be modified by pp65. Preliminary studies with a mutant of IE1 (ΔIE) which lacks the first 85 amino acids of the protein have demonstrated that the presentation of this mutant is not influenced by pp65 coexpression, suggesting that the N-terminus of IE1 may be phosphorylated by pp65. To determine if a direct association between pp65 and IE1 occurs, studies are currently underway using a two-hybrid system to detect protein-protein interactions between this pair of viral proteins *in vitro*. In this system, the proteins to be tested are fused to either the activation or DNA binding domains of the GAL4 transcriptional activator and coexpressed in yeast cells. Direct interaction between the proteins induces the transcription of a lacZ reporter gene, yielding a positive signal. Wild type and mutant forms of the pp65 and IE genes are currently being studied in this system and will be discussed. Determination of how pp65 induces a blockade of IE1 presentation may provide a means of rendering antigens less immunogenic.

C2-476 HIV-SPECIFIC CYTOTOXIC T CELLS IN HIGHLY EXPOSED BUT UNINFECTED GAMBIAN WOMEN, Sarah Rowland-Jones, J. Sutton, K. Ariyoshi, T. Dong, F. Gotch, S. Macadam, S. Sabally, D. Whitby, A. Gallimore, T. Schultz, M. Takaguchi, A. McMichael and H. Whittle. Molecular Immunology Group, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, OX3 7TW U.K.

To design an effective vaccine against HIV, it is crucial to know if protective immunity can follow natural exposure, and one way of addressing this is to study people exposed to HIV who remain uninfected. We have detected MHC class I-restricted HIV-specific CTL in a group of repeatedly HIV-1 & 2 exposed but persistently seronegative female sex-workers in The Gambia, West Africa. We first identified peptide epitopes from HIV-1 & HIV-2 recognised by CTL from infected Gambians with the common Gambian HLA molecule, HLA-B35, which showed cross-reactivity between HIV-1 & HIV-2. These peptides were used to stimulate CTL from peripheral blood mononuclear cells of seronegative Gambian prostitutes. In three out of six women with HLA-B35, high levels of specific CTL were detected against one or more of these peptides. These women have no evidence of HIV infection by highly sensitive techniques, and CTL were not detected by the same stimulation protocol in control donors at low risk of HIV infection. We believe that HIV-specific CTL activity in these highly exposed but apparently uninfected women may represent protective immunity to HIV generated in response to repeated exposure, despite the absence of antibody.

C2-477 CONTROL OF SELF-TOLERANCE BY FREQUENCY OF SELF-REACTIVE T CELLS. Susanne C. Schneider, N. Avrion Mitchison, Deutsches Rheumaforschungszentrum, Berlin, Germany

Mechansisms of self-tolerance of 4-hydroxyphenylpyruvate dioxygenase (HPPD) are heres explored. It is well established that negative selection based on TCR affinity occures in the thymus. We here provide evidence of an auxilary mechanism based on the frequency of self-reactive T cells in the periphery which also mediates self-tolerance. Mice immunized with the self-form of HPPD gave rise to T-cell hybridomas able to recognize self-protein and a synthetic peptide representing the T cell epitope, at a higher Ag concentration than necessary for recognition of allo-protein. The efficiency of negative selection was then reduced by treating neonatal mice with anti-HPPD antiserum. This reduced T-cell tolerance of the self-protein, as judged by in vitro proliferation, and enabled sefl-reactive T-cell hybridomas to be generated at a higher frequency. However, the Ag concentration requirements of these hybridomas for the self-protein and the self-peptide remained unaltered. This provides a situation in which repertoire frequency determines tolerance: tolerance of the self-protein is maintained simply by keeping the frequency of self-reactive T cells low, even though they are potentially able to respond if their frequency rises.

C2-478 OTHER CNS MACROPHAGES BUT NOT FRESHLY ISOLATED ADULT MICROGLIA ACT AS APC FOR MBP-REACTIVE T CELLS *IN VITRO*. Jonathon D. Sedgwick and Andrew L. Ford. Centenary Institute of Cancer Medicine and Cell Biology, Building 93, Royal Prince Alfred Hospital, Missenden Road, Sydney Australia.

Cells of the macrophage/monocyte lineage in the CNS are the cell types thought most likely to be the predominant CNS APC by virtue of their propensity to constitutive or inducible MHC class II expression *in vivo*. But what can the substantial population of CNS-resident macrophages (microglia) do in this context? We previously defined a flow cytometric phenotype for microglia ($CD45^{low}$ CD11b/c$^+$) that distinguished these cells from all blood-derived leukocytes, the latter being $CD45^{high}$. Within the $CD45^{high}$ population are other CNS-associated macrophages, distinct from microglia, which also express the CD11b/c molecules. Cells have been recovered from the normal CNS as well as from rats with graft versus host disease in which the microglia are highly activated (increased number, size and MHC class II expression), and the two CD11b/c$^+$ populations sorted to homogeneity. When added to MBP-reactive T cells *in vitro* in the presence of whole MBP or specific peptide, it is the $CD45^{high}$ CNS macrophages, which exhibit the most potent APC capacity (T cell proliferation and IL-2 secretion). There is also some evidence that this same cell population is presenting 'endogenous' MBP (that is, antigen picked up in the CNS), as T cells proliferate to some extent in the presence of these cells but in the absence of added MBP. Surprisingly, microglia whether activated (ie, high MHC class II expression) or not, are consistently poor in terms of their ability to stimulate T cells to proliferation or cytokine secretion, even in the presence of added MBP or peptide. Thus, unlike 'microglial' cells derived from fetal or newborn CNS by long term cell culture, those directly from the adult CNS show little evidence of antigen presenting cell function, even when MHC class II expression induced *in vivo* is substantial. Conversely, the minority population of 'other' CNS macrophages, which could include perivascular cells or choroid macrophages, are potent APC. Whether microglial cells are actively inhibitory for T cell responses (eg secretion of suppressive/toxic mediators) or induce a state of T cell anergy, is being investigated.

C2-479 MHC CLASS I *TRANS*-REGULATORY DEFECTS IN AUTOIMMUNE DIABETES PRONE NOD MICE. David Serreze, Harold Chapman, and Edward Leiter, The Jackson Laboratory, Bar Harbor, ME 04609

While clearly mediated by T cells, autoimmune diabetes in NOD mice is initiated at the level of hematopoietically derived APC, with the major component of susceptibility residing within the $H2^{g7}$ (K^d, $I\text{-}A^{g7}$, $I\text{-}E^{null}$, D^b) MHC. Transgenic analysis has established that diabetogenesis requires APC homozygously express the unusual $I\text{-}A^{g7}$ molecule in the absence of I-E. However, we have found that diabetes fails to develop in NOD mice congenic for a ß2 microglobulin locus functionally disrupted by homologous recombination. This indicates the common class I alleles of $H2^{g7}$ (e.g. K^d, D^b) also serve a pathogenic function, which most likely is to select and target ß cell autoreactive CD8$^+$ T cells. Thus, we evaluated the regulation of MHC class I expression in peritoneal macrophages (PM) from NOD mice, to those from NOR mice, a diabetes resistant control strain which shares approximately 88% of its genome with NOD, including $H\text{-}2^{g7}$, but carries C57BL/KsJ (BKs)-derived genomic elements on regions of chromosomes 1, 2, 4, 5, 7, 11, 12, and 18. Constituitive levels of MHC class I expression were equivalent in PM from NOD, NOR, and BKs mice. However, following a 6 day exposure to IFNγ, PM from NOD mice failed to up-regulate either MHC class I mRNA or protein, while these parameters were upregulated normally in IFNγ treated PM from NOR mice. This was not due to dysfunctions of the intra-$H2^{g7}$ *Tap* genes shared by NOR and NOD. Gel mobility shift analysis revealed that IFNγ treatment of PM from NOR, but not NOD mice, increased expression of *trans*-acting nuclear proteins binding to two different sites within the $H2K^d$ promoter. One of these was the interferon response element (IRE) at sites -186 to -169, with the second being the A/AP-1 element at sites -234 to -217, which binds *fos-jun* heterodimers. No differences were detected in nuclear protein binding to a second *fos-jun* site (B/AP-1) located a positions -137 to -119. Segregation analysis of (NOD x NOR)F2 probands indicated that the major BKs-derived genetic component contributing to diabetes resistance in NOR is located on Chromosome 2, with weaker modifiers on Chromosomes 1 and 4. We are now investigating whether NOR genome on Chr 2 contributes to the differential *trans*-regulation of $H2^{g7}$ class I expression in PM from NOD and NOR mice. This may provide insight as to whether aberrant *trans*-regulation of MHC class I gene expression is a key event in initiating the diabetogenic process.

C2-480 **THE ROLE OF B CELLS IN LPR/LPR-INDUCED AUTOIMMUNITY**

#*Mark J. Shlomchik, ^Michael P. Madaio, *Donghui Ni, &Mary Trounstein, and &Dennis Huszar. *Fox Chase Cancer Center, Philadelphia, PA; #Department of Laboratory Medicine and Section of Immunobiology, Yale University School of Medicine, New Haven, CT; ^Penn Center for the Molecular Studies of Renal Disease, The Renal, Electrolyte and Hypertension Division, Department of Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA; &GenPharm International, Inc., Mountainview, CA

The primary roles of T cells and B cells in the initiation of systemic autoimmunity are unclear. To investigate the role of B cells, we have crossed the "Jh knockout" mutation onto the autoimmune lpr/lpr background. Animals homozygous for both traits were obtained. As expected, these animals lack B cells. These animals also show no signs of autoimmune kidney destruction nor of vasculitis, in spite of carrying the lpr/lpr mutation. In contrast, lpr/lpr littermates which had B cells had severe nephritis and vasculitis, as well as autoantibodies. These results demonstrate a primary role for B cells and/or (auto)antibodies in initiating several types of autoimmune-mediated tissue destruction. The implications of this finding for models and therapy of autoimmunity are discussed.

C2-481 DIFFERENTIAL SIGNALING OF T CELLS BY CROSS-REACTIVE SELF-DETERMINANTS PRESENT ON DIFFERENT SELF-PROTEINS COEXPRESSED IN THE SAME INDIVIDUAL, Luis R. B. Soares, Patricia L. Orr, Marvin R. Garovoy and Gilles Benichou, Department of Surgery. UCSF Medical School, San Francisco, CA 94143-0508.

T cells are activated through cross-linking of their antigen receptor (TCR) following recognition of the relevant peptide associated with the appropriate MHC molecule. It is still unclear how T cell repertoire directed towards a myriad of foreign peptides can be positively selected in the developing thymus in absence of the corresponding foreign proteins. Similarly, self-reactive T cells are selected to a large variety of self-proteins which apparently never access the thymus during ontogeny (developmentally regulated and tissue specific proteins). Therefore, the number of self-peptides available for presentation to nascent T cells in the thymus is not sufficient to determine the wide variety of T cells that are positively selected during development. On the other hand, while exquisite specificity has always been the hallmark of antigen recognition by T cells, recent evidence has emerged that individual T clones specific for a given peptide can also recognize other structurally and sequentially related peptides. It is therefore possible, that in the absence of certain self- and all foreign antigens, degenerate recognition by developing T cells of the pool of self-peptides presented on thymic APCs represents the main driving force for positive selection. Here, we studied the relationship between a series of sequentially related self- and nonself-peptides corresponding to residues 61 to 80 on different individual MHC class I proteins. Following immunization of B10.A mice (K^k, A^k, E^k, L^d, D^d) with the self-peptide L^d 61-80, T cells could be restimulated to proliferate in vitro either to the priming peptide or to another self-peptide, K^k 61-80, as well as to different foreign class I peptides (residues 61-80). Surprisingly, in contrast to the priming L^d 61-80 peptide, the cross-reactive self-K^k 61-80 failed to induce in vitro γ-Interferon production by primed T cells. Furthermore, restoration of γ-Interferon secretion in the presence of the cross-reactive self-K^k peptide could not be achieved by the addition of anti-CD28 antibodies or activated APCs to the culture. Therefore, although autoreactive T cells could proliferate to both priming and cross-reactive self-peptides, they did not however receive the same signals by each of these peptides as shown by their different lymphokine patterns. The relevance of this finding to both induction and regulation of auto-reactivity will be addressed.

C2-482 **BREAKING IMMUNOLOGICAL IGNORANCE TO SV40 LARGE T ANTIGENIC PEPTIDE REQUIRES PRIMING AND COSTIMULATION**

Gloria Soldevila, Terrence Geiger and Richard Flavell. Section of Immunobiology. Yale University School of Medicine. New Haven, CT 06510.

We have generated transgenic mice that express a non-transforming antigenic fragment of the SV40 large T antigen in the pancreatic beta cells. SV40 T antigen transgenic lines which showed no thymic expression of the transgenic RNA were crossed with T cell receptor (TCR) transgenic mice that recognize the antigenic peptide 559-576 of the SV40 large T oncoprotein in the context of H-$2K^k$. TCR x SV40 double transgenic mice do not respond *in vivo* to the SV40 large T antigenic fragment expressed in the islets, even though they are fully responsive to SV40 T ag, as assessed in *in vitro* proliferation assays. Furthermore, immunisation of double TCR X SV40 transgenic mice with SV40 large T antigen expressing splenocytes does not lead to an autoimmune destruction of beta cells. However, the immunologic ignorance seen *in vivo* is broken and diabetes induced when primed SV40 large T antigen reactive T cells are transferred into mice that co-express the B7 costimulatory molecule and the SV40 large T antigenic fragment. This demonstrates that at least in our model the presence of (primed) reactive T cells is not sufficient to break tolerance and that the B7 costimulatory molecule is required to elicit an autoimmune response.

C2-483 MICE TRANSGENIC FOR HLA-DR4 AND HUMAN CD4 : AN ANIMAL. MODEL TO EXPLORE THE IMMUNOGENIC T CELL EPITOPES OF SELF ANTIGENS IN HUMAN AUTOIMMUNE DISEASE. *Grete Sønderstrup-McDevitt, *Mauro Congia, *Andrew Cope, *Norbert Hain, *Jonathan Rothbard, and #Lars Fugger. #University of Copenhagen, *ImmuLogic, Palo Alto, *Stanford University Medical School, Stanford CA 94305.

Mice transgenic for the human class II gene HLA-DR4 (B*0401) and human CD4 were produced in H-$2^{f,q}$ (I-E negative) animals. Because of the introduction of an I-E equivalent (HLA-DR) these animals deleted a large number of T cell receptor Vβ families according to the retrovirus types build into the genome of these mouse strains. A number of $CD4^+$ T cell hybridomas were generated after immunization with bovine collagen type II (CO II). A majority of the HLA-DR4 restricted hybridomas recognized one single synthetic peptide (SP) epitope, corresponding to a stretch of the CO II molecule, where human and bovine CO II are identical. None of the remaining CO II specific DR4 restricted T cell hybridomas did respond, when they were challenged with pools of SP's covering the entire CO II molecule. Since the CO II molecule normally is glycosylated *in vivo*, we believe, that the specificity of these T cell hybridomas depends on a post-translational modification, such as a glycosylation, of some of the aminoacids in the important peptide epitope(s).

C2-484 HIERARCHY OF ACETYLCHOLINE RECEPTOR ALPHA- DERIVED PEPTIDE BINDING TO COMMON HLA-DR ALLELES, Edward G. Spack, Shrikant V. Deshpande, Nancy L. Corbelletta, Michael McCutcheon, Tien Ha, and Nancy G. Wehner, Anergen, Inc., 301 Penobscot Dr., Redwood City, CA 94063.

Myasthenia Gravis (MG) is an antibody-mediated autoimmune disease dependent upon T cell recognition of peptides derived from the acetylcholine receptor (AChR). In patients of Northern European descent, early onset MG is associated with HLA-DR3, whereas onset after the age of 40 is associated with HLA-DR2. To learn more about the role of AChR-derived T cell epitopes in these patient populations, we measured the relative affinity of a series of overlapping AChR peptides to solubilized HLA-DR2 and HLA-DR3 molecules. The binding of biotinylated peptides with relatively high affinities for DR2 (myelin basic protein 84-102) and DR3 (apoprotein B-100 1273-1291) was measured in the presence of unlabelled AChR peptides by a sensitive europium fluorescence assay. The reactivity of AChR peptides which bound to HLA-DR2 was further delineated by competition with biotinylated peptides specific for DRB1*1501 (influenza virus hemagglutinin 307-319) and DRB5*0101 (HLA-A3 152-166). Quantitation of the unlabelled AChR peptide required to reduce the binding of the biotinylated peptide by 50% (IC_{50}) yielded a relative hierarchy of affinities for the HLA-DR2 and HLA-DR3 alleles. Several peptides with relatively high affinity for HLA-DR have been previously identified as T cell epitopes by proliferation assay. This approach can complement T cell activation assays to develop a better understanding of the role of affinity in epitope selection and immunodominance in autoimmune diseases.

C2-485 CHARACTERIZATION OF DIFFERENCES IN IMMUNE RESPONSES BETWEEN B10 CONGENIC STRAINS THAT BIND THE N-TERMINAL PEPTIDE OF MBP Keri M. Tate, Irina Conboy, Stacy Edelman, John Chang, Yoon-Hee Cha, Bethany B. Moore, Christopher Lee and Patricia P. Jones, Department of Biological Sciences, Stanford University, Stanford, CA 94305-5020.

We have been studying the differences in immune response of the N-terminal peptide of myelin basic protein (MBP) presented by A^u and A^k MHC class II molecules. Examination of these differences at the MHC class II level have revealed that MBP Ac1-11 binds better to A^u than A^k. Using peptide analogs and mutated $A^{k/u}$ MHC class II transfectants, we have shown that the peptide binds slightly differently between A^u and A^k and have identified some residues that are important for these differences. These differences at the MHC class II level and the differences at the T cell level (Davis *et al.*, 1989) may also influence the susceptibility to experimental autoimmune encephalomyelitis (EAE) in mice with these class II alleles. Using B10 mouse strains, congenic for H-2, we are continuing to identify a non-MHC class II difference that is involved in EAE susceptibility/resistance and intend to locate more precisely the locus that is responsible for influencing disease susceptibility in addition to I-A.

C2-486 THE EFFECT OF MF59 ADJUVANT ON THE IMMUNOGENICITY OF RECOMBINANT HEPATITIS B VACCINE IN PRIMATES, P. Traquina[1], M. Morandi[2], M. Contorni[2],and G. Van Nest[1], [1]Chiron Corporation, Emeryville, CA 94608 and [2]Biocine S.p.A., Siena, Italy

We have investigated the effects of MF59 adjuvant on the immunogenicity of an HBV vaccine in baboons. MF59 was compared to alum using a recombinant CHO cell derived vaccine containing PreS2 and SAg (Biocine HBV). Antibody titers were measured by ELISA against recombinant CHO cell derived PreS2-SAg (vaccine material), recombinant yeast derived SAg, and a synthetic peptide representing the PreS2 region. After one immunization, antibody titers against CHO derived SAg and PreS2 peptide were low but detectable in five out of five MF59 immunized baboons. In contrast, only two out of five alum immunized baboons had detectable titers after the first immunization. After two immunizations Biocine HBV combined with MF59 showed anti-SAg (CHO derived) antibody titers which were 38 fold higher than alum, and anti-SAg (yeast derived) antibody titers which were 13 fold higher than alum. In addition, titers against PreS2 peptide were 10 fold higher in animals immunized with MF59 compared to alum. We also monitored HBV-specific T cell proliferation in vaccinated animals. After one and two immunizations, four out of five MF59 immunized baboons had proliferative responses while only two of the alum immunized baboons responded. The MF59/Biocine HBV was compared to two commercially available alum adjuvanted vaccines: Recombivax HB® (yeast derived SAg), and GenHevac B (CHO derived PreS and SAg). Post second immunization MF59/Biocine HBV showed anti-SAg (CHO derived) antibody titers which were 41 fold higher than Recombivax HB® and 160 fold higher than GenHevac B, and anti-SAg (yeast derived) antibody titers which were 8 fold higher than Recombivax HB® and GenHevac B. Antibody titers to PreS2 were 30 times higher with the MF59/Biocine HBV than with GenHevac B vaccine. As expected no PreS2 antibody titers were measured in baboons that had received the Recombivax HB® vaccine. Primary immunization with Recombivax HB® or GenHevac B failed to elicit T cell proliferative response. The secondary immunization only elicited proliferative responses in one out of five Recombivax HB®, or two out of five GenHevac B immunized baboons. The magnitude and duration of the anti-SAg and anti-PreSAg antibody, and cell mediated responses induced by each vaccine, and the results post third immunization will be presented.

C2-487 **COEXPRESSION OF B7 AND VIRAL ANTIGEN IN β-CELLS OF DOUBLY TRANSGENIC MICE CAN INDUCE SPONTANEOUS DIABETES OR ACCELERATE VIRUS-INDUCED DIABETES,** Matthias G. von Herrath[1], Sylvie Guerder[2], Janel Dockter[1], Richard Flavell[2], and Michael B.A. Oldstone[1]

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[2] Yale University, Section of Immunobiology and Howard Hughes Medical Institute, New Haven, CT.

Double transgenic (tg) mice were generated that express the B7.1 molecule and glycoprotein (GP) or nucleoprotein (NP) of lymphocytic choriomeningitis virus (LCMV) in β-cells of their pancreas using the rat insulin promoter (RIP). Two observations were made:

First, single RIP-GP tg mice that do not develop insulin dependent diabetes mellitus (IDDM) spontaneously, but only after viral challenge (10-14 days), developed spontaneous IDDM with generation of CTL specific for LCMV-GP when crossed to RIP-B7 mice. $CD8^+$ MHC restricted GP-specific CTL were found in pancreatic infiltrates and spleens of these mice and upon adoptive transfer into brains of immunosuppressed recipients persistently infected with LCMV they were able to be active and cause disease *in vivo*.

Second, RIP-NP mice normally develop a slow-onset IDDM (4-5 months) after LCMV infection that depends on both, $CD4^+$ and $CD8^+$ lymphocytes. In contrast, RIP-NP x RIP-B7 double tgs developed fast-onset IDDM within two weeks after viral infection. $CD4^+$ cells were not required anymore, as their depletion with antibodies did not abrogate the development of IDDM. However, spontaneous IDDM did not occur.

Thus, costimulation by B7 can break tolerance to a self (viral) antigen removing the need for a secondary viral infection. Additionally, B7 can transform virus induced slow-onset into a fast-onset IDDM by enhancing the generation of anti-self (viral) CTL.

C2-488 T CELL RECEPTOR V-REGION STRUCTURES FROM T CELLS SPECIFIC FOR A DNA-BINDING PEPTIDE, Ming Wang and Tony N. Marion, Department of Microbiology and Immunology, University of Tennessee, Memphis, Memphis ,TN 38163.

When immunized with complexes of DNA and the DNA-binding peptide Fus1, non autoimmune-prone BALB/c mice produce anti-DNA antibody. The induced antibody is both structurally and functionally similar to autoimmune anti-DNA antibody in autoimmune (NZB x NZW)F_1 mice. The sequence for the synthetic 27 amino acid peptide, Fus1, was derived from a 52 amino acid ubiquitin-carboxyl extension protein found in *Trypanosoma cruzi*. Fus1 is highly basic, binds to DNA, is highly conserved among all eukaryotes including mice and humans, and is immunogenic. The mouse homologue of the Fus1 peptide, MFus1, shares 67% identity and 74% similarity with Fus1, and like Fus1, MFus1 is also highly basic and binds DNA. The anti-DNA antibody response induced by DNA-Fus1 immunization of NZW mice is much stronger than that induced by similar immunization of NZB mice. This finding is interesting since autoimmune anti-DNA antibody production in (NZB x NZW)F_1 mice , H-$2^{d/z}$, is linked to MHC class II of NZW, H-2^z.

T-cell hybridomas reactive to the Fus1 peptide presented by syngeneic splenic antigen presenting cells (APC) have been generated from an (NZB x NZW)F_1 mouse previously immunized with DNA-Fus1 complexes. All of the T-cell hybridomas responded to the Fus1 peptide presented by (NZB x NZW)F_1 APC but neither APC nor peptide alone. Interestingly three out of a total of 41 Fus1-responsive , cloned T-cell hybridomas also responded to MFus1. Vα and Vβ cDNA sequences have been obtained from 16 individual hybridomas including all three of the MFus1-responsive hybridomas. Analysis of the T-cell receptor sequences and class II restriction for H-2^d or H-2^z of these T cell hybridomas will allow us to: (1) determine T-cell receptor structures for T cells responsive to MHC-presented DNA-binding peptides, (2) determine the structural differences between T-cell receptors that bind to an MHC-presented foreign peptide and those that bind to MHC-presented foreign and self peptides, and (3) determine the H-2^d or H-2^z MHC restriction for Fus1-responsive T cells derived from (NZB x NZW)F_1 mice. These results may provide important insight about the specificity and T-cell receptor structures for T cells in autoimmunity to DNA.

C2-489 **BLOCKADE OF C5 PREVENTS THE DEVELOPMENT OF COLLAGEN INDUCED ARTHRITIS AND REDUCES ESTABLISHED JOINT INFLAMMATION,** **Yi Wang, Scott Rollins, and Louis Matis, Alexion Pharmaceutical Inc., 25 Science Park, New Haven, CT 06511**

Collagen induced arthritis (CIA) is a chronic auto-immune polyarthritis that has similar features to human rheumatoid arthritis. In the CIA model, both T and B cells are activated after immunizing mice with heterologous native type II collagen (CII). Studies has shown that CII specific antibodies are necessary to initiate CIA. We have hypothesized that when the anti-CII antibodies deposit on joint cartilage surfaces, activated complement components, particularly C5a and C5b-9, initiate joint inflammation by recruiting and activating inflammatory cells. We therefore examined the effects of inhibition of complement component C5 by a C5-specific inhibitory murine monoclonal antibody (mAB) on the onset and progression of CIA. Prophylactic administration of the anti-C5 mAB three weeks following attempted induction of CIA by immunization with CII completely prevented the development of CIA in DBA/1LacJ mice, while 90% of mice treated with a control mAB developed severe polyarthritis. Anti-C5 mAB treatment significantly reduced C5 dependent hemolytic activity in the sera of all treated mice, but did not inhibit CII-specific T cell responses or serum anti-CII antibody levels. Furthermore, when compared to control treated arthritic animals, mice with established arthritis treated with anti-C5 mAB showed significantly reduced joint inflammation. The progression of joint inflammation to additional joints was also blocked. Following treatment of established disease, histological examination revealed that anti-C5 therapy had blocked the recruitment of neutrophils into synovial tissue, preserved joint architecture, and prevented the extensive pannus formation and bone and cartilage erosion uniformly observed in the control mAB treated arthritic mice. These data demonstrate that C5 is critically involved in both the initiation and the progression of joint inflammation in CIA, and suggest C5 as an appropriate target for therapeutic intervention for the treatment of human rheumatoid arthritis.

C2-490 TARGET ORGAN EXPRESSION OF THE MRC OX-40 ANTIGEN ALLOWS FOR THE SELECTIVE DEPLETION OF MYELIN REACTIVE T CELLS IN THE CENTRAL NERVOUS SYSTEM AND AMERLIORATION OF AUTOIMMUNE ENCEPHALOMYELITIS.

Weinberg, A.D.,* Sullivan, T.J.,* Lemon, M.,* Wallin, J.J.,* Bourdette, D.N.,* Fulton, R.J.,~ Offner, H.,* and Vandenbark, A.A.* *Veteran Affairs Medical Center, Neuroimmunology 151-D, Portland, OR 97207. ~Inland Laboratory, 1638 Osprey Dr., Desoto, TX 75115.

The OX-40 protein, a member of the newly described TNF-receptor gene family, provides a costimulatory signal to activated $CD4^+$ T cells. The OX-40 protein is selectively upregulated on encephalitogenic myelin basic protein (MBP)-specific T cells isolated from the central nervous system (CNS) during the onset of experimental autoimmune encephalomyelitis (EAE). The selective expression of this activation marker in vivo allowed us to target and eliminate MBP-reactive T cells within the inflamed CNS, before and during clinical onset of EAE. The in vivo adminsitration of an OX-40 immunotoxin directly and exclusively bound the myelin reactive T cells isolated from the CNS and ameliorated clinical signs of EAE. These results demonstrate the importance of the OX-40 marker for selectively detecting and eliminating autopathogenic T cells at the site of inflammation, thus providing a novel therapeutic strategy that does not require prior knowledge of the pathogenic autoantigen.

C2-491 A MULTINATIONAL STUDY OF HLA-DR AND-DQ FREQUENCIES IN EPIDERMOLYSIS BULLOSA ACQUISITA: ABSENCE OF A PREVIOUSLY REPORTED ASSOCIATION WITH HLA-DR2 IN CAUCASIAN PATIENTS WITH AUTOIMMUNITY TO TYPE VII COLLAGEN. EA Welsh[1], C Prost[2], V Lepage[3], D Charron[3], HO McDevitt[4], and DT Woodley[5]. Depts of Derm[1], and Microbiol & Immunol[4], Stanford Univ Sch of Med, Stanford, CA USA; Dept of Derm[2] and Laboratory of Immunol & Histocompatibility[3], Hôpital St. Louis, Paris, France; Dept of Derm[5], Northwestern Univ Med Sch, Chicago IL USA.

Epidermolysis bullosa acquisita (EBA) is characterized by circulating and/or tissue-bound IgG autoantibodies to anchoring fibril type VII collagen in the basement membrane zone of skin. Two clinical presentations of EBA have been reported. They are the classical or non-inflammatory form (cEBA) and a generalized, inflammatory form (iEBA) of EBA. Autoimmunity to type VII collagen has been reported to be associated with HLA-DR2 (based on serology) in black and white EBA patients in the U.S. Our serologic studies could not confirm this finding in white EBA patients. The purpose of this study was to clarify these results by an analysis of the frequency of DRB1 and DQB1 alleles in a large cohort of EBA patients (n=27, all of Caucasian European decent). We determined the DRB1 and DQB1 genes using sequence-specific oligonucleotide probes hybridized to amplified genomic DNA from 10 white patients from France with cEBA and 17 white EBA patients (6 known cEBA) from the U.S. In all cEBA patients analyzed, we observed an increased frequency of DRB1*0101/02 (31%), and *0301 (25%) versus 22% and 18% in controls, respectively, and a smaller increase in DRB1*08 (13% vs. 7% in controls). Analysis of the DQB1 alleles resulted in an increase in the frequency of the DQB1*0501 allele in cEBA patients (46% vs. 25% in controls). When all EBA patients were analyzed together, a more pronounced increase in the frequency of DRB1*08 (22% vs. 7%) was observed. An increase in the frequency of DQB1*0501 (35% vs. 25%) was still observed. In this study, the frequencies of the DR2 alleles, DRB1*1501 and *1502, were not significantly different from control values in any EBA cohort analyzed. These results suggest that DRB1*0101/02, *0301, 08, and DQB1*0501 may be important risk factors associated with autoimmunity to type VII collagen in EBA. Additionally, different HLA haplotypes may be involved in susceptibility to these two clinically different forms of EBA.

C2-492 ALTERED DIABETES INCIDENCE IN NOD MICE WITH A SELF MHC CLASS II TRANSGENE

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The unique non-obese diabetic (NOD) mouse MHC class II I-A β chain confers much of the NOD susceptibility to diabetes. Class II transgenic NOD mice generally show partial or complete protection from diabetes. We have generated two independent lines of transgenic NOD mice in which their own I-Aβ $g7$ molecule is the transgene. One line shows a similar diabetes incidence in transgenic and non-transgenic females (80% and 94%, n=20, 18) and an increased incidence in transgenic males (64% vs. 40%, n=25, 30) at 35 wks. Transgene expression level, as assesed by FACS analysis, is lower than that of the endogenous allele. The second line shows a reduced incidence in female transgenics (43% vs. 75%, $p<.05$, n=28, 16) at 33 wks. with a divergence of the incidence curves at 17 wks. Transgenic and non-transgenic males have similar diabetes incidence. Transgene expression is higher in this line and is similar to the endogenous allele. This line develops a reduction in splenic B cell number (18% B220 positive cells vs. 30% in non-transgenics at 26 weeks), a defect seen in other MHC class II transgenic mice. Further characterization of this defect and of the immunocompetence of both lines by assesment of T cell proliferation, autoantibody production and antibody isotyping is in progress. The protective effect of the transgene in the second line may be related to the transgene's overall effect as demonstrated by the reduced B cell number.

C2-493 INCREASED EXPRESSION OF B7-1, B7-2 AND CD70 ON T CELLS FROM HIV-INFECTED INDIVIDUALS.

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T cells express CD28 and CD27, which play an important role in T-cell costimulation. Their ligands, B7-1, B7-2 and CD70, are expressed on APC and to a lesser extent on activated T cells. To obtain insight into immune dysfunction in HIV infection, expression of CD28, CD27, B7-1, B7-2 and CD70 on T cells and B7-1 and B7-2 on monocytes was studied in HIV infection.

Upon stimulation, B7-1 and B7-2 expression on monocytes from HIV-infected subjects with normal to low CD4 cell counts was normal, however, in subjects with extremely low CD4 cell counts, B7-1 expression was decreased. On T cells from HIV-infected subjects, CD28 expression was decreased, mainly within the CD8 subset, and an increase in the percentage of CD27-negative cells could be seen in both unstimulated and stimulated T cells.

In contrast, B7-1 and B7-2 expression was increased on T cells from HIV-infected subjects upon stimulation. CD70, normally expressed on 15 to 20 % of activated T cells, was expressed on 50 to 75% of activated T cells from HIV-infected individuals. Interestingly, this upregulation of B7-1, B7-2 and CD70 on T cells also occurred in CD4-depleted PBMC from a non-infected donor, while it did not occur in the CD8 subset of total PBMC from a non-infected donor, suggesting a role for CD4-dependent regulation.

In summary, our data show that upon stimulation T cells from HIV-infected individuals acquire an APC-like phenotype. To what extent this phenotype contributes to immuno-regulatory dysfunctions observed in HIV infection remains to be elucidated.

C2-494 CD8 T CELL LINES DERIVED FROM NOD ISLETS CAN CAUSE DIABETES IN THE ABSENCE OF CD4 T CELLS

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The NOD (Non-Obese Diabetic) mouse is a model for human type I diabetes in which there is lymphocyte infiltration into the islets of Langerhans and later destruction of islet cells leading to glycosuria and diabetes. We have generated oligoclonal T cell lines, C7 and D2, by limiting dilution, from the lymphocytic infiltrate in the pancreatic islets of Langerhans of NOD mice. These T cell lines are cultured *in vitro*, stimulated by pancreatic islet cells derived from (NODXB7)F1 mice, which were generated by crossing NOD mice with transgenic mice expressing the human B7 molecule, driven by the Rat Insulin Promoter, in β cells on an $H\text{-}2^b$ genetic background. The lines both express αβ T cell receptor with Vβ6 and Vβ17 predominating, a CD8 coreceptor, the activation markers CD28 and CD44, and the adhesion molecules ICAM 1, LFA1 and α4 integrin. The cytokines interferon γ, TNFβ and perforin have been detected by RT-PCR. Their ability to cause disease has been tested *in vivo* using adoptive transfer. 7 week old female NOD mice were irradiated with 725 rads 24 hours prior to transfer. 5×10^6 C7 or D2 were injected iv with and without purified CD4+ T cells from the spleen of diabetic NOD mice. Mice were tested for glycosuria and diabetes confirmed by blood glucose measurement >250mg/dl. All the mice injected with C7, with and without CD4+ T cells developed diabetes by 5 days after transfer. In contrast, mice injected with D2+CD4 developed diabetes by 5 days while animals transferred with D2 alone developed disease in 8 days. Immunohistochemistry shows massive infiltration of CD8+ cells and extensive islet destruction. None of the animals transferred with CD4+ T cells alone, CD4+ and CD8+ T cells derived from diabetic spleen, or PBS had developed diabetes by 14 days. The results indicate that CD8+ T cells activated in the presence of the costimulator B7 have a markedly increased cytotoxic potential and are able to cause diabetes in the absence of CD4+ T cells.

C2-495 USAGE OF MULTIPLE TCRBV GENES DURING EXPERIMANTAL AUTOIMMUNE MYASTHENIA GRAVIS PATHOGENESIS IN $H\text{-}2^b$ STRAINS.

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Experimental autoimmune myasthenia gravis (EAMG) is an antibody-mediated autoimmune neuromuscular disease. The production of pathogenic anti-acetylcholine receptor antibodies is dependent on the activation of CD4+ cells after their TCR interact with dominant T cell epitopes within the acetylcholine receptor (AChR) in the context of the MHC class II molecule. One of the dominant T cell epitopes has been mapped within the AChR alpha chain 146-162 amino acids. By in vitro analysis the TCRBV6 gene segment was shown to be the predominant TCR which recognizes the AChR and the dominant epitope alpha 146-162 in C57BL6 ($H\text{-}2^b$) mice. But in vivo depletion of TCRBV6 cells in $H\text{-}2^b$ mice by anti-TCRBV6 mAb failed to suppress the in vitro immune response to AChR and prevent EAMG. Moreover, the B10.TCR^c ($H\text{-}2^b$) mice with a massive germ-line deletion of TCRBV genes including TCRBV6, and B10.TCRBV8S2 transgenic mice with a restricted TCRBV8S2 T cell repertoire, responded to AChR and the dominant peptide alpha 146-162 and developed EAMG after active immunization with AChR. Therefore, even if TCRBV6 cells are involved in EAMG pathogenesis, other TCRBV-bearing cells (e.g. TCRBV8) have the affinity for the AChR-dominant peptide and are involved in disease pathogenesis. Moreover, in the absence of both TCRBV6 and TCRBV8 cells, B10.TCR^c mice have the capacity to develop EAMG, suggesting usage of other TCRAV, TCRBV gene(s). If a common motif in the VDJ regions of TCR among different TCRAV, BV-bearing cells is involved in the recognition of the dominant AChR epitope(s) in the context of the class II molecule, then mAb specific to the common motif in the VDJ region could be expected to block the development of EAMG.

*James W. McLaughlin posdoctoral fellow, and +Osserman fellow of Myasthenia Gravis Foundation.

C2-496 THE DEVELOPMENT OF CONGENIC MOUSE STRAINS FOR THE GENETIC DISSECTION OF INSULIN DEPENDENT DIABETES SUSCEPTIBILITY, Mary A. Yui, K. Muralidharan, Kye Chesnut and Edward K. Wakeland, Center for Mammalian Genetics and Department of Pathology, University of Florida, Gainesville, FL 32610

Insulin dependent diabetes (IDD) is caused by the autoimmune destruction of insulin-producing pancreatic beta cells. The genetics of IDD in both humans and mice has proven to be very difficult to study because of the polygenic nature of its inheritance. Using the non-obese diabetic (NOD) mouse strain, we produced over 1600 backcross female (NOD X C57Bl/6)F1 X NOD mice, in which 20% had significant insulitis (inflammation of pancreatic islets) and 3.1% progressed to diabetes by 1 year of age. For genetic interval mapping 104 simple sequence repeats (SSRs) were typed on 48 diabetics and 120 non-diabetics. We found 6 linkage groups with at least 8 recessive loci correlating with the development of insulitis and/or diabetes and that the mode of inheritance is that of a threshold liability. The NOD $H\text{-}2^{g7}$ on chromosome 17 is required but not sufficient for diabetes while the other genetic intervals contribute to, but are not required for, disease. To further study IDD inheritance, we have bred a series of congenic mouse strains with the NOD-derived diabetes susceptibility genetic intervals from chromosomes 1,2,3,6,11 and 17 onto the C57Bl/6 background. Mice with each NOD-derived interval (19 to 44 cM) were backcrossed 6 times to C57Bl/6 mice and then intercrossed for homozygotes. The goals of this study are to dissect out the pathogenic roles of the diabetogenic loci in each of these intervals, to reconstitute insulitis and diabetes by combining the different susceptibility intervals in polycongenic strains, and to fine map the intervals. Histological examination reveals pancreatic perivascular/periductal infiltrates, the precusor to insulitis, in some chromosome 1 and 3, but not chromosome 6 and 11, single congenic mice.

Immunity to Bacteria, Parasites, Tumors and Allografts; Theoretical Immunology

C2-500 **CYTOKINE GENE THERAPY OF MURINE TUMOURS USING RECOMBINANT VACCINIA VIRUS**, Bruce ACRES, Karin DOTT, Lorette STEFANI, Jean-Marc BALLOUL and Marie-Paule KIENY, Department of Immunology, TRANSGENE S.A., Strasbourg 67082, France

Mutation of the thymidine kinase gene of Vaccinia virus (VV) has been shown to reduce drastically the *in vivo* pathogenicity of VV. We have observed that the *iv* injection of tumour-bearing mice with TK-, Copenhagen strain VV results in the efficient infection of tumour cells, while leaving internal organs with either no detectable infection or barely detectable infection. Infection of tumour cells in euthymic mice lasts for 3 - 4 days while tumour infection in nude mice lasts for over one week.

We have assembled several VV recombinants with a cytokine cDNA inserted into the TK gene, rendering the virus TK-. Infection of BHK cells *in vitro* with these recombinants results in the production of 100 ng to 1µg per ml of cytokine in the culture supernatant per 24 hours.

DBA/2 mice growing P815 tumours and nude mice growing human xenografted tumours were injected with these cytokine VV recombinants and the effect on tumour growth was followed. To date, we have found that the injection of tumour-bearing DBA/2 mice with VV-IL2, VV-GM-CSF or VV-TNFβ results in signifigant retardation of tumour growth, with complete tumour rejection in some mice. VV-IL4, VV-IL5, VV-IL6 and VV-IL7 had no signifigant effect on tumour growth in this system. In nude mice, only VV-IL6 was able to slow the growth of human tumour xenografts, with some complete rejections.

C2-501 PRIMARY IMMUNE RESPONSE INDUCTION AGAINST MELANOCYTE DIFFERENTIATION ANTIGENS BY DENDRITIC CELLS

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Recently, we and others have identified antigens recognized by anti-melanoma cytotoxic T cells (CTL). Several of these antigens are tumor-specific proteins (e.g. MAGE family). Interestingly, other anti-melanoma CTL recognized melanocyte differentiation antigens (gp100, tyrosinase and Melan-A/Mart-I) that are not only expressed by melanoma cells but also by their normal counterpart, melanocytes. This finding indicates that tolerance against these differentiation antigens is not absolute.

To investigate tolerance against aforementioned melanocyte differentiation antigens, we used dendritic cells obtained from PBL of healthy donors to induce CTL responses against peptides derived from these antigens . For these purposes dendritic cells were generated by culturing highly enriched monocytes, obtained by centrifugal elutriation, in the presence of GM-CSF and IL-4. When pulsed with immunogenic peptides derived from melanocyte differentiation antigens, these cells were able to induce a primary CTL response *in vitro*.. These CTL specifically lysed melanoma cells expressing the melanoma differentiation antigens, indicating that they recognized naturally processed antigen. These data demonstrate that CTL reactive with melanocyte differentiation antigens can be induced in healthy donors using dendritic cells and indicate that tolerance against these antigens can be broken. Therefore, melanocyte differentiation antigens constitute potential targets for immunotherapy of melanoma, provided that no unacceptable cytotoxicity against normal tissues is observed.

The mechanisms involved in peripheral tolerance to self antigens expressed by cells of the melanocytic lineage have not been elucidated. We are currently generating transgenic mice that specifically express the human gp100 antigen in their melanocytic cells to investigate the state of tolerance against this antigen. In addition, we will study how apparent tolerance to the gp100 antigen may be ablated through effective immunization schemes and/or presentation by professional antigen-presenting cells such as macrophages or dendritic cells. Understanding the mechanisms of tolerance to melanocyte differentiation antigens may lead to strategies for targeted immunotherapy of malignant melanoma.

C2-502 ANTIGENS ARE TRANSPORTED BETWEEN DIFFERENT SITES OF INFLAMMATION: A MECHANISM FOR GENERATION OF IMMUNE RESPONSES AT SITES DISTANT FROM ANTIGEN ENTRY, Salvatore Albani*, Antonio La Cava#, Helen Tighe#, Carlos J. Carrera#, Stephen M. Baird@, Dennis A. Carson#, Departments of Pediatrics*, Medicine# and Pathology@, University of California San Diego, 9500 Gilman Drive, La Jolla, CA, 92093-0663.

Deposition of antigens on mucosal surfaces induces inflammation that attracts immune response cells. Antigens present at the inflammatory sites are phagocytosed by antigen presenting cells which migrate to regional lymph nodes and initiate specific immune responses. A definition *in vivo* of the fate of the antigen beyond transport to regional lymph nodes has been hampered by difficulties in detecting small amounts of antigen on individual cells. The hypothesis that cells bearing antigens taken up at one inflammatory site can be attracted by inflammation generated at another distant site has not been tested. In many inflammatory diseases specific immune responses can be detected in tissues far from the presumed point of entry of the antigen, without demonstration of the triggering antigen. We have developed a novel gene targeting system to study the migration of antigen loaded cells. First, we generated simultaneous foci of inflammation by injecting intradermally in the neck, and intraperitoneally a pro-inflammatory synthetic adjuvant in rabbits and Balb/c mice. Then a mammalian DNA expression vector that was injected intradermally only. The use of DNA over protein as antigen enabled us to employ a novel system that combines polymerase chain reaction with *in situ* hybridization for amplification of the signal and flow cytometry for its detection. The results showed that antigen loaded cells migrate between different *inflammatory sites. This phenomenon may have relevance in* the generation of physiological as well as disease-associated immune responses.

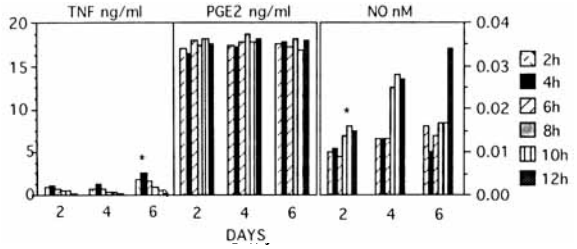
C2-503 T CELL RECEPTOR JUNCTIONAL REGIONS OF PREDOMINANT CLONES FOUND IN MUCIN-SPECIFIC CTL LINES, Mark D. Alter, Y. Kirii, J. M. Blander, Olivera J. Finn, Dept. of Molecular Genetics and Biochemistry, U. of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

We have previously reported the establishment of human cytotoxic T lymphocytes specific for the mucin molecule expressed on pancreatic and breast cancer cells. A study of predominant V_α and V_β genes utilized in mucin specific CTL lines indicated that T cell receptor (TCR) usage is not restricted to a particular V_α or V_β family. Rather, expansion of V_αs and V_βs in culture are indicative of expansion of particular CTL clones as determined by sequencing predominant PCR products directly. We have sequenced the junctional regions of α and β chains of predominant clones in mucin-specific CTL lines. The presence of an asparagine in 5/8 α chains and a glutamine in11/18 β chains in similar positions in many junctional regions has been found in sequences from 4 separate CTL lines. We will continue to investigate this further. The identification of TCR motifs important in recognition of mucin expressed on carcinomas will be useful in the identification of tumor specific T cells and would facilitate monitoring the success of therapy designed to enhance this anti-tumor immune response.

C2-504 LENGTH OF REOXYGENATION PERIOD FOLLOWING HYPOXIA EFFECTS CYTOKINE ELABORATION BY MURINE PERITONEAL MACROPHAGES, Gajra Arya and Victor F. Garcia, Division of Pediatric Surgery, CHMC, Cincinnati, OH 45229

Tissue injury occurs not during the period of hypoxia, but rather during the period when tissues are reoxygenated. To determine whether the length of reoxygenation period following in-vivo hypoxia affects cytokine elaboration by macrophages, adult male CBA mice (20-25g) were subjected to 16h of hypoxia (8%O_2). Immediately thereafter, the animals were reoxygenated by placing them in normoxia. Upon reoxygenation for either 2, 4, or 6 days, peritoneal macrophages were harvested and plated at one and a half million cells/ml. Culture supernatants were collected at 2, 4, 6, 8, 10, and 12h post in-vitro plating and assayed for tumor necrosis factor (TNF), prostaglandin E_2(PGE_2) and nitric oxide (NO).

RESULTS: 1) TNF levels were significantly higher on day 6. 2) NO production on day 2 was significantly lower than that on days 4 and 6. 3) Whereas TNF and NO production changed with duration of reoxygenation, PGE_2 levels were independent of duration of reoxygenation. Data analyzed by two way ANOVA for the interaction of day and hours, $p < 0.01$



CONCLUSIONS: We conclude that the duration of reoxygenation period influences the amplitude of cytokine elaboration. An increase in TNF production on day 6, despite consistently high PGE_2 levels, suggests desensitization of macrophages to PGE_2. This data suggests that early tissue injury during reoxygenation is not mediated by TNF.

C2-505 MITOGENICITY OF STREPTOCOCCAL PROTEINS ON MURINE B LYMPHOCYTES, Karol Axcrona[1], Lars Björck[2] and Tomas Leanderson[1]. 1. Immunology Unit, University of Lund and 2. Department of Medical and Physiological Chemistry, University of Lund, Lund, Sweden.

Streptococci express various Ig-binding proteins on their surface. We tested Protein H and Protein G (both expressed on Group C and G Streptococci) and Protein L (Peptostreptococcus magnus) for mitogenicity on B lymphocytes. We found that Protein H and L coupled to Sepharose induced proliferation of both murine splenic B cells and human peripheral lymphocytes, measured as Tdr-uptake. When labelled with flourochromes Protein H stained virtually all $CD19^+$ cells in human peripheral blood but also a significant $CD19^-$ fraction of cells. The staining pattern of mouse spleen cells was more complex where the majority of the $B220^+$ cells were Protein H^+ but also a big fraction of $B220^-$ cells were Protein H^+. The chimaeric Protein LG, composed of the Ig-binding domains of Proteins L and G, induced proliferation and differentiation to Ig-secretion of murine splenic B cells in both a soluble form and when added as a Sepharose-coupled moiety. These results have implications concerning the modular interactions between bacterial proteins and cell surface receptors and the functional effects of such interactions.

C2-506 CD4 T CELLS ARE MAINTAINED IN AN UNRESPONSIVE STATE BY PERSISTING ANTIGEN,

Eric B Bell, Chun-ping Yang and Mark McDonagh. Immunology Research Group, Biological Sciences, University Medical School, Manchester M13 9PT,UK.

Rats were rendered unresponsive by a pre-operative donor specific blood transfusion (DST) 7,14 or 30 days before cardiac allografting. Surprisingly, CD4 T cells from DST animals induced acute rejection on transfer to cardiac allografted athymic nude recipients. Experiments showed that CD8 T cells were not involved in this tolerance model. CD4 T cell subsets, defined in the rat by mAb OX22 (anti-CD45RC) and obtained from rats made unresponsive by DST, were also examined after transfer to allografted nude recipients. $CD45RC^+$ CD4 T cells (Th1-like and normally capable of inducing prompt rejection on transfer) failed to induce rejection and lacked alloreactive cells. In contrast, $CD45RC^-$ CD4 T cells (Th2-like and normally poor at inducing rejection) were enriched in alloreactivity and on transfer induced acute rejection. This rejection by $CD45RC^-$ T cells was entirely prevented by giving nude recipients a DST two or more weeks before allografting and cell transfer. Apparently, the residual components of the DST (present in the original unresponsive DST animal and in the pre-transfused nude recipient) maintained $CD45RC^-$ T cells in a non-rejecting state.

C2-507 OSP A IMMUNODOMINANT Th CELL RESPONSES AFTER IMMUNIZATION AND INFECTION WITH THE LYME DISEASE SPIROCHETE, Linda K. Bockenstedt and Fred S. Kantor, Department of Internal Medicine, Yale University School of Medicine, New Haven, CT 06520-8031

Lyme disease is a multisystem disorder due to infection with the tick-borne spirochete, Borrelia burgdorferi (Bb). The host adaptive immune response to Bb appears inadequate because protective antibody responses against outer surface proteins (Osps) are a late finding and are ineffective in eradicating the organism from the infected host. The types of T helper (Th) cell responses elicited early in infection may be critical factors in the timely development of protective and disease-modulating antibodies. We have used cathepsin D digests of recombinant Osp A (rOsp A) to define a T helper cell epitope on this Osp which is known to be a target of protective antibodies. Immunization of C3H/HeJ mice with rOsp A resulted in a T cell response against one HPLC separated cathepsin D digest fragment containing amino acids 179-193. Using a panel of overlapping synthetic peptides 20 amino acids in length, peptide 179-193 was found to be the immunodominant T cell epitope, as no T cell responses to any other Osp A peptide were detected after immunization with rOsp A. T cells responding to this peptide were CD4+ and secreted IL-4, indicating that they were Th2-type T helper cells. To determine whether infection alters the T cell response to rOsp A, T cell hybridomas were generated from mice 14 days after intradermal infection with 10^3 Bb. Mice do not seroconvert to Osp A during the first 6 months of infection with this dose of spirochetes, despite the fact that Osp A is a dominantly expressed surface protein on cultured organisms. Of five Osp A-specific T cell hybrids produced, all were CD4+ and reacted only with peptide 179-193. In addition, these CD4+ T cell hybrids secreted IL-2 and not IL-4, suggesting that they have a Th1 phenotype. These results indicate that immunologic exposure to Osp A occurs early in infection despite the absence of early antibody production to this Osp. Moreover, although the immunodominant T cell epitope is the same after immunization and infection, infection may favor the development of a Th1-type response over a Th2. These results suggest one mechanism by which infection with Bb may influence the immune response in favor of organism persistence.

C2-508 CENTRAL AND PERIPHERAL T CELL TOLERANCE TO A TUMOR-SPECIFIC ANTIGEN. Bjarne Bogen, Peter Hofgaard and Grete Lauritzen, University of Oslo, Norway

It is well established that immunoglobulins are processed and presented by MHC molecules. Thus, fragmenting of Ig V-regions yield idiotypic peptides recognized by class II-restricted, $CD4^+$ T cells. As idiotypes are highly tumor-specific antigens, such Id-specific T cells could have a role in immunosurveillance of plasmacytomas and B cell lymphomas. To test this, we have established T cell receptor transgenic mice specific for a 91-101 fragment of the $\lambda 2^{315}$ Ig L-chain; this fragment is presented by the I-E^d class II molecule. Such TCR-transgenic mice were significantly protected against the MOPC315 plasmacytoma producing IgA $\lambda 2^{315}$, indicating that $CD4^+$ T cells indeed have a role in tumor protection (Lauritzen, Weiss, Dembic & Bogen, PNAS 1994 91:5700). However, some mice developed MOPC315 tumors despite of being TCR-transgenic. We have now studied T cell tolerance in such mice. TCR-transgenic MOPC315 bearers delete Id-specific T cells at the $CD4^+CD8^+$ stage. Peripheral T cells are also deleted, and the responses of lymph node T cells to $\lambda 2^{315}$ peptide is greatly reduced. A high concentration of serum M315 (10-100μg/-ml) is needed to elicit both central and peripheral T cell tolerance. In summary, $CD4^+$ T cells can protect against tumors. But if the initial elimination of tumor cells fails, a profound T cell tolerance develops. Therefore, a major challenge in immuntherapy of cancer patients will probably be to reverse and already established T cell tolerance.

C2-509 FATE OF TOXOPLASMA GONDII DURING LYSIS OF ITS HOST CELL BY CYTOTOXIC T LYMPHOCYTES, Charles R. Brown, Randee Estes and Rima McLeod, Department of Medicine, Michael Reese Hospital, Chicago, IL 60616.

Lysis of infected cells by cytotoxic T cells (CTL) is an important defense mechanism against intracellular pathogens. It is unclear, however, what effect this lysis has upon the pathogen. *T. gondii* is an intracellular protozoan parasite capable of infecting virtually every mammalian cell. Cell-mediated immunity is responsible for resistance to this organism with the MHC class I molecule L^d and $CD8^+$ T cells being implicated in early protection. CTL from mice immunized with Ts-4 strain of *T. gondii* have been demonstrated to lyse *T. gondii*-infected cells *in vitro*. Using this model system we investigated the fate of *T. gondii* organisms residing within target cells lysed by CTL.

BALB/c mice were immunized with Ts-4 and immune and control spleens removed 6 weeks later. Following *in vitro* stimulation for 5 days immune, but not control, splenocytes lysed *T. gondii*-infected target cells. This lysis was MHC- and $CD8^+$ T cell-restricted. Following the CTL assay, electron microscopy of infected target cells incubated with immune splenocytes revealed numerous cells with apoptotic morphology, but with apparently healthy *T. gondii* inside them. Infected target cells incubated with control splenocytes exhibited normal morphology.

To determine the growth capability of *T. gondii* following lysis of its host cell by CTL, target cells were treated with a Ca-ionophore which causes egress of *T. gondii*, destroying their host cells in the process. The released organisms were then incubated on P815 (H-2^d) or EL4 (H-2^b) cells and parasite growth measured by ^{3}H-Uracil uptake. There were no differences in parasite growth between organisms incubated with immune or control splenocytes when cultured with either cell line. This indicated that CTL lysis of their host cell had no direct effect on the parasite. However, if the infected apoptotic host cells were placed on naive peritoneal macrophages there was a significant decrease in ^{3}H-Uracil uptake in parasites incubated with immune splenocytes as compared to control splenocytes. This result was not MHC restricted as the growth was inhibited by both BALB/c and B10 macrophages. Growth inhibition was not dependent on activation of macrophages by IFN-γ as anti-IFN-γ or blocking of activation with NMMA had no effect.

Thus lysis of *T. gondii*-infected host cells does not directly affect the parasite but may allow for their destruction by macrophages.

C2-510 Autoimmunity And Its Regulation.Godfrey Caesar, 209 West, 137 th. St. N.Y.N.Y. 10030.

Substances that arouse an immune response are described as antigenic or substantively as antigens. The distinguishing characteristic of an antigen is it foreignness, i.e., its property of being non self. However, even self-constituents can sometimes arouse an immunity reaction, for the foreignness that is a qualifying property for being an antigen means only foreignness to the reacting system, and if some parts of the body have been sequestered throughout life, their liberation through injury or some degenerative process enables them to exercise their antigenic power. Reactions upon such self constituents are referred to as autoimmune reactions. Bodily constituents altered by chemical action or virus infection may also arouse autoimmunity.
Wholesome fetal cells can compensate for the immunological breakdown or genetic defect/s which can result in autoimmune disease/s. This concept is relevant to genetic manipulation of immunity and autoimmunoty. It is further explained in (1-2).

REFERENCES.

1. Caesar, G. Oncogenes, Antioncogenes, and a Hypothesis On Cancer Therapy, i.e., The Origin of Cancer, and The Prevention of Its Activity, Medical Hypotheses, 40,1, 15-18, 1993.
2. Field, L.J., et al, Science, 264, 98-101, 1994.

C2-511 IMMUNOSUPPRESSION OF HUMAN LYMPHOCYTE PROLIFERATION BY *Toxoplasma gondii,* Jacqueline Y. Channon and Lloyd H. Kasper, Departments of Microbiology and Medicine, Dartmouth Medical School, Hanover, NH 03756

Toxoplasma infection in mice is associated with a transient state of immunosuppression. In humans, toxoplasmosis is a major cause of infection in the newborn and the immunosuppressed, especially those with AIDS. In these studies, human monocytes infected *in vitro* with *T. gondii* are shown to inhibit the Con A-induced proliferative response of autologous lymphocytes by 80% compared to uninfected monocytes. The observed lymphocyte inhibition is parasite dose dependent with 50% inhibition occurring at a monocyte:parasite ratio of 20:1. Further, the inhibitory response appears to be due to a soluble factor released from the infected monocytes, as inhibition occurs when monocytes and parasites are separated from lymphocytes by a 0.4 µm pore size transwell. Maximal inhibition (80 to 90%) was achieved only when infected monocytes were present throughout the lymphoproliferation assay. Ten to 20% inhibition was seen in the presence of culture supernatant obtained from infected monocytes. The cytokines, TNF-a, IL-1ß, and IL-10, are measurable at the pg/ml level from monocytes infected for 24 h with toxoplasma. The inhibitory factor is not IL-10, TGF-ß, or prostaglandin E2 as inhibition can still be observed despite the presence of anti-IL-10, anti-TGF-ß, or indomethacin. When monocyte cell surface receptors were examined 24 h after infection, CD40, ICAM-1, and Class II were found to be up-regulated 2- to 3-fold, whereas other cell surface receptors showed little or no modulation. Hence, signaling by these co-stimulatory molecules may be intact in infected monocytes. These observations may help explain why toxoplasma can evade the immune response during an acute infection.

C2-512 AUTOIMMUNE EVENTS ARE RELATED IN MULTIPLE SCLEROSIS (MS) TO CHANGES IN EFFECTOR TARGET (E:T) INTERACTION DURING THE IN VITRO NATURAL CYTOTOXIC REACTION (NCR), G.B.Chekney, Ya.G.Ashmanova, and O.L.Latysheva, Lab. of Immunochemistry, N.F.Gamaleya Epidemiol. & Microbiol. Institute, Moscow, Russia

In context of NK cell deficiency in MS associated with changes in IFN regulatory circuit, NK cell cytotoxicity in patients with MS was measured in parallel with a quantity of E:T conjugates formed in NCR in presence in vitro of mediators influencing on different steps of E:T interaction, such as Hu-rIFN-gamma, C-reactive protein (CRP), or fibronectin (FN). The results of study show that in contrast with an ability of Hu-rIFN-gamma to destroy false E:T conjugates in NCR in healthy subjects, and thereby to enhance NK cell cytotoxicity, this factor increased a quantity of E:T conjugates with following reducing in vitro NK cell activity in patients with MS. In presence of CRP a quantity of E:T conjugates decreased in vitro with following augmentation of NK cell cytotoxicity both in MS patients and healthy subjects. FN was found to increase in MS patients an NK cell cytotoxic activity as an intensity of E:T conjugates formation in vitro. Such an effect of FN was not observed in healthy subjects.

Consideration of NCR as of a process initiated by formation of different types of E:T conjugates allows to suggest that MS represents a pathology characterized by abnormalities in E:T interaction during NCR. Involvement of CRP and FN in regulation of the lytic circle may probably substitute for IFN in vitro in activatory transduction in patients with MS.

C2-513 INDUCTION OF SPECIFIC MEMORY T CELLS REQUIRES EXPRESSION OF B7-1 COSTIMULATORY MOLECULE AND SECRETION OF IL-12 , Peter W. Chen, D. Corey Geer, Eckhard R. Podack, and Bruce R. Ksander, Department of Ophthalmology, Harvard Medical School, Boston, MA 02114, Department of Microbiology, University of Miami Medical School, Miami FL 33136.

Antigen presenting cells provide multiple costimulatory signals that successfully activate tumor-specific T cells. Two important costimulatory signals are provided by B7-1, which stimulates differentiation of precursor cytotoxic T cells, and IL-12, which stimulates differentiation of precursor T helper cells towards the Th1 phenotype. To increase the immunogenicity of tumor cells, we transfected l P815 tumor cells with cDNA for either B7-1 and/or IL-12 p35 and p40 chains. Untransfected P815 cells grow progressively and fail to induce tumor-specific T cells when injected into the flank of syngeneic mice. Transfected P815 that secrete IL-12 alone failed to induce protective immunity against primary tumors. By contrast, P815 cells expressing B7-1 alone or B7-1 + IL-12 inducted tumor specific cytotoxic T cells resulting in the elimination of the primary tumor. However, immunity induced by P815 cells that expressed B7-1 alone was transient and only delayed the growth of a contralateral secondary tumor challenge (100% tumor incidence). By contrast, mice immunized with transfectants expressing B7-1 and secreting IL-12 were protected from a contralateral second tumor challenge (20% tumor incidence). We conclude that expression of B7-1 and secretion of IL-12 by P815 tumor cells induces a long-lasting and protective memory T cell response, and we suspect that this results from the induction of both cytotoxic T cells and memory Th1 type helper T cells. (Supported by NEI-08122)

C2-514 EXPRESSION OF A MUTANT P53 EPITOPE FUSED WITH THE ADENOVIRUS E3 LEADER SEQUENCE IN TUMOR CELLS OVERCOMES γ-IFN DEPENDENCE OF LYSIS BY P53-SPECIFIC CTL. I. Frank Ciernik, Michael Yanuck, Jay A. Berzofsky, David P. Carbone; Simmons Cancer Center, The University of Texas, Southwestern Medical Center, Dallas, Texas 75235 and the Metabolism Branch, National Cancer Institute, NIH, Bethesda, MD 20892

Point mutations are the most frequent cause of inactivation of tumor suppressor gene p53. Peptides surrounding the mutations are potential targets for tumor specific MHC class I restricted T cell immunotherapy. Peptide induced, mutant p53-specific cytotoxic T cells (CTL) can lyse p53-peptide pulsed target cells. We are investigating the conditions under which the mutation specific CTL are able to lyse target cells endogenously expressing the tumor specific T cell epitope. P815 mastocytoma cells expressing the p53 gene fragment coding for the peptide of p53 from amino acid 125 to 145 surrounding mutation C to Y at position 135 are not lysed by mutant-specific CTL unless the peptide is targeted to the endoplasmatic reticulum (ER) with an appropriate leader sequence derived from the Adenovirus E3 gene. P815 mastocytoma cells transfected with the whole open reading frame (ORF) of p53 with mutation C to Y at position 135 present the tumor specific epitope effectively to CTL. In transformed BALB/c 3T3 fibroblasts expressing the whole ORF of the mutated p53, the tumor specific epitope is presented only if the target cells are pretreated with γ-IFN. This difference in presentation of this endogenous tumor specific epitope is not due to different levels of expression of MHC class I, as all cell types express MHC class I by FACS, and expression is only slightly upregulated by γ-IFN. In addition, BALB/c 3T3 fibroblasts efficiently present the mutant epitope of p53 (125-145) to mutation specific CTL in the absence of γ-INF pretreatment if the p53 epitope is fused to an ER-leader sequence. We are currently investigating which aspect of processing and transport of T cell-epitopes is rate limiting in the absence of γ-INF pretreatment in these tumors. Thus, resistance of tumor cells to lysis by mutant oncogene-specific CTL can be overcome either by fusion of the mutant epitope with an E3 leader sequence or by pretreatment with low doses of γ-IFN.

C2-515 BLOCKADE OF CD40/CD40 LIGAND INTERACTION AMELIORATES LETHALITY OF ACUTE GRAFT-VERSUS-HOST DISEASE ACROSS CLASS I AND II BARRIERS IN MICE, Karen K. Clark, Anthony W. Siadak, Mark R. Stebbins, Randolph J. Noelle and Philip M. Wallace, Bristol-Myers Squibb Pharmaceutical Research Institute, 3005 First Avenue, Seattle, WA 98121

The interaction of CD40 on B cells and CD40 ligand (gp39) on activated T cells is necessary for optimal stimulation of Ig secretion in the presence of cytokine. We have studied the role of CD40/CD40L in the activation of T cell function in a model of acute graft-versus-host disease. Blockade of CD40L interaction with a monoclonal antibody, MR1, was capable of reducing the lethality of acute graft-versus-host disease. Control mice, following lethal irradiation and a transplant of bone marrow and spleen cells survive less than twenty days. If given a short course of treatment with MR1, 75-83% of mice survive >100 days. This effect is dose dependent and requires treatment to be initiated within a few days of receiving the transplant. Although lethality is reduced, some hematological and immunological abnormalities associated with acute GVHD were noted. The data suggest that CD40/CD40L interaction plays a role in T cell activation and that more than one costimulatory pathway is involved in the etiology of graft-versus-host disease, as we have previously shown that blockade of the B7-CTLA4/CD28 pathway manifests similar alteration in the course of the disease.

C2-516 EFFECTS OF rhIL-6 IN THE SCIDhu MOUSE MODEL, M.A. Coccia, S.K. Morris, C. Knott and K. Kuus-Reichel, Hybritech, Incorporated, P.O. Box 269006, San Diego, CA 92196-9006.

Human interleukin-6 (hIL-6) is important for human lymphopoiesis and antibody production. We developed a hIL-6 secreting transfectoma - SCIDhu PBL model system to study the effects of rhIL-6 on human lymphocytes. An expression vector was constructed from mouse metallothionein-1 promoter and hIL-6 gene sequences and transfected into SP2/0-Ag14 cells generating the recombinant hIL-6 (rhIL-6) secreting transfectoma cell line SP2/0-hIL6.17. On day one, *scid-/scid-* mice were injected subcutaneously with 10^4 SP2/0-hIL6.17 cells (rhIL-6 positive mice) or 10^4 SP2/0-Ag14 cells (rhIL-6 negative mice). The mice were reconstituted with 10^8 human PBLs on day two and immunized with 100 μg tetanus toxoid. Serum rhIL-6 concentrations in mice injected with SP2/0-hIL6.17 cells ranged between 10 and 100 ng/ml by days 28-35. These mice had greatly enlarged spleens and lymph nodes. Predictably, rhIL-6 negative mice had no measurable hIL-6 in their serum, but did have somewhat enlarged spleens and lymph nodes. FACs analysis detected larger populations of splenic B and T cells in rhIL-6 positive mice compared to control mice. Interleukin-2 receptor (IL-2R) expression was detectable on human lymphocytes isolated from rhIL-6 positive mice but not on human lymphocytes isolated from rhIL-6 negative or control mice. Immunohistology of spleens and lymph nodes confirmed FACS analysis. Despite results of enhanced IL-2R expression and human lymphocyte localization to lymphatic tissues, mice with elevated rhIL-6 serum concentrations produced slightly less non-specific IgM and IgG. No difference in tetanus toxoid specific IgG production was observed.

C2-517 ABROGATED EXPRESSION OF CLASS II ALLOANTIGENS REDUCES $CD4^+$ INFILTRATES AND IMPROVES FUNCTION OF MOUSE RENAL TRANSPLANTS, T.M. Coffman, R.B. Mannon, C. Doyle, R.C. Griffiths, & J.L. Platt. Duke University and Durham VA Medical Centers, Durham, NC 27710

To further examine the role of donor MHC class II antigens in shaping rejection responses, we studied mouse kidney allografts from donor animals that were deficient in expression of MHC class II antigens. These donor mice had been generated from embryonic stem (ES) cells in which expression of the $A\beta^b$ gene was abrogated by homologous recombination. The ES cells are derived from mice of the $H\text{-}2^b$ haplotype that do not express I-E, thus, animals that are homozygous for the targeted disruption of the $A\beta^b$ gene lack expression of both I-A and I-E antigens. Kidneys from these $H\text{-}2^b$ class II^- mice were transplanted into allogeneic $H\text{-}2^d$ recipients. Four weeks following transplantation, glomerular filtration rate in the mice bearing class II^- kidney allografts (5.0±0.7 ml/min/kg) was significantly higher than that of allograft controls (2.2±0.5 ml/min/kg; $p<0.01$). Despite this preservation of renal function, the class II^- allografts contained significant inflammatory cell infiltrates in perivascular and interstitial regions. Substantial numbers of Thy 1^+ cells were present within the infiltrate. However, the grafts were almost completely devoid of $CD4^+$ T cells. To examine in situ T cell function within the class II^- allografts, we assayed intra-graft expression of IL-2, γ-interferon, IL-4 and granzyme B using RT-PCR. Transcripts for each these cytokines could be detected in class II^- allografts, however, the levels of expression of IL-4 were reduced compared to control allografts from class II^+ donors. These studies suggest that direct recognition of class II antigens plays a significant role in regulating $CD4^+$ T cell accumulation in a kidney graft. Furthermore, maneuvers that reduce or inhibit expression of class II alloantigens may ameliorate rejection of kidney allografts.

C2-518 A MINIMAL MODEL FOR T CELL VACCINATION

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We develop a mathematical model for the regulation of autoreactive T cell growth. The model is very simple in that it is only based upon fundamental properties of T cells. Despite this simplicity, it accounts for a variety of phenomena known as T cell vaccination (TCV). These involve obtaining resistance to autoimmune disease by "vaccination" with either a sub-pathogenic amount of autoreactive T cells, or with attenuated autoreactive cells, or with cells that recognize the autoreactive cells.

The results are based upon our assumption that the self antigens involved in TCV are normally not expressed. Thus they fail to stimulate or tolerate the autoreactive T lymphocytes. Self tolerance is therefore "passive" and corresponds to a naive state. TCV then corresponds to attaining an active state of tolerance in which autoreactive T cells are controlled by regulator cells recognising the effectors.

The model predicts a qualitative difference between vaccination with normal and attenuated autoreactive cells. Normal cells may give rise to a permanent switch to the vaccinated state. However, attenuated cells can only provide a dose dependent transient protection. Preliminary experimental data confirm this prediction.

C2-519 BONE MARROW INFUSION WITHOUT LETHAL IRRADIATION PREVENTS AUTOIMMUNE DISEASE.

Conor P. Delaney, Noriko Murase, Melissa Woan, Veronique Fournier, John J. Fung, Thomas E. Starzl, Anthony J. Demetris. Pittsburgh Transplantation Institute, University of Pittsburgh, Pittsburgh, PA 15213.

The success of lethal irradiation and bone marrow (BM) transplantation at preventing autoimmune (AI) disease has lead to this being suggested as suitable therapy for autoimmunity, even though patients would be exposed to the risks of graft failure and graft-versus-host disease. Based on the persistence of rare donor cells and donor-specific tolerance after BM infusion with transient FK506 and using a model of chemical AI vasculitis in the BN rat, we show that irradiation is not required to prevent AI disease.

All animals received 5 subcutaneous injections of $HgCl_2$ (1mg/kg) from d0 to d8 and all transplants were fully allogeneic from Lewis to BN.

Group (G)	n	Pre-conditioning (day)	Surface changes	Mortality
1	11	naive BN	severe	44%
2	7	*HHT (d-20)*	mild	29%
3	6	FK506 1mg/kg x 14d (d-100)	mild	33%
4	7	as G3 + $3x10^8$ BM (d-100)	absent	0%

Surface manifestations, mortality and autoantibody titres were significantly improved in G4 alone, correlating with immunohistochemistry of kidney and skin. A significant B cell increase (on FACS) was prevented only in G4. Protection from AI disease was associated with elevated baseline levels of B cells and an upregulation of MHC class II expression on T cells by FACS analysis. Levels of baseline cell proliferation were significantly increased in G4 lymphocytes. In vitro autoreactive assays, by treating lymphocytes with 10^{-9} and 10^{-10} mol of $HgCl_2$ before use as stimulators in MLR, suggested that the protection is at the level of the initial cellular response to chemical injury.

The results demonstrate that the persistence of rare allogeneic cells after BM infusion without radiation provides sufficient immunostimulation to control the emergent properties of AI-prone BM and prevent AI disease. Furthermore, allogeneic and AI reactions can be regulated through a common controlling pathway. We suggest that this provides new, low morbidity treatment options for human AI disease.

C2-520 PROCESSING AND CLASS II MHC-RESTRICTED *PRESENTATION OF MEASLES VIRUS DETERMINANTS,*

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It is now established that loading of class II MHC molecules with antigen fragments derived from exogenous proteins takes place in a specialized endosomal/lysosomal compartment. By contrast, the processing and presentation pathways followed by determinants derived from cytosolic proteins are still poorly defined. To study this aspect of antigen processing, we are developing a system using measles virus as model antigen since it has been reported that class II MHC-restricted presentation of some measles determinants is not affected by the presence of chloroquine during infection. This observation suggests the possibility that either formation or loading of the antigen fragments takes place in compartments other than an endosomal/lysosomal compartment.

Human T cell clones specific for measles virus-infected B cells have been derived and characterized. We have also developed an assay to measure proliferation of T cells stimulated by isolated DR molecules inserted into artificial membranes. Using cell fractionation techniques in combination with various inhibitors (Brefeldin A), this system will now allow us to determine where loading of DR molecules with measles virus-derived determinants takes place.

C2-521 A CYTOLYTIC T-CELL MEDIATED IMMUNE RESPONSE TO A MURINE MODEL OF BREAST CANCER, Katharine H Dixon, Caryn D Tong, Drew M Pardoll, Daniel Medina, Elizabeth M Jaffee, Department of Oncology, Johns Hopkins University School of Medicine, Baltimore, MD 21205

Investigation of the immune response to tumors will benefit from the use of animal models that are representative of human malignancies. Of critical interest is the identification of proteins that stimulate T-cell mediated immunity and whose expression is associated with either changes in expression of, or mutation of oncogenes and tumor-supressor genes. Mutations in the gene encoding the p53 tumor supressor are associated with a spectrum of human tumors, and have frequently been detected in carcinoma of the breast. The tumor line T-4960R 125-DIM3 belongs to a family of independently-arising hyperplastic outgrowths and tumors established from the immortalized mouse-mammary epithelial line, COMMA 1D. The BALB/C derived COMMA 1D cell line and its derivatives display mutation in the *p53* tumor supressor gene. Injection of 1.10^5 T-4960R 125-DIM3 cells into the mammary glands of either BALB/C or nude mice produces tumors with similar growth kinetics irrespective of mouse strain, indicating that primary tumor challenge illicits no effective immune response. However, vaccination of BALB/C *mice with irradiated T-4960R 125-DIM3 cells transduced to secrete* GM-CSF produces splenocytes with cytolytic activity toward T-4960R 125-DIM3 target cells. This lytic activity was absent from the splenocytes of naive mice and was inhibited when primed splenocytes were preincubated with antibodies that were specific for CD8. Employing expression cloning techniques, this model will be used to identify genes that give rise to epitopes recognized by cytolytic T-cells. It will be of future interest to determine whether these genes also produce epitopes that are recognized by human T-cells.

C2-522 AGE-RELATED PROPENSITY OF Mls-1^a MICE TO DEVELOP Vß6$^+$ T CELLS AFTER IRRADIATION AND SYNGENEIC BONE MARROW TRANSPLANTATION, Gino Doria, Camillo Mancini, Giorgio Leter, Masanori Utsuyama* and Katsuiku Hirokawa *, Laboratory of Immunology, AMB-BIO-MED ENEA, Casaccia (Rome), Italy , * Department of Pathology, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan

Age-related autoimmunity is concomitant with the appearance of autoreactive T cells, suggesting that accumulation of thymic stromal damage may hamper negative selection, leading to ineffective elimination of autoreactive T cell clones. The effects of aging on clonal deletion of mature Vß6$^+$ T cells was investigated in (C57BL/10-Mls-1^b x DBA/2-Mls-1^a)F1 female mice in which negative selection of autoreactive cells specific for Mls-1^a is known to occur in the thymus. Normal mice at the age of 3 mos. (Young) or 12 mos. (Old) were given X-rays (8.5 Gy) and 1x10^7 T cell-depleted syngeneic bone marrow cells from Young or Old donors. Chimeras of the four recipient-donor combinations were sacrificed 9 mos. after irradiation and bone marrow transplantation. Pooled axillary and inguinal lymph node cells from individual chimeras and Young or Old normal mice were analyzed by flow cytometry to determine the percent of CD4$^+$ or CD8$^+$ T cells and the percent of Vß6$^+$ T cells in each subset. As expected, the percent of both subsets in normal mice was decreased by aging whereas the percent of Vß6$^+$ T cells in each subset was negligible and unaffected by aging. The total percent of CD4$^+$ and CD8$^+$ Vß6$^+$ T cells was 0.19 ± 0.05 in Young and 0.19 ± 0.06 in Old mice. Bone marrow cells from Young as compared to Old donors were more efficient in T cell repopulation regardless of the recipient age. The percent of Vß6$^+$ T cells was found increased in both CD4$^+$ and CD8$^+$ cell subsets of all chimeras regardless of the recipient-donor combinations. The increase, however, was much more pronounced in Old as compared to Young recipients. Hence, the total percent of CD4$^+$ and CD8$^+$ Vß6$^+$ T cells was 1.30 ± 0.20 in Young and 3.11 ± 1.08 in Old recipients of bone marrow cells from Young donors while was 0.94 ± 0.36 in Young and 3.54 ± 0.50 in Old recipients of bone marrow cells from Old donors. These findings suggest that the development of Vß6$^+$ T cells in chimeras was a consequence of radiation-induced damage of the thymic epithelium, as already shown in syngeneic bone marrow chimeras undergoing GVHR. The remarkable increase in the percent of Vß6$^+$ T cells in Old recipients suggests that aging had deteriorated the thymic epithelium prior to irradiation leading to a more profound alteration of the negative selection of Vß6$^+$ T cell clones. Furthermore, the same percent of Vß6$^+$ T cells found when bone marrow cells from Young or Old donors were transplanted into recipients of equal age rules out the possibility that aging of stem cells contributed to the generation of autoreactive T cell clones. Whether Vß6$^+$ T cells in the peripheral lymphatic tissues of chimeras were reactive or anergic to Mls-1^a was not determined.

C2-523 RAPAMYCIN SELECTIVELY BLOCKS IL-2 INDUCED PCNA GENE EXPRESSION IN T LYMPHOCYTES: EVIDENCE FOR THE ROLE OF CREB/ATF BINDING ACTIVITIES.

Feuerstein N., Huang D., and Prystowsky M. B., Medical College of Pennsylvania, Philadelphia, PA, and Albert Einstein College of Medicine, Bronx, NY.

Rapamycin, inhibits T lymphocyte proliferation by targeting a distinct step initiated by IL-2 and consequently arrests cells at G1/S. We have recently found that increases in the binding of discrete transcription factors of the ATF/CREB family are associated with cell cycle progression at G1/S and are specifically involved with PCNA promoter activity in T lymphocytes stimulated by IL-2. Inhibition of IL-2 induced proliferation by rapamycin was associated with inhibition of the IL-2 induced CRE binding activities at G1/S. We now show, by using 2D gel electrophoresis, that rapamycin selectively blocked the expression of PCNA, an obligate cofactor of DNA polymerase delta, an important component for DNA replication. Further studies demonstrated that rapamycin inhibited the IL-2 induced PCNA mRNA, and the murine PCNA promoter activity in IL-2 stimulated cells. Using EMSA we demonstrate that rapamycin potently inhibited the binding of CREB/ATF transcription factors to CRE elements in the murine proximal PCNA promoter. These results suggest that PCNA is a preferred target in a rapamycin sensitive transduction pathway, and that the mechanism by which rapamycin inhibits PCNA gene expression may involve the inhibition of the interaction of CREB/ATF transcription factors with CRE elements in the proximal PCNA promoter.

C2-524 IDENTIFICATION OF MHC SUSCEPTIBILITY GENES THAT CONTROL ALLOANTIBODY RESPONSES AGAINST AN HLA CLASS I PUBLIC EPITOPE. Thomas C. Fuller and Anne Fuller, Department of Pathology, University of Utah, Salt Lake City, UT. 84132

The genetic mechanisms that control antibody responses against HLA Class I alloantigens are not understood. One obvious postulate would be that MHC Class II restriction elements interact with HLA allopeptide and regulate the cognate interaction between B lymphocytes and CD4+ T helper lymphocytes. We have analyzed our allosensitized transplant candidates with regard to responsiveness to the serologically-recognized, HLA-Bw4 epitope. All transplant patients were screened monthly for HLA antibody by the antiglobulin cytotoxicity method using >35 HLA-select, panel lymphocytes. We have found that 15 of 17 patients who have produced HLA-Bw4 antibody are HLA-DR1 and/or DR3 positive while the phenotypes of the remaining two responders are both DR4,15 (X^2=20.45, p<<0.0001).

If the above hypothesis is correct, peptide encompassing the Bw4 epitope may have a pattern of binding to HLA Class II consistent with humoral responsiveness. Two synthetic peptides, one representing Bw4 sequence (aa77-85) and the other, Bw6, were biotinylated and assayed for binding to a EBV-LCL panel of homozygous cells using a triple sandwich, fluorescence binding assay with flow cytometry. Three distinct binding patterns evolved: high intensity (DR1$^+$ and/or DR3$^+$), low (DR4,7) and intermediate (DR11,13,15); some LCLs bound Bw6 peptide weakly.

In conclusion, we have identified two human *Ir* genes, HLA-DR1 and HLA-DR3, that confer a high risk for humoral alloimmunization against the Bw4 epitope. These data also suggest that the Bw4 allopeptide serves a dual function as the epitope against which the B cell response is directed as well as the stimulant recruiting T cell help.

C2-525 THE CTLA-4 COUNTER-RECEPTOR IS REQUIRED FOR T CELL IL-4 PRODUCTION DURING THE PRIMARY BUT NOT CHALLENGE MUCOSAL IMMUNE RESPONSE TO A GASTROINTESTINAL PARASITE, William C. Gause*, Xia di Zhou*, S.-J. Chen*, Suzanne C. Morris#, Fred D. Finkelman#, Peter Linsley##, Joseph F. Urban**, and Pin Lu*. Departments of *Microbiology and #Medicine, USUHS, Bethesda, MD 20814, ##Department of Cellular Immunology, Bristol-Myers Squibb Pharmaceutical Research Institute, WA,Helminthic Diseases Laboratory, ARS, USDA, Beltsville, MD 20705-2350.

The costimulatory signal provided through CTLA-4 counter receptor interactions is required for T cell activation resulting in increased IL-2 production in vitro, but its role in IL-4 production is unclear and few in vivo studies have been performed to confirm results of in vitro experiments. We have examined the in vivo effects of blocking CTLA-4 ligands on the primary and challenge Th2-associated mucosal immune responses that follow oral infection of mice with the nematode parasite, *Heligmosomoides polygyrus*. Murine CTLA4-Ig administration on the day of immunization inhibited *H.-polygyrus*-induced increases in CD4$^+$,TCRα/β$^+$T cell IL-4 gene expression and IL-4 protein production as measured by RT-PCR and ELISPOT respectively at 8 days after primary inoculation. In addition, CTLA4-Ig partially blocked increased IL-3, IL-4, IL-5, and IL-9 cytokine gene expression in the Peyer's patch (PP), and completely blocked elevated serum IgE levels, but not blood eosinophils, at 14 days after inoculation. In contrast administration of CTLA4-Ig at days 3 and 4 after immunization did not affect either elevated cytokine production or IgE levels. In addition, CTLA4-Ig administered at the time of a second inoculation with *H. polygyrus* failed to suppress IL-4 gene expression by CD4$^+$,TCRα/β$^+$T cells. These results suggest that stimulation of CD28 and/or CTLA-4 is required for T cell activation leading to IL-4 cytokine production, B cell activation, and IgE secretion during a Th2-like, primary mucosal immune response to a nematode parasite but that IL-4 production by primed T cells or memory T cells does not require CTLA-4 counter-receptor interactions.

C2-526 TCR-AND PEPTIDE CONTACT RESIDUES IN HLA-DR3, Annemieke Geluk*, Krista E. van Meijgaarden*, Xin-ting Fu#, René R.P. de Vries*, Robert W. Karr# and Tom H.M. Ottenhoff*, * Dept. Immunohematology & Blood Bank, University Hospital, Leiden, The Netherlands, and # Dept. of Immunology, G.D. Searle & Co., St. Louis, MO 63198.
Previously, the anchor residues in different peptides that bind to HLA-DR17(3) were determined and slightly different DR17-peptide binding motifs were identified. We have now studied the functional role of polymorphic HLA-DR17 ß1 chain residues in peptide binding and T cell stimulation. Proliferative responses of two unrelated DR17-restricted T cell clones to hsp65 p3-13 and 30/31kD p56-65 were examined using L cell transfectants expressing mutant DR17 molecules as APC. Binding of these peptides to the DR17 mutant cells was analyzed as well.
Differnt residues were important for binding/presentation of the two peptides. This may explain the fact that variability is observed in peptide binding motifs of peptides binding to the same DR molecule.

C2-527 MECHANISM OF NICOTINE INDUCED IMMUNE UNRESPONSIVENESS. Geng, Y.M., Savage, S.M, and Sopori, M.L. Institute for Basic and Applied Medical Research, The Lovelace Institutes, 2425 Ridgecrest Drive, S.E., Albuquerque, NM 87108, and Biology Department, University of New Mexico, Albuquerque, NM 87113
Tobacco smoking is known to predispose individuals to a number of diseases. It has been postulated that such susceptibility has resulted from the negative effects of tobacco on the immune system. The mechanism through which the immune system is affected, however, is not clearly understood. Results from our recent studies have demonstrated that the immuno-suppressive effects of cigarette smoke are associated with the nicotine-containing particulate phase of cigarette smoke. Chronic exposure of animals to nicotine results in inhibition of the plaque-forming cell (PFC) responses to T dependent and T independent antigens. But no significant differences between control (CON) and nicotine-treated (NT) animals were observed in the number or percentages of B cells, T cells, and T cell subsets. These results suggest that nicotine may un-couple antigen receptors from the components of signal transduction pathway. Results from our *in vivo* studies indicate that cells from NT animals have increased basal level of PTK activity and are unresponsive to antigen-induced activation. These findings are further supported by our *in vitro* studies showing that nicotine stimulates lymphocytes, leading to their unresponsiveness to antigen-induced activation.

C2-528 HUMAN NATURAL KILLER CELLS INDUCE THE EXPRESSION OF E-SELECTIN AND INTERLEUKIN 8 mRNA IN PORCINE ENDOTHELIAL CELLS, David J. Goodman and Fritz H. Bach. Sandoz Center for Immunobiology, Harvard Medical School, Boston, MA 02215.
NK cells display a wide spectrum of cytotoxicity towards virally-infected cells, malignant cells and some xenogeneic targets; NK cells are present in rejecting discordant xenografts. Activation and damage of endothelial cells (EC) in the rejecting xenografts appear to underlie the rejection process. Thus, to determine if NK cells may be pathogenetically involved in rejection, we have assessed, in vitro, whether human NK cells would induce EC activation, as measured by induction of the adhesion molecule E-selectin and the chemotactic cytokine IL-8. We studied co-cultures containing human NK cells, used immediately after isolation from blood, and confluent porcine endothelial cell monolayers (pEC's).
After 4 hours of co-culture, RNA was extracted followed by RT/PCR with oligonucleotide primers specific for E-selectin and Interleukin 8. The expression of E-selectin was confirmed by a cellular ELISA. In addition, cellular cytotoxicity of the pEC was determined using a lactate dehydrogenase (LDH) release assay.
NK cells added to pEC's resulted in significant cellular cytotoxicity at effector to target (E:T) ratios ranging from 30:1 to 5:1, demonstrating direct cytotoxicity; the addition of purified human IgG enhanced cytotoxicity, consistent with antibody mediated cellular cytotoxicity. At lower NK:pEC (E:T) ratios, EC were activated with induction of both E-selectin and IL-8 mRNA expression. The induction of E-selectin and IL-8 mRNA was seen with three separate sources of NK cells: purified (sorted) CD56+ve cells, the NK cell clone B22, and the Fc receptor deficient NK cell line, NK92. These effects did not seem to be mediated by TNF since the addition of either anti-TNF-α or human soluble TNF-α receptor failed to inhibit the induction of E-selectin.
Thus, human NK cells, dependent on the E:T ratio can lyse or activate pEC resulting in the expression of E-selectin and IL-8 mRNA and E-selectin protein, apparently independent of TNF-α and at E:T ratios below those associated with cellular cytotoxicity. These findings implicate NK cells in endothelial cell activation and cell mediated xenograft rejection.

C2-529 CHARACTERIZATION OF A MECHANISM OF HOST-PROTECTION IN THE BALB/c MODEL FOR LEISHMANIASIS WHICH INVOLVES TOLERIZATION RATHER THAN IMMUNIZATION, Emanuela Handman, Lynn Morris, Anton Aebischer and Jean-Pierre Scheerlinck, The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia.
Susceptible BALB/c mice can be protected from fatal infection with *Leishmania major* by prophylactic intravenous immunization with irradiated parasites. Protection is dependent on the route of inoculation: intravenous injection is protective while subcutaneous injection is not. We used this BALB/c-*L. major* model system to investigate the mechanism of T cell activation leading to a Th1 response and host protection. We analysed quantitatively the parasite-specific, $CD4^+$ T cell mediated immune responses by limiting dilution. Subcutaneous vaccination resulted in priming of $CD4^+$ precursor T cells, whereas intravenous vaccination was ineffectual. Moreover, intravenous injection prevented the increase in the number of specific precursor cells usually observed after infection of normal mice. This was not due to the elimination of the virulent challenge parasites as a result of immunity, nor to inefficient antigen-presentation of the irradiated organisms after intravenous injection. The data suggests that intravenous injection results in tolerization rather than immunization. Tolerization as a mechanism of host protection is consistent with earlier observations that transient immunosuppression results in cure of *L. major* infection in BALB/c mice. Transfer of antigen-presenting cells isolated from spleens of mice injected previously with irradiated parasites mimicked the effect of intravenous immunization with irradiated parasites. The phenotype and role of these antigen presenting cells in parasite-specific T-cell tolerization will be discussed.

C2-530 HUMAN IMMUNE RESPONSE TO BILAYERED CULTURED ALLOGENEIC SKIN GRAFTS. J. Hardin-Young, C. Isaacs, S. Prosky, C. Nolte, N. Pliss, and N. Parenteau, Cell and Tissue Sciences Organogenesis, Canton, MA 02021

The immune response to non-hemapoietic allogeneic cells is poorly understood. To address this issue human and animal studies using bilayered cultured skin allografts (BLCA) have been conducted to determine: 1) whether BLCA sensitize patients to alloantigen and 2) what role immunology plays in the use of BLCA. The BLCA are composed of bovine type I collagen, human dermal fibroblasts (HDF), and human epidermal keratinocytes (HEP). Both HDFs and HEPs in the BLCA express class I, but not class II HLA antigens as shown by immunocytochemistry. In this report we demonstrate that PBLs from naive individuals fail to proliferate in response to gamma-IFN treated HDF or HEP in vitro and that clinically, 237 patients receiving BLCA for the treatment of venous ulcer or surgical excision wounds did not generate allospecific T cells or cytotoxic antibodies. The patients immune response was evaluated in vitro by testing pre- and post-graft blood in an antibody dependent cytotoxicity assay and by measuring the T cell proliferation (H^3-thymidine incorporation) response to graft cells. Allogeneic HDFs and HEPs, treated with gamma-IFN to induce class II expression, failed to induce a significant proliferative response in patient PBLs as compared to unstimulated PBLs. In contrast, patient PBLs showed significant proliferation when cultured with PHA or allogeneic PBLs as stimulators. A SCID mouse model was also used to investigate the lack of allospecific immune response to BLCA. SCID mice were injected i.p. with 3-8 x 10^7 human PBLs. Reconstitution with huPBLs was confirmed by ELISA to detect circulating human Ig and Alu-PCR analysis of blood and spleen. Reconstituted mice received either BLCA or human skin grafts. Preliminary data show that reconstituted mice reject normal human skin within 10-14 days, while BLCA can persist for at least 5 wks without gross or histologic signs of graft rejection. Immunocytochemistry was used to confirm the persistence of human BLCA. Studies are in progress to quantify the amount of complement-fixing and non-fixing antibody in the serum. In addition, immunocytochemistry is being used to demontrate that persistence of BLCA is not due to inaccessiblity of graft to huPBLs. In conclusion, data from both animal and human studies show that BLCA fail to sensitize host cells to alloantigen and fail to illicit graft rejection in reconsitituted SCID mice.

C2-531 NOVEL PEPTIDE ALDEHYDES ARE PROTEASOME INHIBITORS AND BLOCK THE MHC-I ANTIGEN PROCESSING PATHWAY. Clifford V. Harding[1], John France[1], Rui Song[1], John Farah[2], Mohamed Iqbal[2] and Robert Siman[2], 1. Institute of Pathology, Case Western Reserve University, Cleveland, OH 44106. 2. Cephalon, Inc., West Chester, PA 19380.

The proteasome is a multicatalytic protease complex that is present in the cytosol and has been implicated in cytosolic antigen processing. Novel peptide aldehydes were synthesized and found to inhibit the chymotrypsin-like protease activity of isolated proteasomes. These inhibitors were membrane permeable and blocked intracellular cleavage of a membrane-permeable fluorogenic substrate of the chymotrypsin-like proteasome activity. When OVA was introduced into the cytoplasm of murine B cells by electroporation, the proteasome inhibitors blocked its processing for presentation by class I MHC (MHC-I) molecules, while they had little effect on class II MHC antigen processing. The potency of different inhibitors for blockade of MHC-I antigen processing correlated directly with their potency for inhibition of proteasome activity. These results directly demonstrate that inhibition of the chymotrypsin-like proteasome activity blocks MHC-I antigen processing.

C2-532 EFFECT OF PHOSPHORYLCHOLINE-CONTAINING FILARIAL EXCRETORY-SECRETORY PRODUCTS ON B LYMPHOCYTE SIGNALLING PATHWAYS, W. Harnett, M. Deehan, M.J. Frame and M.M. Harnett*, Department of Immunology, University of Strathclyde, Glasgow G4 ONR and *Department of Biochemistry, University of Glasgow G12 8QQ.

A characteristic of infection with filarial nematodes is the induction of lymphocyte hypo-responsiveness. Although the cause of this is uncertain, increasing evidence suggests a role for products excreted-secreted (ES) by the parasite, and in particular those containing a phosphorylcholine (PC) moiety. We have recently demonstrated that the PC-ES, ES-62, can inhibit anti-Ig induced murine B cell proliferation, and that this can be mimicked by PC-BSA or PC alone. In an attempt to understand the biochemical mechanisms underlying this result, we have investigated the effect of ES-62/PC on B cell signal transduction pathways. We have found no evidence to support an effect on the generation of inositol phosphates, but have witnessed (i) an inhibition of protein tyrosine kinase activity, and (ii) a downregulation of protein kinase C, both at the level of expression and activity.

C2-533 IMMUNITY TO *LISTERIA MONOCYTOGENES* IN THE ABSENCE OF IFN-γ, John T. Harty and Michael J. Bevan* Department of Microbiology, University of Iowa, Iowa City, IA and *Howard Hughes Medical Institute, University of Washington, Seattle, WA.

In vivo and in vitro studies have demonstrated that IFN-γ is a critical mediator of natural immunity to *L. monocytogenes* infection of mice. Consistent with earlier studies, disruption of the IFN-γ receptor gene increases susceptibility of mice to *L. monocytogenes* infection. We show that IFN-γ structural gene knockout (GKO) mice are extremely susceptible to virulent *L. monocytogenes* infection. However, GKO mice immunized with an attenuated *L. monocytogenes* strain contain antigen specific CD8 T cells which can be propagated in vitro. These CD8 T cell lines, which are incapable of producing IFN-γ, transfer similar levels of antilisterial immunity to naive wild-type mice as CD8 T cells which produce IFN-γ. Thus, we conclude that CD8 T cell derived IFN-γ is not required for immunity to *L. monocytogenes*. Furthermore, immunized GKO mice themselves develop significant immunity to rechallenge with virulent *L. monocytogenes*. These studies suggest that specific immunity can overcome the lack of a cytokine critical for resistance to acute infection. The specificity and mechanism of immunity to *L. monocytogenes* in the absence of IFN-γ is currently under investigation.

C2-534 ANALYSIS OF NOVEL THYMIC SUBPOPULATIONS EXPRESSING THE CELL SURFACE MOLECULE GL7: EXPRESSION, GENETICS, AND FUNCTION. K.S. Hathcock[1], C.E.M. Pucillo[1], G. Laszlo[1], L. Lai[2], and R.J. Hodes[1,3] [1]EIB, NCI and [3]NIA, National Institutes of Health, Bethesda, MD 20892, and [2]Pharmingen, San Diego, CA 92121

Expression of cell surface molecules such as CD44, CD69, IL2R, QA-2, and LY6 has been used to characterize thymic subpopulations. Recently we reported a rat monoclonal antibody, GL7, which defines a cell surface molecule expressed by activated B cells and T cells as well as by a subpopulation of $CD4^+CD8^-$ thymocytes. The current study analyzes GL7 expression by adult and foetal thymocytes in greater detail and demonstrates that 1.) the majority of $GL7^+$ thymocytes isolated from adult BALB/c thymuses are mature $CD4^+CD8^-$ cells that are $CD3\epsilon^{hi}$, $Tcr\alpha\beta^{hi}$, HSA^{int}, and bimodal for CD69 expression. 2.) There is variation among mouse strains in expression of GL7 on adult $CD4^+CD8^-$ thymocytes; 10-30% of BALB/c $CD4^+CD8^-$ thymocytes are $GL7^+$ whereas only 1-5% of C57BL/6 $CD4^+CD8^-$ thymocytes are $GL7^+$. 3) Foetal BALB/c and C57BL/6 thymocytes contain 1-3% $GL7^+$ cells which are TcR $\gamma\delta^+$, HSA^{int}, and $CD69^{neg}$. 4) Adult BALB/c $GL7^+$ $CD4^+CD8^-$ thymocytes are functionally competent cells that can be activated by TcR-specific stimuli to proliferate and secrete cytokines. 5) Activation of adult BALB/c $GL7^-CD4^+CD8^-$ thymocytes with TcR-specific stimuli results in GL7 expression on a subpopulation of $CD4^+$ thymocytes. Thus, expression of GL7 defines a novel functional subpopulation of $CD4^+CD8^-$ thymocytes.

C2-535 A POTENTIAL IN-VITRO PARAMETER OF IMMUNE PRIMING FOLLOWING VACCINATION WITH A GM-CSF SECRETING TUMOR. Karen M. Hauda, Christy A. Counts, Christine E. Weber, Audrey J. Lazenby, Fray F. Marshall, Drew M. Pardoll, Herbert Hurwitz, Hyam I. Levitsky, Jonathan W. Simons, William G. Nelson, Michael Carducci and Elizabeth M. Jaffee, Johns Hopkins Oncology Center, Baltimore, MD 21205.

Murine tumor models have demonstrated that autologous tumor cells engineered to secrete cytokines elicit systemic immune responses capable of eliminating small amounts of established tumor. In most cases, the effector arm of this response is mediated by tumor specific cytotoxic T-cells. We are currently investigating the role of T-cells in a Phase I clinical trial evaluating this vaccine approach in patients with metastatic renal cell carcinoma (RCC). T-cells from peripheral blood of patients vaccinated with either irradiated autologous GM-CSF transduced or non-transduced RCC have been examined. Comparisons of T-cell activity pre- and post-vaccination have been made. In addition, cytolytic T-cell responses have been correlated with delayed type hypersensitivity responses to autologous RCC and normal renal cells at time points pre- and post-vaccination. The utility of this assay system as an in-vitro indicator of successful in-vivo anti-tumor immune priming will be discussed.

C2-536 CONTROL OF CANDIDA ALBICANS INFECTION IN CD4-DEFICIENT MICE, Danuta J. Herzyk, Elizabeth V. Ruggieri, Alison M. Badger†, Alem Truneh‡, and Peter J. Bugelski, Dept of Toxicology, †Dept. of Cellular Biochemistry and ‡Dept. of Molecular Immunology, SmithKline Beecham Pharmaceuticals, King of Prussia, PA 19406.

Experimental evidence suggests that defense against Candida involves non-specific as well as cell-mediated and humoral immunity. Multiple arms of immune response were examined in CD4-deficient mice in three models of host defense against *C.albicans* B311 infection . General immune responsiveness was evaluated using survival as the end point following intravenous injection of live Candida. Cell-mediated immunity was studied by assessment of colony forming units (CFU) at the site of a localized intramuscular infection (calf muscle) and humoral immunity was tested by determination of anti-candida antibody in serum after two subcutaneous injections of *C.albicans*. Mice were administered monoclonal anti-CD4 antibody GK1.5 to deplete CD4+ cells. In addition, mice with a null mutation in the gene encoding CD4 (knockout) were evaluated in the models. Treatment with GK1.5 caused a 50 to 75 % depletion of CD4+ cells in the spleen, inhibited antibody responses to ovalbumin immunization and also inhibited an *ex vivo* mixed lymphocyte reaction (MLR). In the host defense models, GK1.5 had no effect on either survival time during *C.albicans* infection or the rate of clearance of local infection, it blocked, however, anti-candida antibody production. In contrast, Cyclosporine A, a T-cell modulating drug, decreased survival, increased muscle colonization of *C.albicans* and inhibited anti-candida antibody production. CD4 knockout mice demonstrated a similar capacity of controlling Candida infection to normal BALB/c mice. Taken together, these results indicate that although depletion of CD4 impaired the humoral response to *C.albicans* it did not deteriorate host defense against systemic and local infection, suggesting that CD4 cells do not play a critical role in host defense against deep *C.albicans* infections.

C2-537 ENDOGENOUS SELECTION OF LISTERIOLYSIN O (LLO)-DERIVED EPITOPES IN ANTILISTERIAL IMMUNITY. David J. Hinrichs and H. G. Archie Bouwer. Immunology Research, VAMC and EACRI, Portland, OR 97201.

A nanomer peptide, corresponding to amino acid sequence 91-99 (aa91-99) from LLO is a major K^d presented epitope for antilisterial cytotoxic cells. Although at least 5 additional nanomer peptides are contained within the LLO sequence which also satisfy the K^d binding motif, aa91-99 is the only target peptide. In order to further investigate epitope selection of endogenously processed LLO-derived peptides, we have introduced a point mutation in *hly* (gene for LLO) which results in a Y to F substitution in the anchor residue at position 2 within the 91-99 sequence. This "92F" *L. monocytogenes (Lm)* mutant produces biologically active LLO with similar kinetics as wildtype *Lm*, and possesses similar growth kinetics extracellularly, or intracellularly within target cell monolayers, as wildtype *Lm*. The LD_{50} of the 92F *Lm* mutant is similar to wildtype *Lm*, and BALB/c mice actively immunized with the 92F *Lm* mutant are protected against challenge with wildtype *Lm*. Stimulated immune spleen cells, obtained from donors immunized with the 92F *Lm* mutant, adoptively transfer antilisterial immunity against subsequent challenge with wildtype *Lm*. This cell population also contains antilisterial cytotoxic cells which lyse target cells infected with either wildtype *Lm*, the 92F *Lm* mutant, or a *Lm* construct which is LLO^- but becomes intracytoplasmic due to perfringolysin O production, demonstrating antilisterial cytotoxic cell recognition of non LLO-derived determinants. Antilisterial cytotoxic cells from 92F immunized donors are not targeted against MHC class I presented LLO-derived determinants as measured using target cells infected with a LLO producing *B. subtilis* construct, or target cells pulsed with the aa91-99 peptide, target cells pulsed with the other LLO derived peptides which satisfy the K^d binding motif, or target cells pulsed with a peptide corresponding to the aa91-92F-99 sequence. It is apparent that in the absence of the endogenously processed LLO derived 91-99 epitope, additional LLO derived epitopes from the pool of processed *Lm* derived products do not stimulate peptide-specific antilisterial cytotoxic cells. Thus, the conservative aa substitution of Y to F does not alter the biology of the LLO molecule, but apparently eliminates the induction of all H2-K^d restricted cytotoxic responses directed against this key virulence determinant.

C2-538 IL-12 MODULATES ESTABLISHED TH2 RESPONSES IN MURINE LEISHMANIASIS, Brian D. Hondowicz and Phillip Scott, Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104

Leishmania major is a protozoan parasite that causes cutaneous leishmaniasis in man. A model of human leishmaniasis has been developed in inbred strains of mice, in which some strains develop a Th1 response causing resolution of the disease, while other strains, such as BALB/c, develop a Th2 response and are unable to control parasite replication. It was previously demonstrated that administration of IL-12, a cytokine which promotes Th1 cell development, during the first two weeks of *L. major* infection of BALB/c mice resulted in a Th1 type response and resolution of the disease. However, treatment with IL-12 at later time points was ineffective. Recently, we found that combined treatment with pentostam, an anti-leishmanial drug, and IL-12 in three week infected BALB/c mice promoted Th1 cell development and healing lesions. We hypothesized that the inability of IL-12 alone to alter the dominant Th2 response in BALB/c mice might be to due to the high antigen level associated with the infection at later time points. Therefore, we transferred 10^8 spleen cells from three week infected BALB/c mice, into syngeneic C.B-17 SCID mice, infected the animals with *L. major* and treated for two weeks with either IL-12 or PBS. As expected, SCID mice that received cells from three week infected BALB/c mice developed non-healing lesions, which at five weeks contained $>10^7$ parasites. In contrast, mice treated with IL-12 were protected against disease. These studies suggest that in the absence of high antigen levels IL-12 alone can down-regulate the Th2 response associated with progressive leishmanial infection.

C2-539 ANALYSIS OF THE IMMUNOLOGICAL EVENTS WHICH LEAD TO THE INDUCTION OF PERIPHERAL TOLERANCE

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We have previously shown that intranasal administration of peptides containing the immunodominant T-cell determinant on the major allergen Der p I can render high responder H2b mice profoundly unresponsive to an immunogenic challenge with the whole antigen. The state of non-responsiveness is characterized by a failure of antigen-specific T cells to secrete IL-2 or proliferate when rechallenged with antigen in vitro. In addition treatment with a single peptide intranasally downregulates responses to four potential epitopes on the antigen.

To understand more about the control of T cell responses following inhalation of peptides we have begun to examine the pattern of cytokines produced following tolerance induction and the time course required to induce specific nonresponsiveness. We have also begun to examine the "infectious" nature of the tolerance induced and the cellular mechanisms responsible for this phenomenon.

C2-540 **PRESENTATION OF TUMOR-SPECIFIC ANTIGEN BY B7-EXPRESSING MURINE EPITHELIAL CARCINOMA IN THE PRIMING OF TUMOR SPECIFIC CYTOTOXIC T LYMPHOCYTES,** Alex Y.C. Huang, Allen T. Bruce, Drew M. Pardoll, and Hyam I. Levitsky, Department of Oncology, Johns Hopkins University School of Medicine, Baltimroe, MD 21205

Many tumors have been shown to express tumor specific antigens capable of being presented to CD8+ cytotoxic T lymphocytes (CTL) by the major histocompatibility complex (MHC) class I molecules. Activation of antigen-specific, MHC class I-restricted CTL can result in potent antitumor immune responses. We have previously demonstrated that, in the case of an epithelially derived tumor cell line, CT26, *in vivo* priming of antigen specific CTL is mediated by the host bone marrow derived antigen presenting cells (APC). Contrary to the current antigen presentation dogma, priming of CD8+ CTL does not occur upon encountering antigens presented on the tumor MHC class I molecules. Several investigators have demonstrated that the expression of the costimulatory molecule, B7-1, on non-immunogenic tumors can lead to tumor rejection *in vivo* in a CD8-dependent manner. We wish to further investigate the role of B7-1 in the priming of tumor-specific CTL. CT26 expressing a model tumor antigen, the influenza nucleoprotein (NP), were co-transfected with B7-1. After one immunization with irradiated B7+ CT26NP, priming of NP-specific CTL is mediated by host bone-marrow derived APC, despite the tumor's B7-1 expression. However, priming of anti-NP CTL by B7+ tumor cells can be detected after multiple vaccinations. This result indicates that host bone marrow derived APC are more efficient in the initial presentation of tumor antigen to naive T cells. Presentation by the B7+ tumor cells may initially occur at a level below the detection limit of the assay, but can be subsequently amplified by a repeated immunization with B7+ tumors. We are currently investigating both the effect of irradiation on B7-1 function, and the mechanisms of MHC class I presentation of exogenous antigens by host professional APC. These results will be presented.

C2-541 INDUCTION OF RECIPIENT UNRESPONSIVENESS WITH HYPERBARIC OXYGEN CULTURE, Debra A. Hullett, PH.D., Debra A. MacKenzie, Ph.D., and Hans W. Sollinger, M.D., Ph.D., Department of Surgery, University of Wisconsin-Madison, Madison, WI 53792

Pretreatment of murine thyroid allografts prior to transplantation with hyperbaric oxygen culture (HOC: 95% O_2, CO_2, 25 psi, 48h) prolongs graft survival, down regulates MHC class I surface expression and induces recipient tolerance to a second non-cultured allograft. Our working hypothesis is that presentation of soluble donor MHC class I by naive splenic B cells results in the induction of unresponsiveness (anergy). To test this hypothesis B10.BR recipients were transplanted (d0) with either a non-treated, a HOC-treated or with both a non-treated and a HOC-treated B10.A thyroid allograft. Recipient spleens removed on d0, 14 or 28 post-transplant. Graft survival was determined on d35 by ^{125}I-incorporation. As expected, removal of the spleen did not affect the survival of the non-treated allograft. In contrast, splenectomy on d0, 14, or 28 completely abrogated the induction of recipient tolerance to the non-treated graft in the cotransplanted group. Additionally, splenocytes adoptively transferred unresponsiveness to naive recipients. LDA analysis provided evidence for a cell able to down regulate the CTL response. Our data suggest that HOC treatment of class I disparate grafts may result in the presentation of donor antigen in context of self class II, possibly by splenic B cells via the indirect pathway leading to tolerance.

Our long-term goal is to successfully apply HOC to the transplantation of endocrine tissues without the use of immunosupppressive therapy. We have used human fetal liver/fetal thymus to reconstitute SCID mice achieving circulating human T cells in greater than 95% of recipients. Two months post-reconstitution, SCID-hu mice were transplanted with fresh human fetal pancreas (HFP) explants or explants that had been HOC-treated. H&E analysis of grafts removed 21d post-transplant showed a significant reduction in HFP surrounded by scar tissue. Importantly there was no difference in the appearance or amount of HFP graft remaining in SCID-hu mice transplanted with HOC-treated tissue compared to that remaining in control non-reconstituted SCID mice. Our data demonstrate the usefulness of HOC for modification of graft immunogenicity and the potential to develop recipient unresponsiveness following transplantation of HFP.

C2-542 ADOPTIVE CELL-MEDIATED IMMUNITY TO THE PURIFIED PROTEIN DERIVATIVE (PPD) OF MYCOBACTERIUM TUBERCULOSIS IN SEVERE COMBINED IMMUNODEFICIENT (SCID) MICE, Richard Jones, Amy Young, Tim Sullivan, Michele Mass, Dennis Bourdette, VA Medical Center and Department of Neurology, Oregon Health Sciences University, Portland, Oregon, 97207.

The immunocompetent C.B-17+/+ (C.B-17) inbred mouse strain possesses normal levels of T and B lymphocytes and develops normal protective immune responses. In contrast, severe combined immunodeficient C.B-17scid/scid (SCID) inbred mice lack T and B lymphocytes and do not develop specific immune responses. Intradermal challenge with the purified protein derivative of M. tuberculosis (PPD) produced a vigorous cell-mediated delayed-type hypersensitivity (DTH) response in C.B-17 mice immunized with complete Freund's adjuvant (CFA) containing heat-killed M. tuberculosis. In contrast, immunodeficient SCID mice immunized with CFA remained unresponsive to challenge with PPD. PPD-specific, CD4+ T lymphocyte cell lines were selected from PPD-immune C.B-17 donors and transferred by injection into SCID mice. Intradermal challenge with PPD produced a strong DTH response in SCID mice following transfer of C.B-17 PPD-specific T lymphocytes. In vitro, PPD-specific cells responded to PPD presented by C.B-17 or SCID syngeneic antigen-presenting accessory cells. Thus, the molecular and cellular interactions required for a PPD-specific, T cell-mediated inflammatory response were sufficient following the transfer of antigen-specific, syngeneic T cells into SCID mice. The transfer of syngeneic antigen-selected, CD4+ T lymphocytes may have beneficial therapeutic potential since acquired immunity to certain microbial antigens might protect against some of the common infectious pathogens found in immunodeficiency. This cell transfer model may be useful for understanding important elements of immune augmentation in immunodeficiencies.

C2-543 ANALYSIS OF T CELL EPITOPES AND ALTERED T CELL EPITOPES EXPRESSED BY *TRYPANOSOMA CRUZI*, Stuart Kahn, Monika Wleklinski, and Ann Colton, Department of Pediatrics, University of Washington, Seattle, WA 98195.

T. cruzi, an obligate intracellular protozoan parasite, is the cause of Chagas Disease. The parasite is capable of infecting all mammalian species, and of evading eradication for the lifetime of the host. Animal studies have demonstrated that following infection initially both T and B cells contribute to the containment of the parasite. However, a T and B cell inhibition ensues, and as the inhibition occurs, a marked decrease in interleukin (IL)-2 production has been observed; administration of IL-2 to infected animals partially corrects the immunodepression. The mechanism of the acquired immunodeficiency is not understood. *T. cruzi* expresses several large families of surface glycoproteins. One of these families is the SA85-1 surface glycoproteins. Over 100 SA85-1 genes exist, and DNA sequence analysis has revealed 70 to 80% homology to one and other at the DNA and amino acid level. The differences are frequently manifested by single amino acid substitutions, small insertions, and deletions. In addition, clustered differences are observed, suggesting that gene conversion contributed to the diversification of the SA85-1 gene family. In addition, many related *T. cruzi* surface glycoproteins are simultaneously expressed by each parasite. An explanation for the large number of surface glycoprotein genes, and their expression pattern has been difficult to formulate. We have begun to investigate the T cell immune response to the SA85-1 glycoproteins by generating T cell hybridomas to a specific SA85-1 proteins which have been expressed in *E. coli*. The T cell epitope of one hybridoma has been mapped. Interestingly, the homologous portion of other SA85-1 glycoproteins are altered, suggesting that a family of altered T cell epitopes are expressed by the parasite. It is possible that the family of altered T cell epitopes may contribute to the acquired immunodeficiency that develops during *T. cruzi* infection.

C2-544 LIMITED REPERTOIRE OF T-CELL RECEPTOR Vβ FAMILIES IN LANGERHANS CELL HISTIOCYTOSIS LESIONS. George Kannourakis, Jonna Hvas, John Wood, Sara Nouri, Rodney Daly, Soong Ling, and Sika Ristevski. L.A.R.C.H. Cancer Research Unit, Department of Haematology and Oncology, Royal Children's Hospital, Melbourne, Australia.

Langerhans Cell Histiocytosis (LCH) is characterised by infiltration of histiocytes and inflammatory cells in many tissues, and may lead to severe organ dysfunction or death in young children with severe multisystem disease. The aetiology of LCH is unknown. We have demonstrated that T cells within LCH lesions are polyclonal as well as constitutively producing IL-1, IL-3, IL-4, IL-8, GM-CSF, TNFα , and LIF. In order to establish whether there is a restricted cellular immune response at the site of the lesions, we have analysed T-cell receptor (TCR) Vβ gene repertoire directly from LCH lesions, using RT-PCR, and compared these to peripheral blood (PBL) of LCH patients and normal controls. We found a restricted usage of Vβ1 and Vβ7 T cells within LCH lesions, compared to PBL, which showed expression of most Vβ families. Sequencing analyses of Vβ1 and Vβ7 TCR chains from LCH lesions confirmed a polyclonal population of T cells within LCH lesions. These data suggest that cytokine production within LCH lesions may occur as a result of stimulation of the Vβ region of the TCR via a non-conventional antigen. Preliminary studies of PCR analysis for known bacterial superantigens have to date been negative.

C2-545 IL-7 PROTECTS AGAINST ACUTE TOXOPLASMOSIS

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Cytokines, in particular IFN-γ, and IL-12, are important in host protection against infection with *Toxoplasma gondii*. This parasite is a major cause of congenital infection and morbidity and death in the immunosuppressed, especially those with AIDS. IL-7, a monomeric protein produced by bone marrow stromal cells and fetal thymus is able to induce the proliferation of pro-B cells, CD4+ and CD8+ T cells and enhanced cytotoxicity of CTL and NK cells. Inbred mice (A/J) were administered 500ng of IL-7 daily. On day 0, the mice were simultaneously infected with LD90 dose of P strain *T. gondii*. Mice treated with IL-7 survived up to day 22 post infection when the experiment was terminated compared to untreated controls that died by day 5-7 post infection. IL-7 therapy initiated at day 0, day 3 or day 6 post infection demonstrated complete protection in the day 0 group, 50% protection in day 3 and 0% protection in both the day 6 treated and untreated control. Phenotypic analysis of the splenocyte population at day 7 post infection and IL-7 treatment indicates a doubling in the NK population. Of interest is a decrease in both the CD4+ and CD8+ T cells in those mice infected, and treated compared to those mice receiving IL-7 alone. There was no change in T cells expressing the g, d heterodimer. Northern blot analysis shows a marked rise in message for IFN-γ in those mice treated and infected compared to controls. Human PBMC were isolated from toxoplasma seronegative (-) and positive (+) individuals. The cells were either treated with soluble toxoplasma antigen or U.V. irradiated parasites (invade but cannot replicate) and IL-7. Marked proliferation of cells as determined by thymidine incorporation was noted in both (+) and (-) individuals treated with parasite antigen and IL-7. These observations suggest that IL-7 may be an important cytokine involved in host protection against *T. gondii*

C2-546 ANTI-CD28 IMMUNOTHERAPY IN MURINE VISCERAL LEISHMANIASIS, Paul M. Kaye, Patricia Gorak, Michaela Murphy, *Peter J. Perrin and *Carl H. June, Department of Medical Parasitology, London School of Hygiene and Tropical Medicine, London, WC1E 7HT and *Immune Cell Biology Program, Naval Medical Research Institute, Bethesda, MD 20889-5607.

Chronic murine visceral leishmaniasis, caused by *Leishmania donovani*, is characterised by parasites infecting macrophages of the spleen and liver. Although Th1 T cell function is absent or severely depressed, there is little evidence for a dominant role of Th2 T cells in maintaining this state. We have recently shown that *L. donovani* infection of macrophages inhibits the regulated expression of costimulatory molecules and immunohistological studies suggest this to be a direct, rather than cytokine mediated, pathway (Kaye P.M. et.al. Eur.J.Immunol 11, in press, 1994). We therefore considered whether costimulation might serve as a potential therapeutic target. BALB/c mice, infected for 28 days with *L. donovani*, were treated with a single i.p. dose of 100μg hamster anti-CD28 (MAb PV1) or with normal hamster IgG. 10-12 days after administration, parasite load in the liver, but not spleen, was significantly reduced in PV1-treated mice compared to controls. Indeed, this treatment was nearly as effective as conventional chemotherapy (Pentostam, 500mg/kg). Cytokine (IFNγ, IL-4) production by spleen cells re-stimulated *in vitro* with leishmanial antigen was equivalent in PV1 and control groups, consistent with the lack of effect of treatment in this organ. We are currently analysing cytokine production in the liver, by RT-PCR, to determine the mechanism for the effects of anti-CD28 in this model.

C2-547 INDUCTION OF T CELL ANERGY BY *Toxoplasma gondii*. Imtiaz A. Khan, Tadashi Matsuura, Lloyd H. Kasper, Department of Microbiology, Dartmouth Medical School, Hanover, N.H. 03755.

Toxoplasma gondii, has been shown to induce a state of immune suppression in the infected host. Studies in our laboratory and others have indicated that maximal suppression can be observed at day 7 post infection in the experimental mouse model. Cytokines associated with this response in mice include production of IL-10, TGF-β and the reactive intermediate, nitric oxide. Further studies on the CD4+ T cell population following acute infection demonstrates that 80% of the CD4+ T cell population increases in volume compared to uninfected controls (17%). The peak increase in cellular volume occurs on day 7 post infection. This increase in size is accompanied by a phenotypic shift in the CD4+ T cells from the naive to active/memory phenotype. This includes the expression of CD44 (high), IL-2R and Mel 14 (low). Analysis of Vbeta usage in this CD4+ subset indicates that there is an expansion of Vβ5 genes. Further, the message for both CTLA-4 and CD28, T cell activation markers was absent in the CD4+ T cells stimulated with ConA. Isolation and stimulation of these "mature" T cells demonstrates that they are unable to proliferate in response to ConA. The addition of exogenous IL-2 partially restored this reduced mitogenic response. Both Northern blot analysis and ELISA show that these cells fail to respond by increasing IFN-γ or IL-2 following ConA stimulation. However, the CD4+ T cells seperated from the infected mice do express mRNA for IFN-γ and IL-10 at day 7 post infection suggesting a TH0 response. These studies indicate that during acute toxoplasma infection in mice, there is a T cell subset that comprises the majority of the CD4+ T cell subset that appear to be anergic. These anergic T cells exhibit poor response to mitogen and may be due to a superantigen present in the parasite.

C2-548 **SURFACE Ig MEDIATES EFFICIENT TRANSPORT OF ANTIGEN TO LYSOSOMAL COMPARTMENTS RESULTING IN ENHANCED SPECIFIC ANTIGEN PRESENTATION BY B CELLS** Byung S. Kim, Ko-Jiunn Liu, Vandana S. Parikh, and Philip W. Tucker
Northwestern University Medical School, Chicago, IL and University of Texas Southwestern Medical Center, Dallas, TX

A BCL_1 Ig transfectant, expressing wild-type sIgM with the TEPC-15 idiotype (T15-Id) and anti-phosphorylcholine (PC) specificity, was previously shown to present PC-conjugated hen egg-white lysozyme (PC-HEL) to a HEL-specific T cell hybridoma at a much lower Ag concentration than that required for native HEL. Two variant Ig transfectants, expressing T15-Id sIgM with substitutions either in the entire spacer, transmembrane (TM) domain and cytoplasmic tail (B186 variant) or in the NH_2-terminal 1/3 of TM domain only (TM2 variant), failed to display this sIgM-mediated, enhanced presentation of PC-HEL at low concentrations. However, prolonged treatment with anti-T15-Id mAb led to a reduction of surface expression of the T15-Id sIgM in the wild-type and TM2 variant, but not in the B186 variant sIgM transfectants. Treatment with anti-T15-Id mAb also resulted in an increased intracellular accumulation of T15-Id sIgM in the wild-type transfectant, but not in the B186 variant. Subcellular fractionation analysis revealed that the ligands bound to the T15-Id sIgM are not efficiently transported to the dense lysosomal compartments in both B186 and TM2 transfectants, as compared to the wild-type sIgM transfectant. A significant increase in tyrosine phosphorylation after crosslinking of the T15-Id sIgM was observed only in the wild-type sIgM transfectant. These results suggest that, while the NH_2-terminal 1/3 of the TM region is not involved in the process responsible for the ligand-induced reduction of surface expression of sIgM, it appears to be essential for subsequent transport of sIgM/ligand complexes to the lysosomal compartments, as well as efficient activation of tyrosine kinases. These results strongly suggest that sIg-mediated enhancement of specific Ag presentation reflects the ability of sIg to efficiently transport Ag to the lysosomal compartments, and possibly, the activation of protein tyrosine kinases. (Supported by NIH RO1 AI15446)

C2-549 *LISTERIA* INFECTION ELICITS $M3^a$-RESTRICTED CTL WITH DISTINCT EPITOPE SPECIFICITIES, Laurel L. Lenz and Michael J. Bevan, Department of Immunology and Howard Hughes Medical Institute, University of Washington, Seattle, WA 98195

Listeria monocytogenes is a gram positive bacterium and a facultative intracellular pathogen. During infection of susceptible host cells *Listeria* resides initially within a vacuolar compartment. However, virulent strains of *Listeria* express a haemolysin, listeriolysin O (LLO), which enables bacteria to escape from vacuoles into the host cell cytoplasm. Two *Listeria* proteins, LLO and p60, provide peptide ligands which bind the "classical" MHC class I molecule K^d. Previous studies in our laboratory have additionally demonstrated that a "non-classical" MHC class Ib allele, $H\text{-}2M3^a$, restricts the response of a $CD8^+$ CTL clone (JI1.4) to a *Listeria*-derived epitope termed "Fr38." To further investigate the role of MHC class Ib molecules in immunity to *Listeria*, we sought to isolate other "MHC-unrestricted" CTL from *Listeria*-infected C57BL/6 mice. We have isolated and cloned $CD8^+$ CTL (Cb10) which recognize an epitope present in supernatants of *Listeria* cultures, and which can be isolated from *Listeria*-infected J774 macrophage-like cells. HPLC fractionation of such antigen preparations reveals that Cb10 CTL respond to an epitope which is distinct from epitopes recognized by previously described *Listeria*-specific CTL. Lysis of antigen-coated targets by Cb10 CTL requires target cell expression of the $H\text{-}2M3^a$ class Ib gene product. We are currently generating an expression library of *Listeria* genes in *Bacillus subtilis*, and will screen this library to identify epitopes recognized by known $H\text{-}2M3^a$-restricted CTL. Identification of bacterial epitopes presented to these CTL may provide insight into the mechanism of antigen presentation by $H\text{-}2M3^a$, and will facilitate investigation of the immunological role(s) played by MHC class Ib molecules.

C2-550 ANTI-TUMOUR IMMUNE RESPONSES GENERATED BY B7 TRANSFECTION IN MURINE MESOTHELIOMA CELLS OF DISPARATE IMMUNOGENICITIES. Clement C. Leong[1,2], Julia V. Marley[1], Bruce W. Robinson[2] and Michael J. Garlepp[1,2], [1]Australian Neuromuscular Research Institute, [2]Department of Medicine, University of Western Australia, QEII Medical Centre, Nedlands Western Australia 6009, Australia.

In order to assess the effectiveness of B7 in generating anti-tumour immune responses, we have transfected the murine B7 cDNA into two murine mesothelioma cell lines. AC29 (H-2^k, CBA derived) is a highly immunosuppressive non-immunogenic cell line while AB1(H-2^d, Balb/c derived) is considered to be weakly immunogenic. Transfectants were generated and cloned by limiting dilution and surface expression of B7 was confirmed by FACS analysis. Four AC29-B7 clones were inoculated into syngenic CBA mice. Three of the four clones showed marked retardation in the relative rates of tumour formation compared to the parent cell line, however, all grew progressively and eventually formed large tumours. Significantly however, immunisation of mice with B7 transfectants generated cytotoxic effectors which lysed both the parental and B7 transfectants specifically. Previous attempts at generating specific effectors using various other protocols have failed and these transfectants provide the first evidence of specific immune recognition of an apparently non-immunogenic mesothelioma cell line. Three AB1-B7 transfectant clones were inoculated into groups of 10 Balb/c mice. The percentage of animals developing tumours varied from 70%-10% depending on the clone inoculated. Further clonal heterogeneity was demonstrated when tumour free mice from each group were challenged with parental AB1. Two groups were resistant to parental tumour growth while the third was not. These data indicate that the expression of B7 on tumour cells can modify anti-tumour immune responses but the efficacy of this approach may be influenced by clonal differences within a tumour.

C2-551 ROLE OF CYTOKINES IN THE FORMATION OF *SCHISTOSOMA MANSONI* EGG-INDUCED GRANULOMAS. Christopher L. Leptak and James H. McKerrow. Department of Microbiology/Immunology, University of California, San Francisco, Veterans Affairs Medical Center, San Francisco, CA.

The granulomatous response directed against *S. mansoni* eggs is best described as a cell-mediated inflammatory reaction controlled by CD4+ T-helper cells. Previous studies using cultured cells stimulated *in vitro* or an *in vivo* lung model of the disease have described an initial Th1 cytokine expression pattern which is subsequently modulated into a Th2 response. Our goal was to extend these observations to the liver, a natural site of granuloma formation, and to analyze the pattern of cytokine expression to determine how this sequence relates to changes in granuloma morphology. For this purpose, quantitative RT-PCR was performed on liver tissue from infected mice. We found that single sex adult worms induce a predominately Th1 response which becomes down regulated with time. Adults also continually stimulate TNF-α production. Exogenous TNF-α has been shown to increase egg production by adult females. When both sexes are present and producing eggs, cytokines characteristic of both Th subpopulations are induced concomitant with egg deposition. This response pattern modulates into a predominantly Th2 pattern with time. This *in vivo* data suggests that adults induce and then utilize the host TNF-α response to enhance their own egg production. In addition, the deposition of eggs in the liver immunomodulates the Th1 response to the adult worms, allowing a Th2 pattern to predominate.

C2-552 *Abstract Withdrawn*

C2-553 EXPRESSION OF β- H^+ATPase ON THE SURFACE OF HUMAN TUMOR CELLS: ASSOCIATION WITH SUSCEPTIBILITY TO NK-MEDIATED CYTOTOXICITY, Qinqhuan Liu, Mary O. Mondragon, Ballabh Das, Shi-Zhen Tao and Allen J. Norin, Departments of Anatomy & Cell Biology, Surgery and Medicine, SUNY Health Science Center, Brooklyn, New York, 11203.

Previous studies have showed that the β-subunit of H^+ Transporting ATP synthase (β- H^+ATPase) is an important ligand in the effector phase of a cytolytic pathway used by naive NK cells (J. Exp. Med. 180:273 1994). To examine the association of β- H^+ATPase expression and susceptibility NK-mediated cytotoxic activity, flow cytometry analysis of β- H^+ATPase expression on the human tumor cell surface was determined using anti-β- H^+ATPase antibody (whch was raised in rabbits against a 21-mer peptide in the ATP binding site of β- H^+ATPase). The results showed that the NK sensitive target K562 cells expressed about 5 times more β- ATPase on their surface than NK resistant Daudi cells. In order to further examine the association of surface β- H^+ATPase and susceptibiliy to NK cytotoxicity, flow cytometry analysis of a NK resistant human fibroscarcoma cell line HT 1080 (H4) and its E1A oncogene transfectant, NK sensitive P2AHT2A (P2) was preformed. P2 cells expressed about 3 times more β- H^+ATPase than the parental H4 cells. This finding suggests that the level of cell surface β- H^+ATPase is associated with cytotoxic activity further establishing the role of β- H^+ATPase in NK-mediated cytotoxicity. These results also suggest that oncogene activation may be required for expression of β- H^+ATPase at the cell surface. Supported by NCI grant CA47548.

C2-554 COSTIMULATOR B7-TRANSFECTED TUMORS INDUCE CYTOTOXIC T CELLS IN VIVO AGAINST A PROTECTIVE TUMOR ANTIGEN (P1A) WHICH IS EXPRESSED ON MULTIPLE LINEAGES OF TUMORS. Yang Liu, Supria Sarma, Min Zhao, Lieping Chen, Maja Maric, and Lakshmi Ramarathinam, Department of Pathology, NYU Medical Center, New York, New York 10016, and Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, WA98021.

Whether tumors of different lineages share common antigens is acritical issue for understanding anti-tumor immune immune responses and for designing an antigen-specific tumor immunotherapy. Based on large numbers of cross-protection experiments and identification of a few tumor antigens, it has been proposed that tumor antigens are specific for individual tumors. Recently, several tumor antigens have been shown to be expressed on multiple tumors. However, it is not known whether these cross-reactive tumor antigens can induce anti-tumor CTL responses in vivo, and if so, whether these antigens can induce protective immunity. Here we show that a plasmocytoma J558 transfected with the costimulatory molecule B7 activates a cross-reactive CTL response in vivo. The major antigen recognized by the cross-reactive CTL is P1A, which is expressed in mastocytoma P815, plasmocytoma J558, and fibrosarcoma Meth A. Immunization with a synthetic peptide corresponding to the major P1A CTL epitope induce a significant protection against B7-transfected plasmocytoma J558. Furthermore, mice which are primed with B7-transfected P815 cells reject not only parental P815 cells, they have also become more resistant to J558. Thus, our study demonstrates that P1A is a cross-reactive tumor antigen which can activate potentially protective CTL in vivo. This type of cross-reactive tumor antigen may be valuable for designing anti-tumor vaccines.

C2-555 IMMUNODOMINANT EPITOPES TO GAD IN IDDM

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Insulin-dependent diabetes mellitus (IDDM) is probably mediated by T-lymphocytes recognizing critical B cell autoantigens. To determine dominant epitopes of a major autoantigen in IDDM, glutamic acid decarboxylase (GAD), we studied the reactivity of peripheral blood T lymphocytes using peptides covering both major isoforms of GAD, GAD 65 and 67. A significant response to GAD 65 or GAD 67 peptides was detected in IDDM patients (13/15) as well as in controls (9/10). Islet cell or GAD antibodies were detected in 12 of 15 IDDM patients but in none of the controls. Controls most frequently recognized the central region of GAD 65 (residues 161 - 243). IDDM patients, in contrast, preferentially recognized a separate region (residues 473 - 555) of GAD 65 ($p<0.03$): the same region described as the immunodominant epitope in the NOD mouse. Response to whole GAD 65 was also observed in IDDM patients. T cell responses to GAD 67 peptides were comparable in IDDM and controls. We established and characterized T cell clones to specific peptides in GAD 65. The functional characteristics of these T cell clones will be presented.

C2-556 CD4+ T CELL MEDIATED DESTRUCTION OF XENOGRAFTS WITHIN CELL-IMPERMEABLE MEMBRANES IN THE ABSENCE OF CD8+ T CELLS AND B CELLS. T. Loudovaris[1], B. Charlton[2] and T. Mandel[3]. [1]Gene Therapy Unit, Baxter Healthcare Corporation, Baxter Technology Park, Round Lake, IL, 60073. [2]Stanford University School of Medicine, Palo Alto, CA. [3]Walter and Eliza Hall Inst. for Med. Res., Melbourne, Australia

Xenogeneic cells encapsulated in macroporous but cell-impermeable diffusion chambers die within three weeks when implanted into immunocompetent animals, but not immunodeficient animals. Two approaches were taken to determine which cells are necessary for the destruction of encapsulated xenografts. In the first approach we depleted normal mice *in vivo* of either CD4+ or CD8+ cells T cells using monoclonal antibodies. In the second approach, we reconstituted the immune system of athymic CBA mice (T-lymphocyte deficient) and C.B17 SCID mice (T and B-lymphocyte deficient) with different cell subsets from normal CBA and Balb/C mice, respectively. Depleted or reconstituted mice were implanted with a diffusion chamber containing COS (monkey kidney) cells. Membrane enclosed xenografts survived in CD4+ T cell depleted mice but not in CD8+ T cell depleted or non-depleted control mice. Encapsulated xenografts survived when implanted into either athymic or SCID mice. In contrast, the encapsulated xenogeneic cells were destroyed in athymic and SCID mice reconstituted with either spleen or lymphoid cell suspensions, respectively. Furthermore, encapsulated xenogeneic cells were destroyed in athymic or SCID mice reconstituted with CD4+ cell preparations depleted of CD8+ cells and/or B cells. Encapsulated xenogeneic cells were not destroyed in athymic or SCID mice reconstituted with CD8+ cell preparations depleted of CD4+ cells. These studies highlight the critical role of CD4+ T cells, in the absence of CD8+ cells and B cells, in the processes leading to the ultimate destruction of encapsulated xenografts. Because of the use of cell-impermeable membranes in these studies, the most likely involvement of CD4+ T cells is in the indirect antigen recognition by these cells and subsequent stimulation of immune and inflammatory cells.

C2-557 THE IMMUNOGENICITY OF MUC1 PEPTIDES AND MANNAN FUSION PROTEIN (MFP). Ian F.C. McKenzie, Vasso Apostolopoulos, Xiang-Pei. Xing, Bruce E. Loveland, Geoff A. Pietersz, Austin Research Institute, Austin Hospital, Studley Road, Heidelberg, VIC 3084, Australia.

Mucin1 (MUC1) is highly expressed in breast cancer, has a ubiquitous distribution and due to altered glycosylation, peptides within the VNTR are exposed. These peptides are the target for anti-MUC1 antibodies, which give a differential reaction on cancer vs, normal tissue. The amino acids APDTR or adjacent amino acids, are highly immunogenic in mice for antibody production (after immunisation with either breast cancer cells, HMFG or the VNTR peptide). In addition, in human studies, particularly from Finn, show that this region of the MUC1 VNTR functions as a target epitope for cytotoxic T cells. We have performed preclinical and clinical studies to examine the immune responses to MUC1 in mice and humans: a) MUC1+ 3T3 or P815 tumor cells in syngeneic mice are rejected with the generation of CTL and DTH responses, and weak antibody production: this type of immunity gave rise to total resistance to challenge with high doses of these tumors; b) immunisation with synthetic peptides (VNTR x 2); a fusion protein (VNTR x 5), or HMFG leads to no CTLs, aDTH and good antibody production and weak tumour protection (to 10^6 cells, but not $5x10^6$ cells); the protection is CD8+ dependent (~ possibly a TH2 type response); c) immunisation with oxidised mannan - fusion protein (MFP) gives rise to good protection (resistance to $50x10^7$ cells), CTL and DTH responses and weak antibody responses (?TH1 type response), similar in magnitude to that obtained after tumor rejection); d) the CTLp response to MFP is higher (x2) than that to tumor cells; e) established tumours can be rapidly rejected by delayed treatment of MFP; e) the CTL responses are MHC restricted (in contrast to the human studies) and MUC1 can be presented equally well by 5 different H-2 haptlotypes; and f) APDTR appears not to be the T cell reactive epitope in mice; fine mapping is in progress. It appears that oxidised MFP can induce a potent cellular (TH1) response vs synthetic peptides which give antibody responses; the TH1 and TH2 cytokines are now being measured. On the basis of these findings, two clinical trials are in progress: a) VNTR x 2 - diphtheria toxoid - which gives rise to some T cell proliferation and weak antibody responses in some patients; b) an MFP trial.

C2-558 MODELLING COMPETITION BETWEEN B CELL CLONES IN VIVO Angela R. McLean, Anne-Claire Viale, Alf Grandien and Antonio A. Freitas, Unite d' Immunobiology, Institut Pasteur, 25 Rue du Dr. Roux. Paris 75015, France.

Experimental reconstitution of lethally irradiated mice with different populations of B cells yields results that are summarised as follows:

repopulating B lymphocyte populations	receptor diversity	outcome
normal v congenic	broad v broad	*population composition* determined by ratios in initial inoculum
normal v transgenic	broad v narrow	diverse population outcompetes the transgenic population
transgenic v transgenic	narrow v narrow	population composition determined by ratios in initial inoculum

One possible interpretation of these results can be expressed through a simple competition model in which two populations, Y_1 and Y_2, compete for some resource, X.

$$\frac{dX}{dt} = \Lambda - \mu X - b_1 X Y_1 - b_2 X Y_2$$

$$\frac{dY_1}{dt} = b_1 X Y_1 - c Y_1$$

$$\frac{dY_2}{dt} = b_2 X Y_2 - c Y_2$$

We discuss ways in which this well established model of competition, is inappropriate for modelling competition as a regulatory force in the immune system.

C2-559 **A novel embryonic stem cell line from an inbred murine model of inflammation, DBA/1lacJ.** John McNeish, Jeff Stock, Bev Koller* & Marsha Roach, Molecular Genetics, Pfizer Central Research, Groton, CT, *University of North Carolina, Chapel Hill, N.C.

An embryonic stem (ES) cell line has been derived from the inbred murine strain, DBA/1lacJ. This ES cell line allows for genetic modification directly in the DBA/1lacJ genome and subsequent derivation of a DBA/1lacJ inbred mouse with the same genetic modification. The DBA/1lacJ mouse is an established small animal model of inflammation demonstrating marked arthritic phenotype with collagen (Kadowaki et al. 1994, *Clin Exp. Immunol.* **97**: 212). A single DBA/1lacJ ES cell line #252 was derived from culturing of 303 blastocyst stage embryos on primary embryonic fibroblast feeder cells (0.3%). Chimeric mice derived from the DBA/1lacJ and C57Bl/6 genomes are identified by mixed coat colors of dilute brown and black, respectively. We have demonstrated that the DBA ES cell line will readily contribute to the germline by backcrossing chimeras with DBA/1lacJ mates, and identifying ES germline derived offspring by their dilute brown coat color (DBA/B6 F1 are black). Our DBA/1lacJ #252 ES cells have transmitted the DBA/1lacJ genotype in >90% of offspring born of different chimeric mice. Of 165 mice born from C57/DBA/1lacJ #252 chimeras, 156 have the dilute brown coat color associated with transmission of the ES cell genotype. We have demonstrated homologous recombination using a replacement vector for the murine 5-lipoxygenase activating protein (FLAP) in positive/negative selected ES cell clones. This targeting vector was constructed with 129 genomic DNA however we observed a similar targeting frequency in the non-isogenic DBA ES cells. We will discuss the role of FLAP "knockout" in the collagen induced inflammation model and the general utility of this new ES cell line.

C2-560 **ENHANCED T CELL REACTIVITY AFTER ANTI-CD4 DEPLETIONS.** William J. Morrison, Norman J. Kennedy, Halina Offner and Arthur A. Vandenbark. Neuroimmunology Research Laboratory, Veterans Administration Medical Center, Portland, OR 97201.

We show that injections of anti-CD4 antibody rapidly depletes $CD4^+$ cells in a dose-dependent manner. Repeated administration of anti-CD4 Mab during CFA-immunization and PPD-priming significantly reduced PPD-stimulated DTH ear swelling. Lower single doses (ranging from 0.2 to 0.6mg/rat) of anti-CD4 Mab injected after priming caused moderate reduction of PPD-stimulated DTH reactivity while a higher single dose (1mg/rat) enhanced it. T cell proliferation, activation-phenotypes and protein tyrosine phosphorylations show a clear difference between the periphery and draining lymph node. T cells from the spleen showed stronger Con A-activation involving $p56^{lck}$ tyrosine phosphorylation ($pp56^{lck}$) that was reduced by anti-CD4 Mab treatments. In comparison, T cells isolated from the draining lymph node showed greater response to PPD-activation and had lower corresponding $pp56^{lck}$ levels which were enhanced and reduced, respectively, by anti-CD4 treatments. Thus, anti-CD4 Mab depleted PPD-specific $CD4^+$ cells from the blood, spleen and draining lymph nodes. T cells which emerged from the lymph nodes draining the site of antigen presentation had enhanced PPD-stimulated responses with lower $pp56^{lck}$ levels.

C2-561 INFLUENCE OF COSTIMULATORY AND CELL ADHESION MOLECULES ON THE AVIDITY OF T CELLS FOR ANTIGEN, Zoltan A. Nagy, Fiorenza Falcioni, Damir Vidovic, Charles Belunis, David Bolin and Chikao Morimoto*. Department of Inflammation and Autoimmune Diseases, Hoffmann-La Roche Inc., Nutley, NJ 07110, and * Division of Tumor Immunology, Dana-Farber Cancer Institute, Boston, MA 02115

The avidity of human Th cell clones, as measured by antigen sensitivity and susceptibility to class II MHC antagonists, is shown to change up to 40-60 fold within a single cycle of restimulation. Typically, it increases gradually from day 7 to 21 after restimulation. Increased antigen sensitivity is accompanied by acqusition of resistance to class II antagonist peptides. Of 21 cell surface molecules tested, 3 exhibited increased expression in "late stage" T cell clones: CD26, LFA-1 (CD11a+CD18), and VLA-1 (CD49a). The remaining markers were either unchanged (e.g., αβTCR, CD4, HLA-DR), or decreased in expression (e.g., IL-2R, MEL-14). Treatment of late stage T cells with mAb to CD26 or LFA-1 decreased antigen sensitivity and partially restored the susceptibility of T cells to class II antagonists. Evidence was obtained that late stage T cells with elevated expression of CD26, LFA-1 and VLA-1 also exist in vivo, within the CD45RO+ subset. Thus, the aviditiy of T cells for antigen is a complex biological parameter resulting from the cooperative activity of several different cell surface receptors, and it can change according to the expression level of molecules involved.

C2-562 INFECTION WITH THE L3 STAGE OF THE FILARIAL NEMATODE *BRUGIA PAHANGI* INDUCES A TH2-POLARIZED REPONSE. HOW?

J.Osborne and E.Devaney, Department of Veterinary Parasitology, University of Glasgow, Glasgow G61 1QH.

Human Lymphatic Filariasis, a major cause of morbidity throughout the tropics, is a good example of an infectious disease where the balance between Th cell subsets plays a critical role in determining the outcome of infection. Acutely infected individuals exhibit a pronounced Th2 bias in their responses. The specific immunological hyporesponsiveness to parasite antigens apparent in a significant proportion of the infected population led to the hypothesis that the parasite induces a form of semi-permanent tolerance (Th1 anergy) which prevents both parasite elimination and progression to disease. However, studies so far have been unable to demonstrate any simple correlations between Th subset usage and host protection or pathology. Here we have used a mouse model to investigate the immunomodulatory potential of the infective 3rd stage larvae (L3) of *Brugia pahangi*. Our results indicate that exposure to the L3 by the sub-cutaneous (natural) route of infection induces a Th2 response while downregulating Th1 responses. By day 12 p.i. lymphocytes from these animals exhibit a dramatic reduction in polyclonal IL2 secretion and proliferative responsiveness but produce elevated levels of parasite specific IL4,IL5 and IL10. This model therefore provides an excellent opportunity to study in detail the mechanisms by which the parasite mediated Th imbalance becomes established. Our current work is focussed on creating a comprehensive picture of cytokine production and mRNA expression during the critical early period post-infection. Initial results show that as early as 24hrs p.i. the only cytokine transcript detectable in the popliteal lymph node is IL4. The essential requirement for IL4 as the basis for the selective expansion of Th2 cells is being tested using IL4 KO mice and studies are underway to identify the cellular source of the IL4.

C2-563 **MHC CLASS II$^+$B7-1$^+$ TUMOR CELLS ARE POTENT VACCINES FOR STIMULATING TUMOR REJECTION IN TUMOR-BEARING MICE.**

S. Ostrand-Rosenberg[#], S. Baskar[#], L. Glimcher[+], and N. Nabavi[*]. [#]Dept. of Biology, U. of Maryland, Baltimore, MD 21228; [+]Dept. Cancer Biology and Medicine, Harvard; [*]Dept. Inflammation and Autoimmune Diseases, Roche.

Activation of T cells is known to require at least two signals: an antigen-specific signal and a costimulatory signal. Several recent studies from this and other labs have demonstrated that a host's tumor-specific immune response can be significantly augmented if tumor cells are genetically engineered so that they deliver both of the requisite signals. These studies demonstrated that tumor cells co-expressing the B7-1 costimulatory molecule are themselves rejected in autologous mice, and function as effective immunogens against later challenge with wild type B7-1 negative tumor. In the present study we demonstrate that in addition to being vaccines for later tumor challenge, SaI sarcoma cells co-expressing syngeneic MHC class II plus B7-1 molecules are potent immunotherapeutic agents for the treatment of mice carrying large established sarcoma tumors. MHC class II$^+$B7-1$^+$ transfectant tumors are significantly more effective therapeutic agents than previously described cytokine transduced tumor cells. Likewise, the double transfectants are effective in the treatment of longer-term established subcutaneous tumor, while transfectants expressing B7-1 without MHC class II molecules are ineffective immunotherapeutic agents. Mixing experiments indicate that the most efficent tumor rejection occurs if both the class II and B7-1 molecules are coexpressed on the same tumor cell. Immunity induced by immunization with class II$^+$B7-1$^+$ transfected sarcoma cells requires CD4$^+$ T cells, suggesting that the increased effectiveness of the transfectants is due to the activation of tumor-specific CD4$^+$ T_h cells.

C2-564 A MODEL FOR T-B INTERACTION IN PALS ASSOCIATED FOCI, Alan S. Perelson and Mihaela Oprea, Theoretical Biology and Biophysics, Los Alamos National Laboratory, Los Alamos, NM 87545 USA

We present a mathematical model for the clonal expansion of B cells in a T cell area of the spleen, i.e. the periarteriolar lymphoid sheath (PALS). In a primary response a few B cells expand into clones of many hundreds of cells, a focus. The focus reaches maximum size at day 8 and then grows smaller so that by day 14 they are virtually absent.

Our model examines the interactions needed between B cells and T cells, and T cells and APC, such as dendritic cells, that are required to generate the observed cellular expansion. We find that T cell help is limiting and that models which require B cells to obtain T help after each round of division lead to markedly too slow B cell clonal expansion. We thus propose that B cells, after obtaining T cell help via direct cellular contact, can go through a large number of division cycles without further need for direct T-B contact. Their growth however may still be dependent on soluble T cell factors.

C2-565 COMPARISON OF CELLULAR IMMUNITY TO MAGE TUMOR ANTIGENS ELICITED IN MICE USING PEPTIDES, PURIFIED PROTEIN, VACCINIA VIRUS, AND POLYNUCLEOTIDE VACCINES, Robert Ralston, Kent Thudium, and Mazie Coyne. Virology Department, Chiron Corporation, Emeryville, CA 94608

Identification and cloning of tumor rejection antigens which are targets of CTL responses in patients with melanoma and other cancers has raised the possibility of developing cancer vaccines of defined specificity. The recently identified MAGE antigens are attractive targets for vaccine development because of their expression in a wide variety of tumor types. As an initial step in the development of recombinant MAGE vaccines, we have expressed the complete MAGE-1 and MAGE-3 proteins in *E.coli*, recombinant baculovirus-infected insect cells, and recombinant vaccinia-infected mammalian cells. Using these systems we have developed several immunological reagents which will be useful in evaluating humoral and cellular immune responses to MAGE-1 and MAGE-3 in experimental animals and in cancer patients. An H-2^b restricted CTL epitope was mapped in MAGE-3 using a recombinant vaccinia virus and consensus H-2^b peptides. Immunization of mice with recombinant MAGE-3 protein plus DOTAP generated a measurable T_h response and elicited CTL responses against MAGE-3 which were comparable to those elicited by the recombinant vaccinia virus. To extend our analysis of the ability of various immunization methods to generate cellular immunity to the MAGE antigens, we are evaluating the responses obtained from polynucleotide MAGE vaccines or from adjuvanted MAGE peptides. Results of these studies will be presented.

C2-566 REGULATION BY NORMAL T HELPER CELLS OF B CELL LYMPHOMA CELL CYCLE PROGRESSION,
Suzanna L. Reid and E. Charles Snow, Department of Microbiology and Immunology, University of Kentucky, Lexington, KY 40536
The murine BCL_1 lymphoma, grown in-vivo, represents an attractive system for studying intracellular events induced by the cell's environment determining its fate in the cell cycle. Intensive work in the cell cycle field has recently revealed components (e.g. cyclins, cyclin dependent kinases and their substrates and cyclin kinase activators) that allow, or push, cells past checkpoints as well as components that restrict or inhibit the cell cycle (e.g. cyclin dependent kinase inhibitors, phosphatases, and transcription factors). The expression and/or function of these proteins are part of intracellular signaling pathways initiated by the cell's environment. Cells derived from BCL_1 tumors react to their environment in a number of different ways. Cultured alone in-vitro they become quiescent. Stimulation through the immunoglobulin receptor induces cell death. When cultured with activated T helper cells, division continues but exposure to γ irradiation induces programmed cell death. The data presented will begin to determine the effects these varying conditions have on cell cycle related proteins and complexes of these proteins that control entry and commitment into the cell cycle.

C2-567 ROLE OF CSF-1 IN THE ACQUIRED IMMUNOLOGICAL UNRESPONSIVENESS OF TUMOUR INFILTRATING MONOCYTES. Susy ME Scholl, P Pouillart. Departement de Médecine Oncologique, Institut Curie, Paris, France

In pregnancy, macrophages are among the most common bone marrow-derived cells in the human decidua, being found near the implantation site (Hunt JS 1992) and near allogeneic trophoblasts at early stages of pregnancy (Beer AE 1974). Grafts to the pregnant uterus survive much longer than grafts in other locations, which favors a local immune suppressive rather than immune stimulatory role for utero-placental macrophages. Efficient presentation of antigens to T lymphocytes seems not to occur on uterine placental macrophages in situ (Lande IJ 1986; Chang MY 1993). Downregulation of monocyte MHC class II antigen expression after exposure to CSF-1 has been documented (Willman CH 1989) and high local concentrations of CSF-1 might therefore decrease macrophage-mediated cytotoxicity and enhance their local immunosuppressive effect.

We have shown that CSF-1 is present at the level of invasive, but not intraductal breast carcinoma (Tang R 1990) and at the invading tumour front (Scholl 1994) by immunohistochemical techniques and that transcripts of CSF-1 mRNA are present in tumour cells (Tang R 1992). High serum levels of CSF-1 are associated with advanced breast tumour stage as well as with disease activity. Furthermore, abundant CSF-1 immunostaining on tumour cells was associated with abundant monocyte infiltrates in the tumour, in the absence of patent signs of tumour cell necrosis. The CSF-1 dependent recruitment of large numbers of monocytes, instead of resulting in tumoricidal activity, may lead to enhanced tumor cell growth via release of trophic cytokines and other growth factors. Thus growth stimulation and immune tolerance could be dominant effects of CSF-1 stimulated monocytes in tumours, with an emphasis on tissue repair rather than destruction.

C2-568 GENE THERAPY OF MELANOMA PATIENTS WITH IL-2-TRANSFECTED MELANOMA CELLS, P.I. Schrier[1], N. Brouwenstijn[1], N. Weijl[1], G.J. Fleuren[2], C.J.M. Melief[3] and S. Osanto[1], [1] Depts. of Oncology, [2]Pathology and [3]Immunohaematology, University Hospital Leiden, P.O. Box 9600, 2300 RC Leiden, The Netherlands

Animal studies have indicated that immunization with cytokine-producing tumor cells results in systemic immunity against the modified as well as the parental cells. In human melanoma, a number of tumor-specific antigens has been characterized, including MAGE-1, -2, -3, Mart-1 (Melan-Aa), gp100 and tyrosinase. Specific peptides derived from these antigens are presented by either HLA-A*0101 or HLA-A*0201 and are target for tumor-specific CTL in melanoma patients. Moreover, the occurrence of CTLs in patients, cross-reactive with allogeneic HLA-A*0101 or HLA-A*0201-positive melanomas, indicates that common tumor antigens presented by HLA-A*0101 or HLA-A*0201 exist in melanoma. These data suggest that melanoma tumors may be suitable targets for immunization with gene-modified tumor cells.

We have initiated a clinical study in which HLA-A*0101 and/or HLA-A*0201-positive metastatic melanoma patients were immunized with $6x10^7$ irradiated cells of an HLA-A*0101, HLA-A*0201, HLA-B*0801, HLA-Cw7-positive melanoma cell line, producing IL-2 after stable transfection with a plasmid containing the human IL-2 cDNA under control of a CMV promoter. The cells produce at least 0.1 μg IL-2/10^6 tumor cells/24 hr. IL-2 secretion is sustained during three weeks following lethal irradiation.

We have immunized 24 patients with $6x10^7$ melanoma cells s.c. at 6 different sites during 3 consecutive weeks. Systemic toxicity was mild or absent. In most patients local swelling of draining lymph nodes was found. In a number of patients a halo of erythema was seen around the vaccination sites. Biopsies of these sites showed vacuolizing melanocytes. All patients showed inflammatory changes and cellular infiltrate of T cells and macrophages at the site of vaccination. Strikingly, in some patients inflammatory reactions and T cell infiltrate were found at the sites of distant s.c. metastases after vaccination. Four patients showed mixed responses, *i.e.* regression of some metastasis. One patient showed a complete remission. The regressing metastases showed a vast necrosis. Moreover, in one metastasis apoptosis was seen, indicative of a specific lysis by infiltrating T cells.

These results indicate that the approach of tumor cell vaccination with cytokine-transfected cells may elicit a tumor-directed immune response in the patient.

C2-569 GROWTH OF A MURINE MALIGNANT MESOTHELIOMA CELL LINE *IN VIVO*: LACK OF IMMUNE AGGRESSION DUE TO IMMUNE DEVIATION. Bernadette Scott, Andrew Jarnicki, David Fitzpatrick, Amanda Marzo, Robyn Himbeck, Bruce Robinson and Helle Bielefeldt-Ohmann. University Department of Medicine, QEII Medical Centre, Nedlands, Western Australia, 6009.

Malignant mesothelioma is an extremely aggressive tumour of serosal origin which is refractive to conventional therapies. We have developed a murine model of this tumour which closely resembles its human counterpart. Initial experiments have looked at the cytokine repertoire of tumour infiltrating lymphocytes (TILS) in an effort to understand why these cells are incapable of rejecting the tumour. Cytokine production was determined by semi quantitative PCR analysis of mRNA isolated from TILS during tumour progression. The results indicated that IL-2 mRNA increased over the time course of the experiment. Interestingly, a reduction in IFN-γ mRNA was noted that corresponded with an increase in IL-4 mRNA. This observation suggests that TILS may be non destructive due to the production of TH-2-like rather than TH-1 inflammatory cytokines. This result puts the immunobiology of solid tumours into a similar perspective with other diseases (such as leishmaniasis and autoimmune diabetes) which have been linked with differential cytokine production. Our next experiments will look at a broader spectrum of cytokines in an attempt to more closely define the role of cytokines in tumour growth/regression.

C2-570 CELL POPULATION INTERACTIONS IN AUTOIMMUNE DISEASE, Lee A. Segel and Eva Jaeger, Department of Applied Mathematics and Computer Science, Weizmann Institute, Rehovot, Israel

In the first part of this theoretical study, a highly phenomenological approach is employed to reproduce some of the phenomena associated with T-cell vaccination in animal models for autoimmune diseases, and thereby to obtain information on the type of effector-regulator cell interactions that might be involved in EAE or autoimmune diabetes. A second approach is based on the specific hypothesis that it is Th1-Th2 cross-regulation, as mediated by interferon gamma and IL10, that lie at the heart of EAE kinetics. The relation between theory and experiment will be stressed.

C2-571 HYPERMUTATION AND AFFINITY MATURATION IN THE B-CELL RESPONSE, Philip E. Seiden, IBM Research Center, Yorktown Heights, NY 10598 and Franco Celada, Hospital for Joint Diseases, New York, NY 10003

We have used a cellular automaton immune system simulation to investigate the behavior of hypermutation and affinity maturation in evolving the B-cell response. Hypermutation and affinity mutation are often referred to interchangeably, however, they actually refer to two quite different aspects of the evolution of the response. We find that our model system can exhibit affinity maturation quite naturally with no mutation at all. Affinity maturation is a case of natural selection among cells of differing affinities. The higher-affinity cells win this competition by virtue of their higher affinity which makes them fitter. The difference between the primary response, which is not dominated by high-affinity cells, and the secondary, which is, is due to the rarity of any B cells capable of responding at all to a new antigen. In order to have competition leading to natural selection there must be a high enough density to force the cells to compete. This affinity maturation without mutation arises when we have all B-cell receptor types available in our system, i.e., there are no holes in the possible diversity. However, the competitive mechanism can only multiply an existing population. If there are no cells to start with it cannot work. In the real animal there are many holes in the diversity so that there is a low probability for having a high-affinity cell for a random new antigen. Hypermutation allows the system to eliminate these holes. We have carried out a number of *in machina* experiments to investigate the effect of affinity maturation and hypermutation on the immune response. We will present results showing conditions necessary to get good affinity maturation in a time short enough to provide an effective response. In particular we discuss how strongly the affinity must increase, how much mutation is necessary, and whether cycling is needed between periods of cell division with and without mutation.

C2-572 **INTRAVENOUS HIGH-DOSE TOLERANCE TO T CELL DETERMINANTS DERIVED FROM AUTOANTIBODIES DELAYS DEVELOPMENT OF AUTOIMMUNITY IN MURINE LUPUS,** **Ram Raj Singh, Jonathan Jacinto, Fanny M. Ebling, Eli E. Sercarz, Bevra H. Hahn, Departments of Medicine/Rheumatology and Microbiology and Molecular Genetics, UCLA, Los Angeles, CA 90024**

Lupus-prone (NZB/NZW) F1 [BWF1], but not MHC-matched normal mice develop spontaneous T cell autoimmunity to V_H region determinants of autologous anti-DNA, but not to peptides from a foreign mAb, prior to the onset of clinical disease. These peptides bind to MHC class II and activate different Th subsets. Immunization with peptides in adjuvant or adoptive transfer of a peptide-specific T cell line in young BWF1 mice upregulates anti-DNA and clinical disease (R.R. Singh et al, submitted). Here we determined the effect of tolerance induction to 3 determinants from one anti-DNA mAb (A6.1) on peptide-specific T cell proliferation and antibodies (Ab), and its influence on anti-DNA production and clinical disease. Three different modes of tolerance were used: i.v. administration of individual peptides in PBS, i.p. administration of peptides in IFA emulsion to neonatal or adult mice, and oral administration of peptides. We found that i.v. treatment with a combination of determinants prolonged survival ($p<0.05$), delayed the onset of nephritis ($p<0.05$) and decreased serum anti-DNA ($p<0.01$) compared to BWF1 mice treated with saline or control peptides. This beneficial clinical effect was associated with profound unresponsiveness in peptide-specific T cell proliferation and Ab. This was in contrast to the level of tolerance found after i.p administration of peptide in IFA, which induced T cell proliferative unresponsiveness, but enhanced peptide-specific Ab production and increased anti-DNA. Oral administration of peptides neither prevented peptide-specific Ab production nor delayed onset of nephritis. Thus, tolerizing T cell helper function for Ab production appears to be essential for therapeutic intervention in this murine model of lupus.

C2-573 LONG-TERM *IN VIVO* EXPANSION OF HLA-B35 ALLOREACTIVE T CELLS WITH HOMOLOGOUS TCR SUGGESTS CROSSTIMULATION VIA A PERSISTENT PEPTIDE/SELF MHC COMPLEX, Alexander Steinle, Carsten Reinhardt, Petra Jantzer and Dolores J. Schendel, Institute of Immunology, University of Munich, 80336 München, Germany

We generated HLA-B35 specific, alloreactive T cell clones that distinguish HLA-B35 variants differing by one amino acid position located in the peptide-binding cleft. Their postulated peptide dependency was confirmed by testing with TAP deficient mutant cell lines. Sequence analysis revealed that three of seven HLA-B35 specific T cells had homologous T cell receptors (TCR) correlating with their similar fine specificities. They expressed Vα2.3/Jα36 and Vβ4/Jβ2.7 TCRα and β chains with highly related CDR3 sequences. This suggests that the specificity of at least some alloreactive T cells is determined by the interaction between CDR3 and the allopeptide. To study the abundance of this particular TCR specificity in vivo, we analyzed the TCRAV2S3J36 repertoire of unstimulated PBL of the responding cell donor who was never sensitized with HLA-B35. Sequencing of the cloned TCRAV2S3J36 junctional regions obtained from two PBL isolates separated by a nine year interval revealed that about 75% of the clones contained only a few transcripts identical or homologous to those of the three HLA-B35 specific T cells. This suggests that an immune response to a persistent antigen (pathogen?) has led to the expansion and maintenance of a set of T cells bearing homologous TCRs that through their crossreactivity dominate the alloresponse against HLA-B35. These findings reveal a new aspect of alloreactivity and could have major implications for particular transplantation settings, but also can serve as a molecular model of antigen mimicry as discussed for the initiation of autoimmune disorders.

C2-574 INTERLEUKIN-12 INDUCES A PROTECTIVE Th1 RESPONSE IN *PLASMODIUM CHABAUDI* AS SUSCEPTIBLE A/J MICE. M. M. Stevenson, M.F. Tam and A. Sher. Centre for the Study of Host Resistance, Montreal General Hospital Research Institute, Montreal, Quebec H3G 1A4 and NIAID, NIH, Bethesda, MD 20892.

Previous results from our laboratory demonstrated that treatment of *Plasmodium chabaudi* AS susceptible A/J mice with murine recombinant IL-12 results in increased survival concomitant with control and resolution of acute blood-stage malaria. A/J mice treated intraperitoneally with 0.1 μg IL-12 on the day of infection and daily during the first week exhibited >75% survival following infection with 10^6 *P. chabaudi* AS parasitized red blood cells, an infectious dose which normally kills 100% of these hosts. In addition, a significant difference in peak parasitemia levels was observed between IL-12 treated and control A/J mice. In the present study, the mechanism of IL-12 induced protective immunity against blood-stage *P. chabaudi* AS in A/J mice was investigated. Interestingly, the dose of IL-12 was found to be critical. Treatment with 0.1 μg IL-12 per day for 6 days beginning on the day of infection was protective while treatment with higher or lower doses resulted in >50% mortality. Furthermore, the development of protective immunity was abrogated in infected A/J mice treated with 0.1 μg IL-12 and depleted of $CD4^+$ T cells by treatment with GK1.5 monoclonal antibody suggesting that $CD4^+$ T cells are necessary for IL-12 induced resistance against blood-stage *P. chabaudi* AS. Levels of IFN-γ and TNF-α, cytokines associated with Th1 responses, and of the Th2 cytokines, IL-4 and IL-5, were determined in the sera as well as the supernatants of spleen cells recovered from IL-12 treated or control *P. chabaudi* AS infected mice. Significantly higher levels of IFN-γ and TNF-α were observed in the sera of infected mice treated with IL-12 compared to control animals. Marked increases in IFN-γ and TNF-α were also observed in the sera of normal, uninfected A/J mice treated with IL-12. In addition, spleen cells from IL-12 treated, infected A/J mice produced significantly higher levels of IFN-γ in response to Con A and specific antigen as well as spontaneously. There was no significant difference in the production of IL-4 or IL-5 by spleen cells from IL-12 treated versus control *P. chabaudi* AS infected mice. Thus, these results suggest that IL-12 regulates the development of protective immunity to blood-stage malaria via a Th1 response which involves $CD4^+$ T cells and the cytokines, IFN-γ and TNF-α. [Supported by MRC and NIH R01 AI35955-01].

C2-575 DEFINITION OF ALTERNATIVE PEPTIDE-BINDING MOTIFS FOR Qa-2 CLASS 1b MHC MOLECULES, Iwona Stroynowski, Piotr Tabaczewski, Maile Hansen. Department of Microbiology and Center for Diabetes Research, U.T. Southwestern Medical Center at Dallas, TX 75235-8854.

It is widely believed that the nonclassical class 1b MHC antigens have evolved to function as highly specialized receptors for presentation of structurally unique ligands. Biochemical analyses of Qa-2 complexes (Joyce et al. 1994, J. Exp. Med. 179:579) revealed that it can bind at least 200 naturally processed different nonameric self-peptides. The sequence of the pooled Qa-2 associated peptides is enriched for His at P7, hydrophobic amino acid (Leu, Ile or Phe) at P9, Gln/Leu at P2 and Leu/Asn at P3.

To identify structural requirements for the peptide binding to Qa-2 we tested two sets of single substitution synthetic analogs of Qa-2 binding peptides. A series of Ala-backbone peptides carrying defined residues at the predicted anchor positions was also analyzed in Qa-2 specific quantitative ELISA binding assays. We find that requirements for binding to Qa-2 vary among different peptides. In most cases His at P7 and Leu at P9 serve as the two primary anchors. Several different residues at P2, P3 or P5 can substitute for each other in their function as the third auxiliary anchor. Thus the structural requirements for peptide binding to Qa-2 are very similar to those defining peptide binding to classical class 1a antigens.

We conclude that the class 1b Qa-2 molecule is not a receptor of higher stringency than the ordinary class 1a antigens. It can bind a wide array of nonameric peptides carrying various combinations of three anchor residues at P2, P3, P5, P7 and /or P9.

C2-576 T-INDEPENDENT RESPONSE OF B LYMPHOCYTES TO POLYMERIC ANTIGEN, Bernhard Sulzer and Alan S. Perelson, Theoretical Biology and Biophysics, Los Alamos National Laboratory, Los Alamos, NM 87545

The valency of a polymeric antigen and the spacing of its antigenic determinants has a strong influence on the T-independent response of B cells. It has been argued by Dintzis et al. that a polymeric antigen has to have at least ten appropriately spaced epitopes in order to elicit a B cell response in the absence of T cell help. In order to identify the receptor-ligand aggregates providing the signal for T-independent B cell activation we calculate the equilibrium distribution of receptor-ligand aggregate sizes when multivalent ligands bind reversibly to membrane immunoglobulin. Unlike the immunon model of Dintzis et al., we do not assume the formation of a special, locked signaling aggregate. In agreement with previous results, we still find that signaling aggregates must contain at least ten receptors. This implies that polymers must carry at least ten accessible sites to be antigenic. The maximum response occurs for a far smaller ligand concentration than in the case where every crosslink contributes to the activation signal. Moreover, we find that the concentration of receptors, i.e., the B cell density, has a major influence on the equilibrium aggregate size distribution. Increasing the cell density at a given ligand concentration reduces the number of signaling aggregates per cell and, consequently, the activation signal. The polymer concentration which yields the maximum response may be determined entirely by the cell concentration. Thus, as the cell density changes during the course of an immune response the stimulus the cells experience will change even if the concentration of the antigen remains constant.

C2-577 T-CELL RECEPTOR Vß GENE USAGE AND SEQUENCE IN TUMOR INFILTRATING LYMPHOCYTES FROM OVARIAN CANCER PATIENTS.

Sohel Talib, Bharati Sanjanwala, Farshid Oshidari, Lydia Kilinski, Jane Lebkowski, Thomas B. Okarma, and Ramila Philip, Applied Immune Sciences, Inc., Santa Clara, CA 95054.

Tumor infiltrating lymphocytes (TIL) are often associated with human tumors and are believed to be enriched for tumor-specific cytotoxic T-cells. Molecular characterization of the variable region of the T-cell receptor (TCR) in infiltrating lymphocytes may provide insight into the mechanism of anti-tumor immunity. We have analyzed the T-cell receptor repertoire expressed in TIL derived from ovarian carcinoma by multiprobe RNase protection assay. This analysis demonstrated limited the heterogenicity of Vß repertoires: Vß2, Vß6 and Vß13.3 predominating. *Ex vivo* propagation of TIL in the presence of irradiated autologous tumor or tumor transduced with the IL-2 gene showed enhanced expression of Vß restricted repertoires. TCR restricted T-cells were predominantly of the CD8 type as indicated by phenotype and functional assays. The presence of dominant clonotypes in the ovarian cancer TIL was further demonstrated by cloning and sequencing the VDJ region of Vß restricted T-cells. Dominance of very similar VDJ sequences in cDNA clones from Vß6, Vß2, and Vß13.3 were observed. This clonal dominance of T-cells with conserved CDR3 region sequences is further enhanced in TIL samples cultured in the presence of irradiated autologous tumor. These results show that unique T-cell subpopulations are clonally amplified in ovarian cancer patients, possibly as a consequence of antigen-driven selection.

C2-578 MECHANISMS OF IMMUNOSUPPRESSION BY THE MURINE IgM ANTI-T CELL MONOCLONAL ANTI-BODY (mAb) T10B9.1A-31 (T10B9), John S. Thompson, Stephen A. Brown and Shirrish Barve, Department of Medicine, University of Kentucky, Lexington, KY 40536

Whereas T10B9 has been demonstrated to be a very effective treatment for acute human kidney graft rejection, relatively little is known about the cellular functions it effects to acheive immuno-suppression (IS). Both T10B9, which reacts with the α/β chains of the T-cell receptor (TCR) and OKT3, which binds with the CD3 epsilon chain, rapidly induce modulation of the CD3-TCR complex from the T-cell surface, effectively blinding these cells from the recognition of antigens. This response does not account, however, for the fact that rejection does not commonly rebound as soon as these drugs are withdrawn and that no further acute rejection episodes occur in the majority of patients so treated. The following results, which will be presented, indicate other possible mechanisms which may contribute to T10B9 IS:

1. T10B9 induces apoptosis in Jurkat cells.
2. Whereas it is not mitogenic in vitro and induces much lower levels of pro-inflammatory cytkines in-vivo than OKT3, both mAb stimulate the release of high levels of IL-10 in vivo.
3. T10B9 suppresses OKT3 in vitro stimulation of PBL, even when added to the culture 21 hours after OKT3. This IS can be over-come by the addition of either anti-IL-10 mAb or IL-2 to the culture.
4. Not only does T`0B9 suppress the primary mixed lymphocyte response, but the responder lymphocytes become anergic tore-challenge with the specific stimulating cells in the absence of the mAb, yet they are fully capable of responding to third party cells.

Therefore, there are at least 5 mechanisms whereby T10B9 may exert IS, i.e., modulation of CD3/TCR, apoptosis, stimulation of IL-10, inhibiton of IL-2 and induction of specific anergy.

C2-579 MODULATION OF CTL MEDIATED IMMUNITY AGAINST Ad5E1-TRANSFORMED TUMOR CELLS BY ACTIVATED RAS AND Ad5E1-ENCODED PEPTIDES. René E. M. Toes, Rienk Offringa, Ria J. J. Blom, Remco M. P. Brandt, Cornelis J. M. Melief and W. Martin Kast. Department of Immunohaematology and Bloodbank, University Hospital, POB 9600, 2300 RC Leiden, The Netherlands

Mouse embryo cells (C57BL/6, H-2^b) transformed by the E1A and E1B genes of Adenovirus type 5 (Ad5E1 MEC) are highly immunogenic. Previously, cytotoxic T lymphocytes (CTL) were cloned from mice immunized with Ad5E1 MEC. These CTL clones were capable of tumor-eradication in nude mice, and were directed against the Ad5E1A-encoded decapeptide SGPSNTPPEI, presented by the H-$2D^b$ MHC molecule.

We have now generated Ad5E1 MEC containing a mutated Ad5E1A-encoded epitope. The mutant Ad5E1 MEC induce a strong CTL response when injected into immunocompetent mice. CTL clones generated against mutant Ad5E1-transformed tumor cells recognize an Ad5E1B-encoded epitope (VNIRNCCYI) in the context of H-$2D^b$. Since this epitope is also present on wild-type Ad5E1 MEC, it is concluded that Ad5E1-transformed tumor cells express at least two CTL epitopes. Ad5E1 transformed tumors expressing an activated ras oncogene are tumorigenic in immunocompetent animals. Interestingly, the lysis of Ad5E1 MEC by the Ad5E1B-specific, but not by the Ad5E1A-specific, CTL clones was strongly diminished by the action of the EJras oncogene. CTL clones directed against this epitope were, like Ad5E1A-specific CTL, able to eradicate established Ad5E1-induced tumors in B6 nude mice, demonstrating that CTL activity directed against different CTL epitopes expressed by the same tumor can be exploited for immunotherapy of cancer.

With the use of peptide based vaccines protective immunity has been estabilised in several murine tumor-, and virus-infection models against a lethal dose of tumor cells respectively virus. In order to establish the effect of peptide immunization in this tumor model, we immunized B6 mice with the Ad5E1-encoded peptides. Peptide immunization, did, unlike immunization with Ad5E1-transformed tumor cells, not result in protective immunity, but instead in an increased rate of tumor growth. Moreover, the protective immunity established with tumor cell vaccination was abrogated by subsequent injection of Ad5E1-encoded synthetic peptides. This suggests that, in this model, the CTL mediating protective anti-tumor immunity were functionally depleted by administration of the peptides

C2-580 ROLE OF IL-12 IN THE INDUCTION OF INSULIN-DEPENDENT DIABETES MELLITUS, Sylvie Trembleau and Luciano Adorini, Roche Milano Ricerche, via Olgettina 58, 20132 Milano, Italy

A major role in the development of insulin-dependent diabetes mellitus (IDDM) in non-obese diabetic (NOD) mice is played by T cells. Administration of IL-12, a key cytokine which guides the development of Th1 CD4+ T cells, induces rapid onset of IDDM in NOD, but not in BALB/c mice. IL-12 administration induces disease in all NOD female mice tested, whereas only about 60% of control female littermates eventually develop IDDM. Histologically, IL-12 administration induces massive infiltration of lymphoid cells, mostly T cells, in the pancreatic islets of NOD mice, accompanied by selective destruction of islet β cells. CD4+ pancreas-infiltrating T cells, after activation by insolubilized anti-TCR antibody, secrete high levels of IFN-γ and low levels of IL-4. Therefore, IL-12 administration accelerates IDDM development in genetically susceptible NOD mice, and this correlates with increased Th1 cytokine production by islet-infiltrating cells. These results hold implications for the pathogenesis, and possibly for the therapy of IDDM and of other Th1 cell-mediated autoimmune diseases. The possibility that the spontaneous development of IDDM may also involve IL-12-dependent generation of Th1 cells is currently being tested by administration of anti-IL-12 antibodies and IL-12 antagonists to NOD mice.

C2-581 CTLA4IG ESTABLISHES TRANSPLANTATION TOLERANCE THROUGH SPECIFIC INDUCTION OF TH2 CYTOKINES, Laurence A. Turka*, Enver Akalin, Wayne Hancock, Charles B. Carpenter, and Mohamed H. Sayegh, *University of Pennsylvania, Philadelphia, PA 19104-6069, and Brigham and Women's Hospital, Boston, MA 02115.

Administration of CTLA4Ig (to block interactions between CD28/CTLA4 and B7-1/B7-2) 2 days following transplantation permits long-term survival of vascularized cardiac allografts in rats primed by receipt of donor lymphocytes at the time of transplantation. Here, we have examined the utility and mechanisms of CTLA4Ig in rat renal allografts. LEW rats underwent bilateral nephrectomy and received full MHC-mismatched grafts from BN donors. Control animals all died within 6-9 days, while animals receiving a single dose of CTLA4Ig on day 2 survived for >100 days with normal renal function as assessed by serum creatinine (1.05 ± 0.04 vs. 1.08 ± 0.18 for age-matched normal rats). Donor lymphocytes were not needed for graft survival. In contrast, only 29% of animals receiving CTLA4Ig on day 0 survived >9 days. Long-term survivors from the day 2-CTLA4Ig group appeared to be tolerant to donor antigens based on acceptance of BN cardiac grafts but rejection of 3rd party Wistar-Furth grafts. Surprisingly, histologic evaluation of day 7 allografts from control and day 2-CTLA4Ig animals showed similarly intense mononuclear cell infiltrates. However, only the control grafts had active tissue destruction with tubulitis and vasculitis, and expression of activation markers such as ICAM-1 and MHC class II.

Immunohistochemical analysis reveal intense staining for Th1 cytokines (IL-2 and IFN-γ) in control grafts with little or no Th2 cytokines (IL-4 and IL-10). In contrast, day 2-CTLA4Ig grafts had marked staining for IL-4 and IL-10, with little if any Th1 cytokines. These grafts also had marked increases in intragraft deposition of IgG1. Administration of rIL-2 to day 2-treated animals induced rejection, with death in 50% of the cases and a rise in serum creatinine in the remainder.

These results indicate that: 1) a single dose of CTLA4Ig 2 days after transplantation is sufficient to induce transplantation tolerance to vascularized organs; 2) this is associated with a Th2-type immune response; 3) delaying administration of CTLA4Ig is critical for its efficacy in this model. Priming of cells in vivo (perhaps through local IL-2) prior to exposure to CTLA4Ig may be required to allow for the emergence of Th2 cells and/or may render Th1 cells more susceptible to costimulatory blockade.

C2-582 A WILD TYPE AND A MUTANT P21RAS PEPTIDE ELICIT CTL RESPONSES IN THE HUMAN, Andrea van Elsas, Hans Nijman[1], Carolien van der Minne, Jacqueline Mourer, Cees Melief [1], and Peter I. Schrier, Dept. of Clinical Oncology and [1] Dept. of Immunohaematology and Bloodbank, University Hospital Leiden, PO Box 9600, 2300 RC Leiden, The Netherlands. Using the well-defined peptide motifs for HLA-A*0201, we selected a number of peptides derived from wild type human p21ras containing two possible anchor amino acids, and from p21ras mutants containing at least one anchor and a substituted amino acid. We showed that strong binding to HLA-A*0201 in the T2 assay is only found for two peptides, a wild type peptide (51-59) and a mutated peptide (51-61, Leu). This mutation is frequently observed to occur in human cancer, and we were able to induce primary CTL responses *in vitro* against this peptide loaded onto T2 cells, using PBMC from normal healthy donors. CTL were found to be of low affinity, and did not recognize 61-Leu transfectants. However, when autologous dendritic cells (DC) were pulsed with the 61-Leu peptide and used as primary stimulators, we did obtain clones that showed cross-reactivity. We also were able to induce a bulk CTL response to wild type p21ras, using the 51-59 peptide pulsed onto DC. This CTL line reacted with all melanoma cell lines carrying HLA-A*0201, and showed enhanced lysis of peptide pulsed melanoma target cells. We speculate that precursor T-cells against wild type p21ras are during T cell selection not deleted entirely, but may be anergized and circulate in healthy individuals.

C2-583 RESISTANCE OF INTESTINAL INTRAEPITHELIAL LYMPHOCYTES TO APOPTOSIS INDUCED BY IMMUNOSUPPRESSIVE AGENTS, Nancy Van Houten and Gordana Gasic. Departments of Internal Medicine and Pediatrics, University of Texas Medical Branch, Galveston, TX 77555-0366.

The immune system associated with the gastrointestinal tract is distinct from the systemic immune system, and consists of diverse sequestered compartments. Intestinal intraepithelial lymphocytes (IEL) are incompletely characterized with regard to their functional responses to immunosuppressive agents. Both phenotypic diversity as well as microenvironmental differences define a uniqueness of these populations. Many phenotypic characteristics of IEL are similar to immature lymphocytes in the thymus, in contrast to peripheral T lymphocytes from the spleen, lymph nodes (LN) or blood. Clearly the signalling mechanisms of IEL differ from those of peripheral T cells, yet the underlying mechanisms have not been defined. Data is presented to show that IEL do not respond to the same immunosuppressive signals as their peripheral counterparts. When mice were treated with 4 mg hydrocortisone acetate for 12, 24, or 48 hr prior to harvest of thymus, LN, or IEL, minimal deletion of $CD4^+CD8^+$ IEL was observed. Other signalling molecules involved in induction of differentiation and death of IEL were examined. Although IEL have many characteristics of activated lymphocytes, $CD4^+$ IEL fail to express the CD40 ligand , and also do not express Fas, the mouse homologue of APO-1. Together these data demonstrate that activation and signalling mechanisms of IEL differ from peripheral T lymphocytes as do their responses to immunosuppressive agents.

C2-584 INHIBITION OF IgE SYNTHESIS BY AN IgE SPECIFIC MONOCLONAL ANTIBODY, Nicki J. Vasquez and Paula M. Jardieu, Department of Immunology, Genentech Inc., South San Francisco, CA 94080

It is a well established observation that *in vivo* administration of antibodies reactive with IgE, result in the loss of serum IgE. While it is clear that antibodies against IgE are capable of inhibiting IgE synthesis, the mechanism for this inhibition is unknown. It is possible that suppression of IgE synthesis is due to a block in development, or a selective deletion of IgE secreting B cells. Alternatively, loss of synthesis may be due to induction of B cell anergy.

In order to elucidate the mechanism by which anti-IgE inhibits IgE synthesis, an Epstein Barr Virus transformed human B cell line has been established that expresses both membrane and secreted IgE. Overnight culture of these cells with an IgE specific monoclonal antibody results in a partial suppression of IgE synthesis, in the absence of any effects on cell viability or growth. These results suggest that the loss of serum IgE seen with *in vivo* administration of anti-IgE, may be due in part to the induction of B cell anergy. In order to study this observation further, we are currently investigating the biochemical and molecular events mediated by ligation of membrane bound IgE.

C2-585 PROTECTION OF LYMPHOMA GRAFTS BY DIFFERENT DOMAINS OF THE MOUSE MHC CLASS I MOLECULE D^d

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In former studies, we have shown that introduction of an allogeneic MHC class I gene (D^d) inte the germline of C57Bl/6 (B6) mice (K^b, D^b) brings about a redefinition of the in vivo NK cell repertoire. A panel of H-2^b lymphomas that grew readily in the syngeneic strain (B6), was rejected by NK cells in the H-$2D^d$ transgeneic strain D8. The rejection could be abrogated by transfection of the lymphoma cells with the D^d gene, a finding consistent with the "missing self" hypothesis for NK cell mediated rejection of H-2 deficient or mismatched grafts. Furthermore, this protective effect could be defined to the $\alpha1/\alpha2$ domains of D^d. We have continued this study to elaborate the role of the $\alpha1$ and $\alpha2$ domins separately. Preliminary results suggest that the $\alpha2$ domain contains protective motifs: chimeric molecules harboring the $\alpha1$ from D^b and $\alpha2/\alpha3$ form D^d protected lymphomas from elimination in the D8 strain, whereas its counterpart ($\alpha1/\alpha3$ from D^d and $\alpha2$ from D^b) did not protect to the same extent. Further studies using site-directed mutagenesis to investigate the role of specific amino acid residues are in progress.

C2-586 ROLE OF T AND B LYMPHOCYTES IN IMMUNITY TO COCCIDIAL INFECTION, Li Wen, Scott J. Roberts, Amy Dudenhofer, Roxanne McDaniel, Nicola Schweitzer, Charles A. Janeway, Jr., Craig Findly and Adrian C. Hayday, Department of Biology & Section of Immunobiology, Yale University School of Medicine, New Haven, CT 06510.

Coccidial infection of intestinal epithelium induces specific resistance to subsequent challenges. Both humoral and cellular factors have been implicated in protective immunity against coccidial disease. However, the precise role of each component of the immune response in conferring protection against coccidial infection is not clearly defined. In the present study, T cell receptor α(TCRα)-/- and immunoglobulin (Ig) μ chain -/- mutant mice (μMT) were employed to further investigate the role of T and B lymphocytes in protection or immunity to Eimeria infection. Our previous studies demonstrated that αβ T cells are important for both primary and secondary immune responses. To ask whether αβ T cells act by inducing a local humoral response, we examined the levels of mucosal Igs post primary and secondary infection. Primary infection of both TCRα+/- and TCRα-/- mice did not elicit dramatic increases in gut lavage antibody of either A or G subclasses. Essentially the same was true for secondary infection. Moreover, μMt -/- mice that lack B cells were fully resistant to secondary infection. We have, likewise, determined that mice lacking γδ T cells are fully resistant to secondary infection. To further define the mechanism of the immunity, cytokine mRNA profiles were investigated using competitive PCR. Similar levels of IFNγ and IL4 mRNA expression were detected from both MLN and IEL of TCRα-/- and TCRα+/- mice at different time points tested post infection. These data suggest that αβ T lymphocytes have an important role in the development of immunity and that the mechanism the immunity is possibly a unique one, neither Th1 nor Th2 mediated.

C2-587 **CHOLERA TOXIN MODULATES MITOGENESIS OF SWINE BLOOD LYMPHOCYTES DEPENDENT ON THE TYPE OF PRIMARY STIMULUS; MECHANISM IS PKC AND AND TYROSINE KINASE DEPENDENT AND INDEPENDENT OF PKA ACTIVATION**

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Cholera toxin, the toxin B subunit and dibutyryl cAMP all enhance the *in vitro* proliferative response of swine blood lymphocytes to the lectin mitogen Con A. In contrast, the proliferative response to the superantigen Staphylococcal enterotoxin A, the memory response to specific antigen (KLH), and growth induced by phorbol ester/ionophore are all inhibited by holotoxin, with more variable influences exerted by the B subunit and dibutyryl cAMP. IL-2 mediated growth of blast cells is unaffected by toxin suggesting that toxin mediates it's influence on growth signals prior to IL-2 release (G1 stage of cell cycle). The mechanism underlying the toxin enhancement of the Con A response involves positive growth signals stimulated by the toxin B subunit since enhancement is independent of cAMP concentrations. The holotoxin effect is equivalent to that generated by purified B subunit and mitogenesis is not reduced by PKA-specific inhibitors, H-8 and H-89. PKC and tyrosine kinase inhibition completely abrogates all growth. Differences in response to mitogens that stimulate growth by known, but incompletely defined pathways, provides a unique model to study the immune-modulating effects of cholera toxin as well as the mechanisms of signal transduction stimulated by commonly used mitogens.

C2-588 **FORMATION OF SPECIFIC LOW AFFINITY PEPTIDE/CLASS II DOES NOT REQUIRE PEPTIDE SIDE CHAIN INTERACTIONS WITH CLASS II GROOVE.**
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Kinetic studies using techniques for rapid separation of peptide/class II complexes from unbound peptide has provided evidence for two thermodynamically distinct kinetic states of the specific interaction of peptides with mouse class II, I-E^k, I-E^d or human HLA-DR1; a fast binding, short lived complex, and a slow accumulating, long lived complex. In addition, it is well documented that class II molecules gain SDS resistance in an SDS-PAGE upon peptide binding. We have demonstrated that the short lived complex correlates with SDS unstable form, and the long lived complex with the compact dimer state for class II molecules. Crystal structure of hemagglutinin peptide residues 306-318 and HLA-DR1 indicates both side chain interactions with the binding pockets and H-bond to the main chain of peptide contribute to the stability of the complex. We examined the possibility that low affinity complex of peptide/class II complex may involve contacts between peptide main chain and DR1 as opposed to the stable peptide/class II complexes that involve peptide side chains within the binding pockets of the DR1 groove. To test this idea, a polyalanine peptide with lysine at the same relative position as HA peptide, (AAAAAAAAAKAAA) was compared to YAK (AAYAAAAAAKAAA) and an HA analogue with a substitution at tyrosine 308 to alanine (HA 308A) in binding to HLA-DR1. All three peptides, AK, YAK and the HA 308A did inhibit formation of low affinity complexes in a peptide competition assay using spun column assay. In addition, YAK inhibited binding of DR1 to immobilized HA peptide on the dextran-modified gold surface of a biosensor flow cell detected by Surface Plasmon Resonance. When a SDS-Gel assay followed a 72 hour incubation of DR1 with peptides at neutral pH, we observed formation of compact dimers for YAK/DR1 and lack of it for AK or HA 308A. These results suggest that contacts between peptide main chain and DR1 binding groove and not the peptide side chains may contribute to the formation of the low affinity interactions in binding of peptide to DR1.

C2-589 ***RECOMBINANT ANTIBODIES IN BIOACTIVE PEPTIDE DESIGN***. W.V. Williams, C. Monfardini, T. Kieber-Emmons, J.M. VonFeldt, K. Kaushansky, C.B. Brown, D. Voet, D.E. McCallus, and D.B. Weiner. University of Pennsylvania, Philadelphia, PA, 19104, and the University of Washington, Seattle, WA, 98195.

Granulocyte/macrophage colony stimulating factor (GM-CSF) binds to specific cellular receptors which represent potential targets for pharmacologic design. Such design depends on a molecular understanding of ligand-receptor interactions. One approach to dissecting out critical intermolecular interactions is to develop analogs of specific interaction sites of potential importance. Here we present application of recombinant antibody (rAb) technology to the development of analogs of a site on GM-CSF important for biological activity and receptor binding. A murine kappa light chain V region expression library was developed following immunization with neutralizing polyclonal anti-GM-CSF. This library was screened with an anti-GM-CSF neutralizing mAb, 126.213, which competes with GM-CSF for binding to GM-CSF receptor alpha chain (GM-CSFRα). Several binding clones were isolated. One clone (23.2) which inhibited 126.213 binding to GM-CSF was sequenced, revealing a murine kappa light chain of subgroup III. Comparison of the 23.2 sequence with the human GM-CSF sequence revealed little sequence similarity, but molecular modeling suggested strucural similarity with a site previously implicated in GM-CSF binding to the GM-CSFRα. Synthetic peptides were developed based on the sequences of the 23.2 complementarity determining regions (CDR). The CDR I peptide was preferentially bound by the 126.213 mAb, while the CDR II and CDR III peptides displayed lower reactivity. Only the CDR I peptide inhibited binding of 126.213 to GM-CSF. This peptide also inhibited GM-CSF dependent cell growth, but had no effect on interleukin-2 dependent cell growth. These studies indicate the feasibility of using recombinant antibodies as sources of interaction site analogs. This strategy allows preliminary contact residue identification and development of specific analogs for pharmacophore design.

C2-590 ANTIGEN-SPECIFIC CYTOTOXIC T-LYMPHOCYTE (CTL) RESPONSE INDUCED BY TUMOR-SPECIFIC MUCIN PEPTIDE IN BREAST CANCER. Stephen E. Wright,* Karen E. Lowe, Sohel Talib, Lydia Kilinski, Kenneth E. Dombrowski,* Jane S. Lebkowski, and Ramila Philip. *Veterans Administration Medical Center and Dept. of Internal Medicine, Texas Tech University Health Sciences Center, Amarillo, TX 79106 and Depts. of Molecular Biology and Cancer Biology, Applied Immune Sciences, Inc. Santa Clara, CA 95054.

One of the challenging goals in breast cancer immunotherapy is to increase tumor-specific cell-mediated immune response. Mucins are glycoproteins expressed by ductal epithelial cells of a variety of tissues, including breast, ovary and pancreas. Breast, ovarian and pancreatic carcinomas produce an abundance of highly immunogenic, aberrantly glycosylated mucins with shorter carbo- hydrate side chains than those of mucins expressed by nonmalignant cells. Tumor-specific epitopes are exposed in the hypogly-cosylated mucin, as it has been shown that deglycosylated normal mucin can induce tumor-specific monoclonal antibodies. Our approach is to use IL-2 and immunogenic mucin peptide ligands to activate and expand CTL from breast cancer patients that will recognize the tumor-specific epitope. It has been observed that the T-cell receptor (TCR) Vβ repertoire may be restricted in breast cancer patients. Since the TCR is involved in antigen recognition, T-cell activation, and the triggering of effector functions, it is of considerable significance to determine the TCR Vβ repertoire expressed by cells which have been treated with IL-2 and mucin peptides. In a preliminary study, we have expanded peripheral blood lymphocytes from a breast cancer patient using native mucin peptide, T16 to N16 mutant peptide or immobilized anti-CD3, in the presence of IL-2 for a period of 14 days. The data indicate that the peptide-stimulated and expanded T cell show antigen specific changes which were not induced by polyclonal activation and expansion.

C2-591 VACCINATION WITH PARASITE (EGG) ANTIGEN PLUS IL-12 SWITCHES HELMINTH-INDUCED CYTOKINE RESPONSES FROM A TH2 TO A TH1 PATTERN AND BLOCKS PATHOLOGY, Thomas A. Wynn, Dragana Jankovic, Robert Poindexter, Pat Caspar, Allen Cheever, and Alan Sher, Laboratory of Parasitic Diseases, NIH, Bethesda,MD

Egg granuloma formation and fibrosis are the primary pathological manifestations in schistosomiasis mansoni. Like most helminth elicited immune responses, the egg-induced reactions are associated with a Th2 profile of cytokine production. Cytokine neutralization studies demonstrated a primary role for Th2 cytokines in mediating granulomatous inflammation, while IFN-γ and IL-12 were shown to suppress this response. More importantly, we have shown that sensitization of mice with eggs and IL-12 can effectively suppress egg-induced pathology upon secondary challenge in the lung. To investigate further the feasibility of this "anti-pathology" vaccine, we sensitized mice with parasite eggs in the presence or absence of IL-12 and then challenged the animals with cercariae to assess the effects of this immunization protocol on Th1/Th2 cytokine expression, granuloma formation, and fibrosis during a natural infection. We observed a nearly 30% reduction in the size of egg-induced lesions in the livers of mice pre-sensitized with eggs plus IL-12. More importantly, hydroxyproline levels were reduced to background levels as were the mRNAs for pro-collagen I and III, indicating a near complete suppression of fibrosis in these animals. These changes were associated with marked increases in IFN-γ and IL-2 expression, while the Th2 cytokines IL-4, IL-5, and IL-13 were nearly completely suppressed. Together, these data demonstrate that helminth-induced Th2 cytokine responses can be switched to a Th1 pattern by prior sensitization with IL-12 and suggest that this approach might be applicable to the prevention of human disease.

C2-592 T CELL STIMULATION BY FILARIAL ANTIGENS: PROLIFERATION, CYTOKINE PRODUCTION AND Vβ USAGE, Maria Yazdanbakhsh, Erliyani Sartono, Yvonne C.M. Kruize, Felix Partono and Rick M. Maizels, Department of Parasitology, University of Leiden, The Netherlands. Department of Parasitology, University of Indonesia, Indonesia. Department of Biology, Imperial College, UK.

Infection with filarial parasites is associated with a spectrum of clinical manifestations and immunological responses. Individuals with patent infection, microfilaremics, show T cell hyporesponsiveness in terms of proliferation and IFNγ production. However, despite lack of antigen specific proliferation, cells from microfilaremics release IL4 following stimulation with parasite antigens. To assess how parasites are involved in the observed skewing of these immune responses, filarial patients were treated with DEC and their antigen specific responses were evaluated post therapy. T cell proliferation and IFNγ production were enhanced substantially, but to a different extent in the distinct clinical categories. IL4 release was not affected significantly. Another approach to determine how filarial parasites influence the immune responses of their host is to study fluctuations in microfilarial densities and associated T cell responses longitudinally in the absence of drug treatment. Seasonal fluctuations in parasite density in the endemic community resulted in variation in T cell proliferation and in IL4 and IFNγ release in a manner that supports results obtained in drug treatment study. Towards identification of T cells that respond (in terms of proliferation) to parasite antigens, TCR Vβ usage was determined in PBMNC stimulated with filarial antigens. A limited number of Vβ genes were used in response to parasite antigens; Vβ 8 and 23 were used relatively more frequently. T cell clones from these Vβ-bearing cells will have to be propagated for the identification of target antigens.

C2-593 CD8+ AND CD4+ T CELL CLONES THAT RECOGNIZE A NORMAL SELF PROTEIN CAN BE ISOLATED AND EXPANDED FROM MELANOMA PATIENTS, Yee C, Gilbert M, Boon T, Fefer A, Thompson J, Greenberg PD. Ludwig Institute, Brussels, Belgium, Clinical Research Division Fred Hutchinson Cancer Research Center, and Div of Oncology, Univ of Washington, Seattle, WA 98195.

The use of antigen-specific tumor-reactive T cells offers an attractive modality for the treatment of cancer with high specificity and minimal toxicity but requires the identification of possible target antigens. Evidence suggests that tyrosinase is such an antigen since tyrosinase-reactive HLA-A2-restricted CTLs have been detected from the peripheral blood of melanoma patients and from heterogenous TIL populations expanded from melanoma patients that mediated tumor rejection. The routine isolation of tyrosinase-specific T cells for the treatment of melanoma may be limited however because tyrosinase is a self protein also found on some normal tissues such as melanocytes. Therefore, we evaluated the frequency with which CD8+ T cell clones specific for tyrosinase could be isolated from the peripheral blood of patients with melanoma. Of eight patients studied, tyrosinase-specific and autologous tumor-reactive CD8+ CTL clones were isolated from the peripheral blood of five patients. The Class I restricting elements presenting tyrosinase to our clones were found to include HLA-A28, B8 and B60 suggesting that this protein contains epitopes that can be expressed and recognized in the context of many Class I alleles. The efficacy in tumor therapy of cytolytic CD8+ T cells is partly limited by a dependency on exogenous IL-2 or IL-2-producing CD4+ T cells. Tyrosinase-specific CD4+ T cells have recently been found in the TIL population of a melanoma patient and we therefore evaluated the frequency with which tyrosinase-specific CD4+ T cell clones could be isolated from the peripheral blood of melanoma patients. Tyrosinase-specific CD4+ T cell clones were isolated from all five of the patients whose peripheral blood also yielded tyrosinase-specific CD8+ CTL clones. The Class II restricting elements for these responses are now being defined. Thus, T cell tolerance to tyrosinase is limited since both CD8+ and CD4+ tyrosinase-specific T cell responses can be isolated from a high proportion of melanoma patients. Based on this ability to reliably isolate and expand tumor-reactive tyrosinase-specific CD4+ and CD8+ T cell clones from melanoma patients of diverse HLA types, we have designed a Phase I clinical trial involving the infusion of tyrosinase-specific autologous T cell clones containing a TK-suicide gene to permit ablation if autoimmune tissue injury develops.

C2-594 INHIBITION OF *I. SCAPULARIS*-INDUCED *BORRELIA BURGDORFERI* INFECTION IN MICE TREATED WITH TNFα, IL-2 AND IFNγ, Nordin S. Zeidner[1], Matthew J. Dreitz[1], Debra L. Belasco[1], and Durland Fish[2], Department of Immunology, Paravax, Inc., Fort Collins, CO.[1], Department of Epidemiology and Public Health, Yale University, New Haven CT.[2]

Down regulation of cytokine production and resultant systemic immunosuppression has been demonstrated during tick feeding upon murine, canine, and bovine hosts. To examine the hypothesis that reconstitution of cytokines during *I. scapularis* feeding would circumvent tick-induced immunosuppression and facilitate a host immune response against *B. burgdorferi*, the following experiments were performed. C3H/HeJ mice were treated with IFNγ, IL-2, TNFα, or combinations of these cytokines at the time of placement of infected ticks, and for a period of 10 days after tick placement. Ear punch biopsies were taken at day 21, cultured for 21 days and then analyzed for the presence of *B. burgdorferi* by polymerase chain reaction (PCR). PCR analysis indicated a 95% protection rate of those mice receiving TNFα, or TNFα in combination with IFNγ or IL-2 (1/16 mice infected). Those mice receiving IL-2, IFNγ or the combination of IL-2 + IFNγ were infected at rates of 30% (IL-2, 3/10 infected), 33.3% (IFNγ, 4/12 infected) and 45% (IL-2 + IFNγ, 5/11 infected) resepectively. Eighty-three percent (10/12) of untreated control mice exposed to infected *I. scapularis* were positive for *B. burgdorferi* by culture or PCR analysis. No significant difference in infection rate was noted in culture and PCR analysis of target tissues (heart, bladder, ear, joint, and spleen) harvested at day 50 after tick placement. Moreover, no significant neutralizing antibody activity was detected in either infected or uninfected mice, and Western blot analysis did not indicate any specific serum reactivity associated with protection from infection. *In vitro* culture of *B. burgdorferi* in cytokine conditioned media indicated that all of these cytokines, alone or in combination, enhanced the growth of *B. burgdorferi* in cell culture. These data suggest that cytokine-induced protection from natural *B. burgdorferi* infection *in vivo* is an immune-mediated event and not a result of direct killing of spirochetes by cytokine treatment. Thus cell-mediated immunity, rather than a specific humoral response to spirochete antigens, appears most likely associated with protection from infection. We are currently using this model to investigate the role of specific host cellular immune responses in the control of early tick-induced *B. burgdorferi* infection in mice.

C2-595 STUDIES ON GLUCOCORTICOID RECEPTOR AND GLUCOCORTICOID IN PATIENTS WITH APLASTIC ANEMIA. Yu-Sheng Zhang. The Pharmaceutical Industrial Company of Henan Province, Zheng Zhou, Henan 450052, P.R. China.

In this study of 36 patients with aplastic anemia (AA), glucocorticoid receptor (GCR) in peripheral leukocytes was detected by radioligand binding method. Their plasma glucocorticoid (GC) was measured by radio-immunoassay. Thirty healthy volunteers were examined simultaneously as the control. GCR in AA patients was significantly lower than that in healthy control (3074 ± 1071 binding sites/cell vs 4134 ± 1258 binding sites/cell, $p<0.05$). GCR in 4 acute AA patients was significantly higher than that in 32 chronic AA patients (4744 ± 689 binding sites/cell vs 2866 ± 864 binding sites/cell, $p<0.005$), and it had no difference from that in control. GCR in chronic AA was significantly lower than that in control ($p<0.005$). Plasma GC concentration was 134 ± 47ng/ml in AA, showing no difference from that in control (130 ± 55ng/ml). There was no difference either in GC between acute AA and chronic AA (132 ± 35 ng/ml and 136 ± 49 ng/ml respectively). GCR density had no correlation with the plasma GC concentration in AA patients ($r=0.077$, $n=36$, $p>0.05$). The decrease of GCR in chronic AA likely explain why the therapeutic effect of GC on acute AA is better than that in chronic AA. The higher GCR in acute AA than that in chronic AA suggests that there may be a different pathological mechanism between acute AA and chronic AA (immunopathogenesis). The assay on GCR may have reference value for administration of GC in AA treatment. In addition, GC concentration may have no effect on GCR density in AA patients since plasma GC was in the normal range and GCR change was not related to the GC concentration in AA patients.

Key Word: aplastic anemia, glucocorticoid receptor, glucocorticoid

C2-596 CYTOKINE DELIVERY BY BIODEGRADABLE BEADS AS ALTERNATIVE TO GENE-TRANSDUCED TUMORS FOR AUGMENTING ANTI-TUMOR IMMUNE RESPONSES, Zhong Zhao, Elizabeth Jaffee, Drew Pardoll, Kam Leong, Departments of Oncology and Biomedical Engineering, The Johns Hopkins University School of Medicine, Baltimore, MD 21205

Cytokine based vaccine strategies have gained increasing recognition as a potential cancer treatment modality due to its tremendous success in animal tumor models. The central principle of this approach is the local delivery of cytokines at the site of the tumor to either provide the required co-stimulatory signal or to enhance antigen recognition and presentation. However, a strategy in which delivery of the cytokines can be uncoupled from the cells presenting the antigen should provide a more versatile system to maximally manupulate the anti-tumor immune responses. We have explored an alternate approach for local delivery of sustained dose of cytokines. In this approach, biodegradable microspheres made of gelatin and chondroitin sulfate coacervate effectively encapsulate high doses of cytokines such as GM-CSF and release their content in a sustained manner upon in vivo degradation of the matrix by proteases. Preliminary studies from our lab showed that GM-CSF containing microspheres together with irradiated tumor cells offered comparable protection to the GM-CSF gene transduced tumor cells in the B16 murine melanoma model. This microsphere system possesses a number of convenient features. First, a patient's excised tumor cells can be irradiated and mixed with cytokine microspheres before re-injection, obviating extensive labor involved in culture and transduction of individual tumor cells. Second, the pharmacokinetics of local cytokine delivery can be varied by modifying the parameters of the microsphere such as crosslinking density of the matrix and the cytokine loading level so that maximal biological effects can be achieved. In addition, co-encapsulation of different cytokines in the microsphere system may offer us a convenient tool to dissect the local synergistic effects of the cytokines. Recent advances in the strategy will be discussed, including characterization of the system and results of in vivo experiments.

Late Abstract

EXAMINATION OF THE BINDING OF A NON-PEPTIDIC LIGAND TO CLASS I MHC ANTIGEN

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Class I MHC antigens present peptides to T-cell receptor (TCR) complexes which, when recognized by the TCR, trigger cytolysis of the presenting cell. This process is important and necessary to protect against viral infection and tumorigenesis, but detrimental in the case of recognition of self-peptides as in the case of autoimmune disease. An examination of the molecular surface presented to the TCR by class I MHC antigens suggests that only the central portion of the peptide is able to interact with the TCR. In the hope that this information may be useful in inhibiting the binding of self-reactive peptides, we have synthesized a ligand lacking a peptidic central region. The substitution (phenanthradine) may be placed into a variety of peptide sequence backgrounds and thus bind to a variety of allotypes. These peptides appear to bind in the conventional fashion and generate a similar amount of structural stability to the class I MHC antigen complex. An examination of the binding of these ligands will be presented along with a discussion of the crystallographic structure of one such ligand/ HLA class I complex.