IMMUNOGENICITY

Organizers: Charles Janeway, Jonathan Sprent and Eli Sercarz
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Poster Sessions

(Poster Sessions Continued)

Generation of an Effective Ligand

C 001 MOLECULAR BASIS OF ANTIGEN-MHC-T CELL RECEPTOR INTERACTION, Ronald N. Germain, Franca Ronchese, Takashi Saito, Ronald H. Schwartz*, and Ned S. Braunstein#, Laboratory of Immunology and *Laboratory of Cellular and Molecular Immunology, NIAID, NIH, Bethesda, MD 20892; #Dept. of Medicine, Columbia University College of Physicians and Surgeons, New York, NY 10032

The clonally distributed αβ T cell receptors (TCR) recognize peptides bound to cell-surface products of the major histocompatibility complex (MHC). Using a combination of assays following transfection of murine class II MHC genes into L cells (efficiency of cell surface expression of class II MHC dimers, reactivity of expressed recombinant or mutant Ia molecules with large panels of monoclonal antibodies, and effective presentation of various peptide antigens by such molecules), we have attempted to gain an understanding of the functional molecular architecture of murine Ia molecules with respect to their roles as peptide receptors and target structures for TCR interaction. Particular attention has been paid to distinguishing between local and distant effects of amino acid substitutions on Ia function and to determining which residues interact with peptide antigen and which (if any) with the TCR. This experimental approach has led to the identification of several regions of the polymorphic amino-terminal domains of the α and β chains as playing critical roles in chain-chain association and quaternary Ia conformation. The α1 and β1 putative helical regions have been found to have distinct degrees of structural lability, with the α1 helix showing much greater susceptibility to conformational change due to allelic variation in other regions of the molecule. Allelically polymorphic residues in the α1 and β1 domains have been shown to play important roles in the activity of the assembly/folding control regions, and hence, analysis of local binding roles of specific residues in Ia molecules must take this additional effect of substitutions at these positions into account. By controlling for large scale conformational effects, individual residues in the β chain have been assigned to desetopic (peptide interaction) and histotopic (TCR interaction) roles. In the cytochrome *c* model, a putative peptide binding "pocket" involving residues from both the postulated β1 α helix and also the β-strand floor has been defined, residues controlling both the extent of binding and the orientation of the bound peptide have been identified, and at least one residue with TCR interaction potential without obvious peptide binding properties has been localized. Combining these data with those of other investigators leads us to propose a general model of class II MHC structure-function relationships.

C 002 HOW ACCURATE IS THE BROWN/WILEY MODEL FOR THE STRUCTURE OF MHC CLASS II MOLECULES? Diane Mathis, Jean Peccoud, Caroline Waltzinger and Christophe Benoist, Laboratoire d'Immunologie Moléculaire, L.G.M.E., 11, rue Humann, 67085 Strasbourg cédex, FRANCE.

The structure of an MHC class I molecule has been elucidated by X-ray crystallography; based on this, a model for the structure of MHC class II molecules has recently been presented (the Brown/Wiley model). To test this model, we propose to mutate to alanine each amino acid of the A^k_α and A^k_β first domain α-helices, to introduce the mutated molecules into L cells, and to assay antibody binding, T cell recognition and (for certain of them) antigen binding. The A_α chain mutants have all been created, and L cell lines carrying each of them in association with a wild-type A_β chain produced. The antibody binding data have permitted a detailed definition of several epitopes, providing some indication of which amino acids are accessible to large molecules. The initial T cell data suggest that surprisingly few, scattered residues are absolutely required for recognition.

T Cell Recognition of Ligand on the APC

C 003 RECOGNITION OF PEPTIDE:CLASS II MHC LIGANDS BY CD4 T CELLS: LIGANDS, RECEPTOR SUBSITES, CORECEPTOR MOLECULES, AND THE ORIENTATION OF THE COMPLEX. Charles A. Janeway, Jr., Jose Rojo, Pilar Portoles, Junji Yagi, and Donal B. Murphy. Section of Immunobiology, Howard Hughes Medical Institute at Yale University School of Medicine, New Haven, CT. 06510. USA.
The initiation of most immune responses requires the activation of CD4 T cells by specific peptide:class II MHC ligands. We have studied this problem from several points of view. The following points will be made: 1. A ligand comparable to that recognized by a T cell receptor is detectable with a monoclonal antibody. This antibody can be used to probe antigen processing and T cell receptor (TCR) repertoire selection. This implies that the T cell receptor will have a structure analogous to that of an antibody molecule. 2. The T cell receptor appears to have distinct subsites for antigen and for MHC. An MHC subsite appears to be on the Vß domain, suggesting that Vß contacts MHC directly. 3. Studies of *Mls* and *Mls*-like bacterial proteins suggest that Vß binding to self MHC may play an important role in this response as well, but that *Mls* is not a peptide but rather a protein that binds the TCR and the MHC class II molecule at sites outside the conventional peptide binding sites on these structures. This has important implications for models of TCR:MHC interaction. It also provides an explanation for how *Mls* can select TCR based on Vß alone. 4. The CD4 molecule is a coreceptor for class II MHC, associating physically with the TCR during antigen recognition. This association potentiates T cell activation by about 100 fold, readily explaining the association of CD4 expression and class II MHC recognition. These data can be assembled to provide a model for the interaction of the TCR:CD4 complex with the peptide:self class II MHC complex, or with the *Mls*:class II MHC complex leading to T cell activation.

C 004 CHARACTERISATION OF VICIA VILLOSA BINDING T CELLS AND THE INHIBITORY EFFECT OF IgA IN AUTOIMMUNITY OF PRIMATES, Lehner T, Fortune F, Brines R, and Fellowes R. Department of Immunology, United Medical & Dental Schools of Guy's and St Thomas's Hospitals, London SE1 9RT UK.

The mechanism responsible for autoimmunity involves augmentation of the immune response or a loss of tolerance to self antigens. A Vicia villosa (VV) binding subset of $CD8^+$ cells is capable of augmenting the immune response by preventing suppressor activity of $CD8^+VV$ non-adherent cells. Reconstitution experiments with B cells, CD4 helper CD8 VV^- and CD8 VV^+ cells have shown both in naturally acquired immunity in human subjects and actively immunized rhesus monkeys that the VV-adherent CD8 cells augment antibody production in the face of suppression. We have pursued the investigations in human autoimmune diseases and found a very significant increase in CD8 VV^+ cells in the circulation of patients with systemic lupus, especially during disease activity. However, in rheumatoid arthritis (RA) we have not detected a significant increase in circulating blood CD8 VV^+ cells but these were found in very significant numbers in synovial fluid. The lack of CD8 VV^+ cells in the circulation of patients with RA was due to IgA_1 binding T cells which might prevent access of the lectin VV to N acetyl D galactosamine on the T cells for which VV is affinity purified. We are now investigating the hypothesis that augmenting the immune response by VV binding T cells in the circulation of SLE and synovial fluid of RA patients is prevented by circulating IgA_1 bound T cells in RA, thereby protecting the central organs from autoimmune damage.

Trans-Membrane Signalling in Lymphocyte Sets

C 005 ION CHANNELS AND CALCIUM SIGNALLING IN INDIVIDUAL T LYMPHOCYTES

Michael D. Cahalan and Richard S. Lewis, Department of Physiology and Biophysics, University of California, Irvine, CA 92717.

Using the patch clamp technique and optical monitoring of intracellular calcium, $[Ca^{2+}]_i$, we have characterized ion channels which modulate Ca^{2+} signalling in T lymphocytes. Video imaging techniques were used to measure $[Ca^{2+}]_i$ in individual Jurkat T leukemia cells loaded with fura-2. Stimulation with phytohemagglutinin (PHA, 10 ug/ml) elicits oscillations of $[Ca^{2+}]_i$ with variable latency (100-300 s) but relatively consistent period (80-100 s). Removal of extracellular Ca^{2+} or addition of high $[K^+]$ rapidly reduces $[Ca^{2+}]_i$ to below resting levels, implicating influx of Ca^{2+} ions through a membrane-potential-sensitive mechanism. Upon restoration of normal ionic conditions the cells initially oscillate synchronously, and $[Ca^{2+}]_i$ transiently exceeds previous stimulated levels, implying feedback regulation of $[Ca^{2+}]_i$. Under whole-cell voltage clamp conditions, depolarization activates a set of voltage-dependent K^+ channels and also reduces $[Ca^{2+}]_i$, consistent with the responses of intact cells to elevated $[K^+]$. Two additional current components were seen which may contribute to $[Ca^{2+}]_i$ oscillations measured concurrently. Immediately preceding and during periods of $[Ca^{2+}]_i$ rise, a small inward Ca^{2+} current (1-6 picoamperes/cell) becomes activated. Unlike Ca^{2+} channels in electrically excitable cells, the Ca^{2+} channels in T cells are not activated by membrane depolarization. As $[Ca^{2+}]_i$ continues to rise toward uM levels, a Ca-activated K^+ current turns on while Ca^{2+} current declines. Upon sequestration and removal of $Ca^{2+}{}_i$, K^+ current at negative potentials is then reduced. The observed Ca^{2+} channels may mediate Ca^{2+} influx following mitogen stimulation, while K^+ channels activated by depolarization or by $[Ca^{2+}]_i$ keep the membrane potential polarized, thus enhancing the Ca^{2+} signal. These results suggest that periodic fluctuations in membrane Ca^{2+} channel activity may be responsible for $[Ca^{2+}]_i$ oscillations.

C 006 ENHANCED TRANSMEMBRANE SIGNALLING ACTIVITY OF MONOCLONAL ANTIBODY HETEROCONJUGATES SUGGEST MOLECULAR INTERACTIONS BETWEEN RECEPTORS ON THE T CELL SURFACE.

Jeffrey A. Ledbetter, Nancy A. Norris, Laura S. Grosmaire and Wesley L. Cosand, Oncogen, 3005 First Avenue, Seattle, WA 98121.

Signal transduction occurs through multiple receptors expressed on mature, resting T cells. In addition to the CD3/T cell receptor complex, the CD2, CD4, CD5, CD7, and CD28 receptors mobilize cytoplasmic calcium within minutes after binding with monoclonal antibodies, and additional crosslinking on the cell surface. As an approach to study the interactions between these receptors and their transduced signals, monoclonal antibodies to each of these receptors were covalently coupled as heteroconjugates and investigated for activity in cytoplasmic calcium mobilization using indo-1 and flow cytometry. Of a total of thirty-five conjugates studied, there were seven heteroconjugates that showed an increase in activity, and these consisted of either certain conjugates of anti-CD3 or certain conjugates of anti-CD5. The CD3/CD2, CD3/CD4, CD3/CD6, and CD3/CD8 heteroconjugates each gained two to three orders of magnitude in titer in calcium mobilization compared to unconjugated CD3 or CD3/CD3 conjugate. The increase in activity was not accompanied by an increase in binding titer, indicating that signal transduction occurred at lower levels of receptor occupancy. The increased activity was dependent in each case on the relevant second receptor, since unconjugated CD2, CD4, CD6, or CD8 MAb could block the activity of the corresponding heteroconjugate. Neither CD3/CD5, CD3/CD28, or CD3/CD3 conjugates gained activity, whereas CD3/CD7 heteroconjugates gained slightly in activity.

The heteroconjugates with CD5 that acquired ability to mobilize calcium at low concentrations were CD5/CD4, CD5/CD8, and CD5/CD6. Their activity could be inhibited by either CD5 MAb or the second MAb of the heteroconjugate. The increased activity of CD3 or CD5 heteroconjugates was observed in the absence of extracellular calcium. Size exclusion chromatography of heteroconjugates demonstrated that 1:1 ratios were optimal, but larger conjugates were also active. These results suggest that certain receptors are capable of molecular interactions on the cell surface to form complexes with enhanced activity in signal transduction leading to calcium mobilization.

APC Requirements for Immunogenicity

C 007 STIMULATION OF $CD8^+$ T CELLS. Jonathan Sprent, Department of Immunology, IMM4A, Research Institute of Scripps Clinic, 10666 North Torrey Pines Road, La Jolla, CA 92037.

$CD8^+$ T cells from B6 and B10 congenic mouse strains give strong $CD4^+$-cell-independent primary mixed-lymphocyte reactions (MLR) to stimulator cells expressing H-2 class I differences. The issue of which particular cells present class I alloantigens to unprimed $CD8^+$ cells is controversial. Studies from this laboratory suggest that, although H-2 class II $(Ia)^+$ cells, especially dendritic cells, have strong antigen-presenting cell (APC) function for $CD8^+$ cells, these T cells also respond well to class I alloantigens presented by various types of Ia^- cell populations. Ia^- cells with APC function for $CD8^+$ cells (in the absence of added lymphokines) include macrophages, various tumor cells and T cells; some of these cells reveal APC function only after treatment with neuraminidase. The significance of these findings will be discussed.

Manipulation of Immunogenicity/Tolerogenicity

C 008 TOLERANCE AND AUTOIMMUNITY IN TRANSGENIC MICE EXPRESSING CLASS II MHC ON PANCREATIC ACINAR CELLS, David Lo[1], Linda Burkly[2], Richard A. Flavell[2], Richard D. Palmiter[3] and Ralph L. Brinster[1], [1]U. of Pa. School of Vet. Med., Philadelphia, PA 19104, [2]Biogen, Cambridge, MA, 02142, [3]U. of Wash., Seattle, WA 98195. To study the nature of tolerance to antigens not expressed by cells of the lymphoid system, expression of class II MHC I-E was targeted to the acinar cells of the exocrine pancreas in transgenic mice (El-I-E). Despite the absence of detectable I-E in the thymus of El-I-E transgenic mice, both thymocytes and peripheral T lymphocytes were tolerant to I-E in mixed lymphocyte culture. Reduction in the numbers of $V\beta17a$ bearing T cells (normally predisposed to I-E reactivity) was not observed, suggesting that the thymus did not delete I-E reactive T cells. The pancreas of unmanipulated El-I-E transgenic mice appeared to be free of autoimmune infiltrates, but nontolerant T cells adoptively transferred into irradiated El-I-E mice rapidly destroyed the I-E positive components of the pancreas. Adoptive transfer of nontolerant T lymphocytes into nonirradiated El-I-E mice did not result in destruction of the I-E positive pancreas, suggesting that tolerance in El-I-E mice is maintained by some peripheral tolerance mechanism. However, cell mixing experiments both in vivo and in vitro suggested that tolerance in El-I-E mice is not maintained by suppressor T cells. Our working hypothesis is that potentially autoreactive T cells encountered I-E on acinar cells and were paralysed for lack of a second signal.

Memory: Generation and Differences in Activation Requirements

C 009 IDENTIFICATION AND CHARACTERISATION OF HUMAN VIRGIN AND MEMORY T LYMPHOCYTES. Peter C. L. Beverley, Matthias Merkenschlager and Diana L. Wallace. I. C. R. F. Human Tumour Immunology Group, University College and Middlesex School of Medicine, The Courtauld Institute of Biochemistry, London, W1P 8BT. The monoclonal antibody (mAb) UCHL1 identifies the 180kDa polypeptide of the leucocyte common (CD45) antigen. UCHL1 antigen is expressed on a subset of peripheral T cells, complementary to the subset expressing the high molecular weight (CD45R) polypeptides of CD45. The UCHL1+ subset contains cells able to provide help for an in vitro antibody response as well as precursor cells for proliferative responses to recall antigens. In contrast UCHL1-, CD45R+ cells cannot provide help and have a very low frequency of cells responsive to recall antigens (1). Stimulation of CD45R+ cells with mitogens or alloantigens leads to expression of UCHL1 and loss of CD45R (2). These data strongly suggest that CD45R+ cells are virgin T cells, while UCHL1+ cells are a memory population. Experiments using further mAbs have delineated heterogeneity of the memory compartment and the repertoire of virgin and memory cells has been explored by stimulation with MHC class II expressing transfected cells.

1. Merkenschlager M. et al., Eur. J. Immunol. (in press).
2. Akbar A. et al., J. Immunol. 140: 1-8 1988.

C 010 T AND B CELL MEMORY ARE SHORT-LIVED IN THE ABSENCE OF ANTIGEN. David Gray, Basel Institute for Immunology, Grenzacherstrasse 487, CH-4005 Basel, Switzerland. We have shown previously that memory B cells transferred into K-allotype distinct congenic rats in the absence of any priming antigen are deleted from the adoptive host within a matter of weeks (half-life of 1-2 weeks). In contrast co-injection of antigen with the cells facilitates their survival and the maintenance of a donor response for periods in excess of one year. In the experiments reported here we ask if the persistence of T cell memory is also dependent on antigen. 10^8 carrier (KLH) primed T cells were transferred in the presence or absence of antigen into irradiated, K-allotype distinct adoptive host. At various times after transfer these rats were injected with $2x10^7$ hapten-carrier (DNP-KLH) primed B cells together with 50 µg of soluble DNP-KLH. This limiting number of B cells makes a secondary type response only if carrier-specific memory T cells survive in the adoptive host. We found that already at 6 weeks following transfer without antigen, no memory T cell help was available for these B cells. In contrast T cells transferred together with 10 µg KLH provided help for secondary type donor responses at 6 and 12 weeks after transfer. We conclude that longterm memory at both the T and B cell levels does not reside with small, very long-lived, resting cells but with active clones that are maintained by small amounts of antigen that may persist for long periods. Once antigen is lost from lymphoid tissues both T and B cell memory wanes within a relatively short time.

C 011 FUNCTIONAL PROPERTIES OF MEMORY T CELLS, H. Robson MacDonald, Ralph C. Budd and Jean-Charles Cerottini, Ludwig Institute for Cancer Research, Lausanne Branch, 1066 Epalinges, Switzerland

T cell memory is characterized by accelerated kinetics and higher peak levels of effector function; however the cellular basis of this phenomenon has remained largely obscure, because of lack of specific markers for memory cells. We have made the chance observation that monoclonal antibodies (mAbs) directed against Pgp-1 (Ly24), a cell surface glycoprotein with a wide tissue distribution, detect in certain mouse strains a subpopulation of T cells that have the expected properties of memory cells. Thus Pgp-1$^+$ cells in both the CD4$^+$ and CD8$^+$ T cell subsets are dramatically enriched for antigen-specific precursor cells following in vivo priming with the appropriate antigen, whereas the Pgp-1$^-$ subsets are correspondingly depleted. Furthermore, in the case of the CD8$^+$ subset, Pgp-1$^+$ precursors appear to express relatively high affinity antigen receptors, since specific cytolysis by the progeny of these cells is highly resistant to inhibition by mAbs directed against CD8. Analysis of sorted Pgp-1$^+$ and Pgp-1$^-$ subsets of CD4$^+$ or CD8$^+$ lymphocytes further demonstrated an unexpected dissociation in lymphokine production: whereas both subsets produce equivalent amounts of IL-2 in response to an antigenic or mitogenic stimulus, the Pgp-1$^+$ subset was found to secrete much higher levels of IL-4 and IFN-γ than Pgp-1$^-$ cells. This latter phenomenon may be relevant to the role of memory T cells in inflammation and as helper cells for antibody formation by B lymphocytes.

Studies of the development of T cell memory suggest that Pgp-1$^-$ ("virgin") T cells are produced in the thymus and migrate to the periphery. When stimulated by antigen, Pgp-1$^-$ cells acquire Pgp-1 rapidly and maintain Pgp-1 expression thereafter in a stable fashion. Although the function of the Pgp-1 molecule remains obscure, it remains a useful marker for the identification and further characterization of memory T lymphocytes.

C 012 NAIVE AND MEMORY HUMAN T CELL SUBSETS: DIFFERENCES IN EXPRESSION OF ACCESSORY MOLECULES, ACTIVATION REQUIREMENTS AND LYMPHOKINE SECRETION, Stephen Shaw, Kevin J. Horgan, M. William Makgoba, Martin E. Sanders, Experimental Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892.

Three concepts will be presented: 1) Differential expression of multiple markers distinguish two major subsets of human peripheral blood T cells; 2) A variety of lines of evidence establish that these subsets represent naive and memory T cells; 3) There are marked functional differences between naive and memory cells.

First, there are two major reciprocal subpopulations of adult peripheral blood T cells (distinct from CD4 and CD8) which differ in levels of expression of six distinct molecules -- all six of which are known or inferred to be involved in T cell adhesion and/or activation. Two dramatic differences are: expression of different molecular weight forms of CD45 (T200, LCA) on the two subsets and expression of the CD2-ligand LFA-3 on one subset but not the other. In addition, one of the subsets expresses higher levels of four molecules known to be adhesion receptors (LFA-1, CD2, CD44) or inferred to be (CD29).

Second, various approaches demonstrate that the LFA-3$^+$ subset (which expresses the lower molecular weight form of CD45 and higher levels of CD2, LFA-1, CD29 and CD44) includes memory T cells and the reciprocal subset is composed of naive cells. Only the LFA-3$^+$ cells proliferate in response to antigens to which the donor has been primed. Furthermore, activation of LFA-3$^-$ cells in vitro results in their conversion to LFA-3$^+$. This conversion is apparently irreversible and is accompanied by changes in expression of the other markers (the CD45 markers, CD2, LFA-1, CD44, CD29) to levels characteristic of the LFA-3$^+$ subset. Only the LFA-3$^-$ subset is found in newborns, who should lack memory cells because of minimal past antigenic exposure.

Third, the functional programmes of naive and memory cells are markedly different. Memory T cells proliferate much more than naive cells when stimulated with CD3 mAb or pairs of CD2 mAb. Enhanced responsiveness to receptor-mediated triggering is a novel mechanism for T cells which could facilitate memory cell response to specific antigen. Furthermore, when triggered via either CD2 or CD3, memory T cells produce substantial amounts of gamma interferon while naive cells produce virtually none; this suggests that differentiation from naive to memory cells is accompanied by a stable change in regulation of the gene for gamma interferon. We conclude that naive and memory cells are not just slightly different, they are dramatically different in a variety of ways.

Closing Lectures

C 013 IMMUNOGENICITY

G.J.V. Nossal
The Walter and Eliza Hall Institute of Medical Research,
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Two worlds co-exist for the study of immunogenicity. On the one hand, we have mainstream academic immunologists interested in lymphocyte activation and the regulation of the resultant immunoproliferative cascade. They do most of their work in tissue culture, frequently using cloned cell lines. On the other, we have vaccinologists eager to bring the fruits of history's most cost-effective public health tool, the vaccine, to bear much more extensively on a whole range of communicable diseases, particularly those of the tropical developing countries. They are the inheritors of a great empirical tradition inherited from Freund and others. Until a decade ago, the worlds were poles apart.

Mutual self-interest is drawing the camps much closer together. The reductionist student of immunoregulation is blocked at many levels of understanding, e.g. of isotype patterns in immune responses, and has to draw on the experiences from real infections and infestations. The vaccinologist is drawing increasingly on the store of basic knowledge generated through lymphokine research, gene cloning, MHC structure and function and many other facets of fundamental immunology. Furthermore, idealism and the catalytic help from W.H.O. and various Foundations has also helped to close the gap.

This closing lecture will concentrate on recent highlights from the work of The Walter and Eliza Hall Institute, which, it is hoped, can extend the bridge still further. The approach will involve analyses of both B and T lymphocyte immunogenicity in model studies and real life situations.

Late Additions

C 014 TOLERANCE IN LYSOZYME/ANTI-LYSOZYME TRANSGENIC MICE, Christopher C. Goodnow, David Y. Mason*, Robert A. Brink, Stephen Adelstein, Jeffrey Crosbie, Helle Jorgensen, Helen Pritchard-Briscoe and Antony Basten, Clinical Immunology Research Centre, University of Sydney, NSW 2006 Australia, and *Nuffield Department of Pathology, John Radcliffe Hospital, Oxford, U.K. OX3 9DU

The phenomenon of self-tolerance involves two key issues: what variables influence whether an autologous antigen is recognised as "self", and what is the fate of self-reactive cells following self-recognition. To address these issues, two types of transgenic mice have been produced. The first type carries one of two gene constructs encoding a "neo-self" antigen, hen egg lysozyme (HEL). Expression of the HEL transgene is driven by a heterologous promoter, derived from either the mouse metallothionein or mouse albumin genes. Multiple lines of HEL transgenic mice have been produced on a C57BL/6 inbred background, and through the combination of position effects, different promoters, and zinc induction it is possible to compare tolerance to HEL in groups of mice expressing a range of levels of serum HEL, from less than 0.2 ng/ml to 100 ng/ml. Tolerance to HEL is manifest in all lines with detectable basal levels of HEL.

The second type of transgenic mouse carries rearranged immunoglobulin heavy (μ-δ) and light chain genes encoding a high affinity anti-HEL antibody. Greater than 90% of the B-cells in these mice express only the transgene-encoded anti-HEL antibody as cell-surface IgM and IgD. These B-cells are localised primarily in the follicular areas of lymph node and in both the follicular and marginal zones of the spleen. Six lines of μ-δ + κ Ig-transgenic mice have been characterised, and mated to various HEL-transgenic lines, to produce "double-transgenic" mice carrying both the HEL transgene and the antibody transgenes. Secretion of anti-HEL antibody is curtailed in most combinations of HEL-transgenic and Ig-transgenic lines, accompanied by a marked and selective reduction in the levels of surface IgM but no change in IgD. Intriguingly, HEL-binding B-cells are depleted from the marginal zones but not from the follicles in double-transgenic spleen. By contrast, in double-transgenic mice expressing 20-fold lower levels of HEL, secretion of anti-HEL antibody continues to a significant extent and there is only a slight reduction in surface IgM on the B-cells. B-cell tolerance in this model therefore appears to be antigen-dose dependent and intimately linked to surface IgM downregulation.

C 015 TRANSMEMBRANE SIGNAL TRANSDUCTION IN REGULATION OF LYMPHOCYTE FUNCTION, John C. Cambier, Division of Basic Sciences, Department of Pediatrics, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO 80206

The humoral immune response is regulated by a large number of soluble and cell associated ligands including (in the mouse), antigen, Tcr/CD4 (through binding of mIa), IL1, IL2, IL4, IL5, IL6, IFN_γ, TGF_β, TNF and perhaps other less well defined factors such as BCAF. Each of these agents stimulates a distinct biologic response in B cells, suggesting that each must interact with a distinct receptor and each of these receptors must be coupled to the biologic response by a distinct transmembrane signal transduction cascade. This requirement for multiple signaling pathways may be lessened somewhat if 1) all receptors are not expressed throughout B cell activation, proliferation and differentiation, and 2) if the cell is programmed to respond differently to the same intracellular second messenger at distinct stages of activation, proliferation and differentiation. Clearly, however, resting B cells express receptors for antigen, Ia binding ligands, IL5, IL4, IL2, TGF_β, IFN_γ, potentially necessitating the use of an equivalent number of transduction cascades. As far as is known, this complexity is unparalleled in any other biologic system.

Available evidence indicates that antigen receptors on both T and B cells transduce signals via activation of polyphosphoinositide hydrolysis, Ca^{++} mobilization, and protein kinase C (PKC) activation. T cell antigen receptor mediated signaling may involve, in addition, the activation of a tyrosine kinase activity. IL4 receptors appear not to transduce via perturbation of phospholipid metabolism of Ca^{++} mobilization, but may activate a membrane associated kinase. In B cells, signaling mediated by Ia, and TGF_β and IFN_γ receptors appears to involve cAMP generation. IL2 receptor mediated signaling involves tyrosine kinase activation and PKC translocation to the plasma membrane in the apparent absence of phosphoinositide hydrolysis and Ca^{++} mobilization. This PKC translocation response also occurs following IL1 stimulation of cells, which, like IL2, does not stimulate phosphoinositide hydrolysis of CA^{++} mobilization, but leads to hydrolysis of phosphatidylcholine liberating diacylglycerol. Thus, diglyceride generation by this mechanism may be an important aspect of signal transduction by both IL1 and IL2 receptors.

This presentation will consist of a review of our current knowledge regarding the molecular bases of signal transduction by receptors for regulators of the immune response. As time permits, transmembrane signal transduction by mIg will be considered in greater detail, with particular reference to the role of receptor associated proteins in signal transduction.

Antigen Processing; Accessory Cell Types and Characteristics; Cell Adhesion Molecules and their Expression; Immunodominance; (Antigen: MHC Effects and Cell Interactions); Immunogenicity and Tolerogenicity in Tissue Transplantation

C 100 IN VIVO COMPETITION BETWEEN PEPTIDES FOR PRESENTATION BY MHC CLASS II MOLECULES INFLUENCES THE IMMUNODOMINANCE OF T CELL DETERMINANTS, Luciano Adorini and Zoltan A. Nagy, Preclinical Research, Sandoz Ltd., CH-4002, Basel, Switzerland.

T cells recognize antigen in the form of short peptides associated to class I or class II MHC molecules. Each MHC molecule has the ability to bind a large number of peptides and peptides with unrelated sequences can compete for binding to the same MHC molecule, in vivo as well as in vitro. In vivo competition strictly correlates with the capacity of the competitor peptide to bind to the MHC molecule presenting the antigenic peptide and its extent dependes on the molar ratio between antigen and competitor. In vivo competition among different peptides derived by processing of hen egg-white lysozyme (HEL) appears to exert a major influence on the immunodominance of antigenic determinants recognized by T cells. Thus, the HEL peptides 1-18 and 25-43 are both generated by HEL processing and are both able to bind to the I-E^k molecule but only 1-18 becomes immunodominant because it has the ability to compete in vivo with other HEL peptides, such as 25-43, for the available sites on the I-E^k molecule. However, two immunodominant T cell epitopes, such as those in HEL peptides 51-66 and 112-129, both interacting with I-A^k molecules, do not compete with each other when injected together at equimolar concentrations. Such a coexistance is anticipated between peptides that bind with relatively high affinity to the presenting molecule and thus have both the chance to occupy a number of binding sites sufficient for T cell activation.

C 101 CELL MEDIATED IMMUNITY IN XENOGENEIC TRANSPLANTATION. Hugh Auchincloss, Jr., David J. Conti, Richard N. Pierson, III, and Henry J. Winn. Department of Surgery, Massachusetts General Hospital, Boston, MA 02114.

Experiments have been performed to study the cell-mediated immune response in xenogeneic transplantation. In vivo investigation using monoclonal antibodies revealed that monkey skin graft survival on mice was significantly prolonged by anti-CD4 antibody treatment but not by anti-CD8 antibody. Neither antibody alone prolonged survival of whole-MHC disparate allogeneic tissue on the same animals but anti-CD4 antibody did prolong minor antigen-disparate allografts. In vivo studies revealed that primary proliferation and IL-2 production by mouse T cells in response to monkey stimulators was weak compared to allogeneic responses. Secondary responses to xenogeneic stimulation were strong after in vivo priming but required the presence of responder APC's. Assays for cytotoxic T cell effectors in mice which had rejected monkey skin revealed few such cells. These results suggest that widely disparate xenogeneic MHC antigens are recognized, like minor allogeneic antigens, only after antigen processing and presentation. Since xenogeneic antigens require that such presentation be in association with the MHC antigens of responder APC's, the xenogeneic grafts have a functional similarity to APC-depleted allografts. Cytotoxic effectors are therefore difficult to generate for targets expressed in the xenogeneic graft and xenograft rejection would seem to require DTH-like mechanisms.

C 102 FETAL KIDNEY AND FETAL AND NEONATAL TESTIS CONGENIC GRAFT SURVIVAL CORRELATES WITH REDUCED MAJOR HISTOCOMPATABILITY COMPLEX (MHC) BURDEN, Edward M. Barksdale, Jr., M.D., Mindy B. Statter, M.D., Daniel Parks, B.A., Patricia K. Donahoe, M.D. Pediatric Surgical Research Laboratory, Massachusetts General Hospital, Boston, MA 02114. In previous studies we showed that fetal renal and fetal and postnatal testis allografts survived longer than corresponding adult tissue in non-immunosuppressed outbred rat hosts. The current study asks whether the difference in survival between renal and testicular grafts and between grafts of different ages is related to differential tissue expression of Class I and Class II mRNA transcripts or surface antigens, and if these patterns change with transplantation. Using congeneic mice we found that prolonged survival of C57BL/6 fetal renal (n=42; $p<0.008$) and fetal (n=14; $p<0.05$) and postnatal (n= 8; $p<0.05$) testis mouse allografts transplanted beneath the renal capsule of adult recipient B10.A mice and this survival correlates inversely with the expression of Class I and Class II mRNA (northern analysis) and proteins (immunohistochemistry) and that both protein and mRNA increased throughout ontogeny for both the testis and kidney. After transplantation there was a marked induction of MHC mRNA transcripts for both testis (n=207) and kidney (n=320). Implanted fetal kidney tissue that survives, however, failed to express detectable MHC protein, indicating that some post-transcriptional modification in this tissue occurs, to afford it protection from rejection. Implanted testis showed induction of both mRNA and protein well above its much lower baseline, indicating that its regulation, in contrast to the kidney may be transcriptional. Thus the fetus may lower the MHC burden as a strategy to escape rejection either by posttranscriptional modification of protein expression as in the kidney or by transcriptional modification of mRNA as in the testis.

C 103 PERSISTENT IMMUNOGENICITY OF RAT THYMIC EPITHELIUM, Donald Bellgrau and Harry Georgiou, Department of Microbiology and Immunology, University of Colorado School of Medicine, Denver, CO 80262

Culture of thymus tissue in 2-deoxyguanosine (2dGua) is thought to reduce tissue immunogenicity by selectively depleting highly immunogenic, thymic immigrants of bone marrow origin. In the mouse 2dGua treated thymus tissue survival is markedly enhanced compared to untreated tissue when transplanted under the kidney capsule of allogeneic recipients. These experiments were repeated in the rat. As expected, strain DA neonatal thymus tissue was rejected when transplanted under the kidney capsule of normal allogeneic strain PVG rats. Surprisingly, acute rejection occurred even when the tissue was cultured for 14 days in 4 mM 2dGua (3x the effective dose in mice). By in vitro criteria this dose was very effective in destroying thymocytes. To test whether residual marrow derived cells that escaped 2dGua treatment were responsible for inducing rejection we "parked" the 2dGua treated DA tissue in T cell depleted PVG rats. Our working hypothesis was that the few remaining donor derived cells of marrow origin would be overgrown by host type cells. When 2dGua-treated DA thymus tissue was transplanted into T cell depleted PVG recipients graft rejection did not occur. However DA 2dGua treated thymus tissue, parked for as long as 200 days in T cell depleted PVG rats, was acutely rejected when retransplanted into normal PVG recipients. We interpret these results to suggest that rat thymic epithelium devoid of marrow derived cells is innately immunogenic.

C 104 EXPRESSION OF CD11a, CD18 AND A CONFORMATIONAL EPITOPE OF LFA1 ON PERIPHERAL BLOOD LYMPHOCYTES IN AIDS, Marie C Béné, Corinne Amiel, Violaine Guérin, Thierry May, Philippe Canton, Gilbert C Faure, Laboratoire d'Immunologie and Maladies Infectieuses, CHU de Nancy, Faculté de Médecine, 54500 Vandoeuvre les Nancy, France.

LFA1 is a dimeric membrane molecule composed of a specific alpha chain (CD11a) and a beta chain (CD18) common to three members of the LFA family. LFA1 is physiologically expressed on all white blood cells, while other molecules of the LFA family (with CD11b and CD11c alpha chains) are restricted to cells of myeloid lineage. A defective expression of LFA1 has been described in some congenital immune deficiency and in AIDS. We investigated the LFA1 defect on peripheral blood lymphocytes from 100 HIV+ patients. Three different monoclonal antibodies were used, respectively directed to chain-specific epitopes of CD11a (SPVL7, Sanbio) and CD18 (IOT18, Immunotech) and to a conformational epitope involving both chains (IOT16, Immunotech). Cell suspensions were stained in indirect immunofluorescence and a flow cytometer (Epics Profile, Coultronics) was used to assess the percentages of stained cell, the fluorescence intensity and the shape of fluorescent peaks. Our data suggest that LFA1 expression is impaired in HIV+ patients both through the quantitative expression of each chain and through conformational alterations.

C 105 THE ROLE OF THE CYTOPLASMIC DOMAIN IN SIGNALING THROUGH CD2, B.E. Bierer, H.L. Wolff, R. Bogart, S.J. Burakoff. Dana-Farber Cancer Institute, Brigham & Women's Hospital, and Harvard Medical School, Boston, MA 02115. CD2 (T11) appears to play a role in signal transduction and T cell activation. Functional studies and direct binding experiments have demonstrated that LFA-3 is a natural ligand for CD2. To investigate the structural requirements for signal transduction via the CD2 pathway, we have expressed a number of cytoplasmic domain deletion mutants of CD2 in an antigen-specific murine T cell hybridoma that produces interleukin-2 (IL-2) in response to MHC class II molecules. Hybridomas expressing a deletion mutant, CD2ΔB, in which the carboxyterminal 100 amino acids have been deleted, were able to bind LFA-3 comparably to the wild type CD2 protein. However, unlike the wild type $CD2^+$ hybridoma, $CD2\Delta B^+$ hybridomas were unable to produce IL-2 in response to pairs of stimulatory anti-CD2 MAbs nor to LFA-3 plus one anti-CD2 MAb. It appears that the cytoplasmic domain is necessary for the delivery of an activating signal but is not required for adhesion. The complexity of the CD2 pathway of activation is demonstrated by expression of a second cytoplasmic domain deletion mutant, CD2ΔS, in which the terminal 42 amino acids have been removed, including the region with greatest conservation between mouse, rat, and human species. $CD2\Delta S^+$ hybridomas were able to respond to antigen, to LFA-3 plus an anti-CD2 MAb, and to certain pairs of anti-CD2 MAbs comparably with the wild type $CD2^+$ hybridomas. These $CD2\Delta S^+$ hybridomas were markedly deficient, however, in their ability to respond to other pairs of stimulatory anti-CD2 MAbs. These data suggest that the cytoplasmic domain may have several functional regions, as this deletion results in a partial, not an absolute, defect in T responsiveness.

C 106 ACCESSORY MOLECULES AUGMENT TRANSMEMBRANE SIGNALING BY THE T CELL ANTIGEN RECEPTOR, Linda K. Bockenstedt and Arthur Weiss, Howard Hughes Medical Institute, Departments of Medicine and of Microbiology and Immunology, University of California, San Francisco, San Francisco, CA 94143.
In order to investigate the role of accessory molecules in T cell activation, we studied the effect of monoclonal antibodies (mAb) against these molecules on transmembrane signaling events initiated by mAb against the T cell antigen receptor CD3/Ti in human peripheral T cells. Phosphatidylinositol (PI) second messenger generation could be induced by immobilized anti-CD3 mAb OKT3 in a dose-dependent fashion, whereas no significant PI turnover could be measured in response to immobilization of any single mAb against accessory molecules (CD2, CD4, CD5, CD8, CD28, HLA or LFA-1) in highly purified T cells. When saturating amounts of OKT3 were immobilized along with a single mAb against any one of these accessory molecules, no difference in PI turnover could be measured over the response to immobilized OKT3 alone. However, under conditions in which the amount of immobilized OKT3 is insufficient to induce PI hydrolysis, addition of a second immobilized mAb against any of the accessory molecules results in substantial production of inositol phosphates. In addition, a requirement for immobilization of both OKT3 and the mAb against the accessory molecule can be demonstrated. This augmentation of PI hydrolysis correlates with cellular activation as measured by cell proliferation. These results demonstrate that one function of accessory molecules may be to stabilize interaction of CD3/Ti with its ligand, thereby augmenting suboptimal transmembrane signals initiated by ligands to the T cell antigen receptor. This may provide a model for the function of intercellular adhesion molecules in T cell-antigen presenting cell interaction.

C 107 THE EFFECT OF STEROIDS ON LYMPHOCYTE ADHESION, Christian Bremicker, Karen Murley, Robert Rothlein(*) and Eric Martz, Department of Microbiology and Program in Molecular and Cellular Biology, University of Massachusetts, Amherst MA 01003 and (*) Boehringer Ingelheim Pharmaceuticals Inc., Ridgefield CT 06877 Glucocorticoids are clinically used as suppressants of inflammatory responses. One of the identified routes of action is the inhibition of leukocyte adhesion. A principal portion of the latter is mediated by LFA-1 and its counter-structures. We have studied LFA-1 dependent homotypic adhesion in the Epstein-Barr virus transformed human B-cell line JY. Growth of JY-cells in medium containing the clinical drug dexamethasone resulted in a substantial decrease of homotypic aggregation. The number of LFA-1 molecules per cell remained unaffected by the hormone; but the density of ICAM-1, the major counterstructure of LFA-1 identified so far, was reduced by 40-50 %. We are investigating whether the downregulation of ICAM-1 is the cells' sole relevant response to the steroid or if other effects contribute to the observed loss of adhesion.

C 108 BIOSYNTHESIS AND MATURATION OF THE MOUSE CD8 α AND β CHAINS IN L CELLS TRANSFECTANTS, Claude Bron, Dominique Blanc*, Bernard Malissen* and H. Robson MacDonald+, Institute of Biochemistry, University of Lausanne, CH-1066 Epalinges; *Centre d'Immunologie INSERM-CNRS, Marseille-Luminy and +Ludwig Institute for Cancer Research, Lausanne Branch, CH-1066 Epalinges.

Recent gene transfer experiments aimed at obtaining information concerning the possible structure-function relationship between the α(Ly2) and β(Ly3) chains of the mouse CD8 molecular complex revealed that the Ly2 polypeptide is required for the cell-surface expression of Ly3 (S.D. Gorman et al. 1988; D. Blanc et al. 1988). Rabbit antisera to synthetic peptides corresponding respectively to the N- and C-terminal segments of the Ly2 and Ly3 chains have been prepared and characterized. The biogenesis, the maturation and the assembly of these polypeptides has been studied with these reagents in Ltk⁻ cells transfected with the Ly3 gene alone or in combination with the Ly2 gene.

S.D. Gorman et al. J. of Immunol. 140, 3646-3653, 1988.
D. Blanc et al. Eur. J. Immunol. 18, 613-619, 1988.

C 109 ICAM-1 EXPRESSION ON T CELLS, Anne-Marie Buckle and Nancy Hogg, Macrophage Lab. ICRF, Lincolns Inn Fields, London, WC2A 3PX, U.K.

The adhesion molecule LFA-1 is known to be important in antigen presentation. We have previously shown that both monocyte and T cell LFA-1 play a role in the interaction between these two cells (EJI 17; 943,1987). Antibody to ICAM-1 (known to act as a ligand for LFA-1) also inhibits antigen presentation, although ICAM-1 is not thought to be expressed on resting T cells (EJI 18: 35,1988). We have looked at the expression of ICAM-1 on T cells after incubation with 12 cytokines and found that only IL-2 consistently effects an increase in both the percentage of ICAM-1 positive cells and in the level of expression. In addition we have found that a proportion of resting T cells express very low levels of ICAM-1. Double labelling experiments have shown that these cells are part of the memory T cell population as defined by antibodies to UCHLI, LFA-3 and LFA-1, and furthermore that ICAM-1 negative cells are unable to respond to to antigens such as PPD and Flu but are able to respond to PHA. This suggests that ICAM-1 represents an additional marker on the memory T cell population which more precisely defines the subset able to respond to recall antigens

C 110 INDUCTION OF OVALBUMIN-SPECIFIC CYTOTOXIC T CELLS BY *IN VIVO* PEPTIDE IMMUNIZATION, Francis R. Carbone and Michael J. Bevan, Department of Immunology, Research Institute of Scripps Clinic, La Jolla, CA 92037.

CTL recognize peptide forms of processed, foreign antigens in association with class I molecules of the MHC and are usually directed against endogenously synthesized "cellular antigens" such as those expressed by virus-infected cells. *In vitro* studies have shown that small exogenous peptides can directly associate with class I molecules on the cell surface and mimic the target complex derived by intracellular processing and presentation. We have recently generated OVA-specific, H-2K^b-restricted CTL by immunizing C57BL/6 mice with a syngeneic tumor line transfected with the OVA cDNA. The CTL recognize the OVA transfectant E.G7-OVA and the synthetic peptide $OVA_{258-276}$, but fail to recognize the native protein. We reasoned that given the potential for direct peptide/class I association observed *in vitro*, $OVA_{258-276}$ may induce CTL after *in vivo* priming. However, we found that this is not the case. $OVA_{258-276}$ and peptides of increasing lengths up to $OVA_{242-276}$ and $OVA_{242-285}$, which are all able to form the target complex *in vitro*, are inefficient at priming E.G7-OVA specific CTL responses following intravenous injection. This is also true for both native and denatured OVA. In contrast to these results, the synthetic peptide $OVA_{229-276}$ corresponding to a peptide in a partial tryptic digestion of OVA can efficiently prime C57BL/6 mice *in vivo* following intravenous injection. This peptide elicits CTL which appear identical to those derived from animals immunized with syngeneic cells producing OVA endogenously.

C 111 THE ROLE OF ASPARTIC AND CYSTEINE PROTEASES IN ANTIGEN PROCESSING, B.M. Chain, Department of Biology, (Medawar Building), University College London, Gower Street, London WC1E 6BT. In order to be recognised by a CD4$^+$ T cell, the majority of protein antigens must be processed by antigen presenting cells (APC), and this is believed to involve some limited form of proteolysis. On the basis of earlier studies, we have suggested that processing may involve proteases at the cell surface. We have now identified a number of classes of membrane associated proteases in enriched plasma membrane preparations from a murine antigen presenting B cell lymphoma, A20. These enzymes include a cysteine and an aspartic protease. We have also developed a cell free system in which these membrane preparations can effectively process antigen. Results from this system, as well as from studies on processing by intact A20 cells suggest that both cysteine and aspartic proteases may be necessary for antigen processing.

C 112 **STABLE EXPRESSION OF THE HUMAN T LYMPHOCYTE SPECIFIC CD2 cDNA IN MURINE L FIBROBLASTS**, Neil A. Clipstone and Michael J. Crumpton, Cell Surface Biochemistry, Imperial Cancer Research Fund Laboratories, London WC2A 3PX, England. It is now well established that human T lymphocytes can be activated via the T cell specific CD2 antigen. In order to determine if a factor(s) other than the single CD2 polypeptide is involved in CD2 mediated signal transduction, we have stably transfected murine L cells with the human CD2 cDNA. We report that such transfectants expressed high levels of CD2 at the cell surface, formed sheep erythrocyte rosettes and expressed the three CD2 epitopes previously defined on human T lymphocytes, including the "activation associated" $T11_3$ epitope. The latter observation unequivocally demonstrating that expression of the $T11_3$ epitope, in contrast to a previous report, is entirely independent of T cell specific factors. Combinations of CD2 mAbs that are potent stimulators of human T cells, however, failed to elicit either an increase in the concentration of intracellular free calcium or augment [^{3}H]-thymidine incorporation in the transfectants. These results provide both formal identification of the CD2 cDNA and clearly demonstrate that the single CD2 polypeptide expressed in an heterologous cell system devoid of T cell specific factors, cannot alone transduce intracellular signals in response to stimulatory combinations of CD2 mAbs. The results are therefore consistent with the notion, that the functional CD2 antigen expressed in human T lymphocytes, requires the association of another, as yet, undefined factor(s).

C 113 CD8 (LYT-2) RECOGNITION OF RESIDUES IN THE CLASS I α3 DOMAIN IN ALLOGENEIC CTL RESPONSES. Janet M. Connolly, Terry A. Potter, and Ted H. Hansen. Washington University School of Medicine, St. Louis, MO 63110 and Albert Einstein College of Medicine, Bronx, N.Y. 10461. It is generally accepted that the CD8 accessory molecule on class I-specific CTL can facilitate the specific interaction between the TCR α and β chains and the polymorphic α1, and α2 domains of the MHC class I molecule, presumably by binding to monomorphic determinants of the class I molecule. We have recently obtained evidence that the CD8 molecule recognizes residues in the monomorphic α3 domain of the class I molecule (Connolly et al., J. Exp. Med. 168:325). This conclusion was based on several lines of evidence including the observation that mAbs specific for the class I α3 domain of either H-2L^d or H-2D^d interfered with the generation of CD8-dependent (low affinity) CTL. In addition, cells expressing a D^d molecule with a single amino acid substitution at position 227 in the α3 domain are not lysed by CD8-dependent primary CTL but are lysed by secondary CD8-independent (high affinity) CTL generated in the presence of antibody to the α3 domain. We are extending these observations using clonal populations of CTL. We have isolated and characterized a CD8$^+$, CD4$^-$ D^d-specific CTL line. This line is CD8-independent and is capable of lysing the α3 domain mutant cell line. In addition, we are currently generating clones from primary D^d-specific CTL cultures to obtain CD8-dependent (low affinity) CTL. Additional α3 domain mutants obtained by site-directed mutagenesis are being tested with the CD8-dependent and CD8-independent clones to define additional residues important for CD8 recognition. The comparison of CTL clones with different CD8 dependencies will allow us to more precisely define the role of CD8 in T cell recognition.

C 114 A TWO STEP METHOD FOR ISOLATING FROM BLOOD AN ENRICHED POPULATION OF ANTIGEN PRESENTING CELLS (APC). T.G.J. de Boer, G.B. Humphrey, E. Boersma, H.H. Spanjer, W.A. Kamps. University of Groningen, The Netherlands; supported by SKOG) A mononuclear cell population is first isolated by Percoll® from the buffy coat of one unit of blood. These cells (≅400x10^6) are introduced into a Curame 5000 elutriation centrifuge (rotor speed of 3000 rpm; loading flow of 10 ml/min). Nine fractions can be obtained. The first three containing >90% lymphocytes; fraction 4 (3000 rpm-18 ml/min) and fraction 5 (2900 rpm-18 ml/min) contain both lymphocytes and monocytes and the next three fractions contain >90% monocytes; the final fraction (rotor off) contains monocytes + granulocytes. Cells from each fraction (5x10^4/well) are incubated for five days with tetanus toxoid (1.5LF/well) and an enriched population of T cells (5x10^4/well). Quadriplicate samples are then pulsed for 16 hours with ^{3}H methyl thymidine. Maximum APC activity is found in fractions 4 and 5 representing 4 to 7% of the mononuclear cells. APC activity for these two fractions can be further purified by selective absorption of the cells onto gelatin coated surfaces that have been preincubated with plasma. The non adherent lymphocytes are removed after two hours. After overnight incubation spontaneously released cells (1-5x10^6) can be harvested which have a higher APC activity than cells rotated by elutriation alone. These methods are now highly reproducible in our laboratory, so we can now begin to characterize and study these cells.

C 115 H-Y KILLING OF HUMAN CULTURED KERATINOCYTES, Cecile A.C.M. van Els, Marleen M. de Bueger, Johanna Kempenaar, Maria Ponec and Els Goulmy, Departments of Immunohematology, Dermatology, University Hospital Leiden, Rijsburgerweg 10, 2333 AA Leiden, the Netherlands. The male specific H-Y antigen has been shown to behave as a minor Histocompatibility antigen in man and mouse. In transplantation, male tissue may trigger the clonal expansion of H-Y reactive MHC restricted effector cells of female origin. Although male epidermal cells (EC) can induce an anti-H-Y T cell response in female mice, so far in vitro techniques have failed to identify the cell-defined H-Y antigen on murine EC (1). Here we developed a ^{51}Cr release assay to use human cultured keratinocytes (K) as target cells for HLA-A2 specific and HLA-A2 restricted H-Y specific T cell clones. HLA-$A2^+$ but not HLA-$A2^-$ K were lysed by anti-HLA-A2 CTLs in a dose dependent manner. Low but detectable levels of anti-H-Y killing were found against HLA-$A2^+$ male K but not against HLA-$A2^-$ male or HLA-$A2^+$ female K. Both levels of alloreactive and H-Y specific lysis were dramatically enhanced after exposure of K to IFN gamma. These results strongly suggest that human male skin cells are directly susceptible for H-Y directed T cell killing through the expression of functional H-Y/HLA complexes on their cell surface. In view of these findings, together with our recent studies on the expression of H-Y CTL determinants on human hematopoietic progenitor cells (2), the role of H-Y as a target structure for cell mediated immunity in clinical transplantation should be seriously taken into account.

1. Steinmuller D. and Burlingham W.J. Transplantation 1984,37,1,22.
2. Voogt P.J., Goulmy E., Fibbe W.E., et al. J. Clin. Invest. sept.1988.

C 116 DIPHTERIA TOXOID (DT) PRESENTATION BY HLA DR7 TRANSFECTED MURINE FIBROBLASTS TO SPECIFIC HUMAN T CELL CLONES, Patrice Debré, Hélène Gouy, Robert Karr*, Georges Bismuth, Laboratory of Cellular and Tissular Immunology, CHU Pitié Salpétrière, Paris, France and * Veterans Medical Center, Iowa city, USA.

L transfectants expressing single type of human MHC class II molecules produced by DNA mediated gene transfection were used to test the function of DR7 restricted DT specific human T cell clones. Both DR7α : DR7 β_1, and DR7α : DR7 β 4/7 IV (DRW53) gene products were functionnal, their expression being necessary and sufficient for DT presentation. Data also indicate that a) the two DR7 molecules share cross reactive epitopes b) the accessory cell function was inhibited with selected anti DR monoclonal antibodies c) DT processing was required to fully activate T cell clones. Transmembrane signalling with HLA DR transfected L cells and anti TcR antibodies will be compared.

C 117 THE DYNAMICS OF CONJUGATE FORMATION AND DISSOCIATION BETWEEN HELPER T CELLS AND ANTIGEN PRESENTING B CELLS, Dutton, Richard W., Swain, Susan L. and Rowe, Douglas B.,Department of Biology and UCSD Cancer Center, University of California San Diego, California,92093.
Conjugate formation has been studied with cloned T cell lines and a B cell hybridoma and with T cells and B cells from normal mice. Resting T cells and B cells do not form appreciable numbers of conjugates but conjugates are formed between T cells stimulated with alloantigen for four days and B cells activated by 24 hour culture with LPS. Irrelevant lymphocytes do not affect the rate of specific conjugate formation in suspensions of cells agitated by gentle rocking but impair conjugate formation when cells are allowed to settle in round bottom tubes. In further experiments, it was shown that the conditions for the induction of lymphokine secretion by the T cell were not indentical to the conditions for conjugate formation.The significance of these and other observations for the interaction of T cells and B cells in vivo will be discussed.

C 118 *AN H-2L^d HYBRID MOLECULE WITH Q7 COMPONENTS AT THE C-TERMINAL END IS NOT RECOGNIZED BY H-2L^d SPECIFIC CTL*, James Forman, Donald W. Mann, Leroy Hood*, and Iwona Stroynowski*, The Department of Microbiology, University of Texas Southwestern Medical Center at Dallas, Dallas, TX and *California Insititute of Technology, Pasadena, CA. L^d/Q7^d, a hybrid molecule consisting of α-1 and α-2 domains from H-2L^d and α-3 and carboxy-end components from Q7^d, was expressed on the surface of CRL-3A rat liver cells. This molecule retained serologic H-2L^d epitopes. The antigen is attached to the cell membrane through a phosphatidyl-inositol (PI) linkage, characteristic of Qa-2 molecules, and can be removed by treatment with PI specific phospholipase C.

Both bulk cultured and cloned H-2L^d alloreactive CTL as well as H-2L^d restricted VSV specific CTL lyse CRL-3A cells which express H-2L^d but show little or no lytic activity on cells which express the L^d/Q7^d hybrid. These cells also fail to act as cold target competitors for alloreactive anti-H-2L^d CTL. However, cells expressing L^d/Q7^d are not resistant to CTL mediated lysis since they can be killed in the presence of lectin.

This data indicates that recognition of polymorphic class I CTL epitopes in the α-1 and α-2 domains are influenced by the structure of the carboxy-end of the molecule.

C 119 DENDRITIC CELL-CD4$^+$T CELL CLUSTER FORMATION AND FUNCTION IN THE HUMAN PRIMARY MIXED LEUKOCYTE REACTION, Peter S. Freudenthal and Ralph M. Steinman, The Laboratory of Cellular Physiology and Immunology, The Rockefeller University, New York, NY 10021. It is known that human peripheral blood dendritic cells are potent stimulators of the primary mixed leukocyte reaction (MLR) and that this reaction occurs in multicellular dendritic cell-CD4$^+$ T cell clusters [Cellular Immunology 111, 183-195(1988)]. Dendritic cells are able to contact, cluster, and retain allogeneic T cells and induce these alloreactive cells to proliferate and divide. Using purified dendritic cell populations labeled with a vital fluorescent dye, we show that only dendritic cells efficiently form stable clusters. Labeled monocytes and B cells do not form clusters with T cells. When labeled monocytes and unlabeled dendritic cells are used to stimulate T cells, unlabeled clusters form. Labeled monocytes do not move into the clusters until the third day of the MLR. Significant levels of IL-2 and γ-IFN appear in the culture supernatant by the first or second day. The majority of clustered T cells have already undergone blast transformation by the second day of the MLR as demonstrated by Giemsa staining of cluster cytopreps. The distribution of certain adhesion molecules within clusters has also been studied by immunoperoxidase staining.

C 120 COMPETITION BETWEEN DOMINANT AND SUBDOMINANT EPITOPES OF MYELIN BASIC PROTEIN FOR THE I-A^s MOLECULE, Robert B. Fritz and Marianne J. Skeen, Department of Microbiology and Immunology, Emory University, Atlanta, GA 30322. Immunization of SJL/J mice with myelin basic protein (MBP) induces the T cell-mediated autoimmune central nervous system disease, experimental allergic encephalomyelitis. Response against a dominant epitope (residues 89-101) leads to disease. Lymph node T cells from MBP-immune mice react against several epitopes in addition to 89-101 indicating that the I-A^s molecule is able to form immunogenic complexes with several MBP peptides. The question asked in these studies was whether subdominant epitopes from the same molecule would compete with the dominant epitope for binding sites on the I-A^s molecule. To address this question two T cell clones, one specific for 89-101 (SP4.2) and a second specific for a second epitope present in peptide 89-170 (SP4.7) were tested for responsiveness when cultured with the dominant epitope alone or with mixtures of peptides containing dominant and subdominant epitopes. Reactivity of SP4.2 against peptide 89-101 was inhibited by peptides 1-37 and 43-88. Reactivity of SP4.7 against peptide 89-170 was not inhibited by peptide 89-101 although peptides 1-37 and 43-88 were inhibitory. Controls indicated that inhibitory reactivity was not due to toxicity at high concentrations of peptides. These findings imply that subdominant epitopes are able to compete with dominant epitopes of MBP for binding sites on I-A^s molecules. (Supported by U.S.P.H.S. grant NS 10721 and National Multiple Sclerosis Society grant RG 1511-B-2.)

C 121 THE NUCLEAR PROTEIN E1A IS THE DOMINANT TARGET ANTIGEN FOR ADENOVIRUS SPECIFIC CYTOTOXIC T LYMPHOCYTES. Linda R. Gooding, Frances C. Rawle, David I. Kusher, William S. M. Wold[+] and Barbara Knowles*. Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, GA 30322, [+]Institute for Molecular Virology, St. Louis University School of Medicine, St. Louis, MO 63110 and *The Wistar Institute, Philadelphia, PA 19104.

In several virus systems early non-structural proteins localized predominantly in the nucleus of infected cells are major target antigens for cytotoxic T lymphocytes (CTL). Whether early synthesis or nuclear localization are important factors in immunodominance is not known. We have recently developed a system for studying the CTL response to human group C adenoviruses in mice. By using both transfected targets and virus deletion mutants we have shown that, in C57BL/10 (H-2^b) mice, E1A encodes the only target antigen recognized in the polyclonal CTL response to wild type AD5. There are two E1A transcripts, 12S and 13S, which both encode major early nuclear antigens differing by a 46 amino acid insertion; both antigens are recognized equally well by CTL. The E3 encoded 19K glycoprotein (gp19K) of Ad5 binds to MHC class I antigens in the endoplasmic reticulum preventing their translocation to the cell surface and strongly inhibiting lysis by Ad5 specific CTL. However, the presence of gp19K in the priming virus does not affect the specificity of the CTL generated for E1A, so the immunodominance of this protein cannot be due to the fact that it is the only major protein synthesized before gp19K in the course of infection. Using virus deletion mutants we are investigating whether CTL specific for other Ad5 antigens can be induced in the absence of E1A, and whether E1A is also the dominant antigen recognized in mice of other MHC haplotypes.

C 122 CLASS II MHC-RESTRICTED T CELLS SPECIFIC FOR AN INFLUENZA VIRUS STRUCTURAL PROTEIN WHICH RECOGNIZE INFECTIOUS BUT NOT NON-REPLICATIVE VIRUS, Charles J. Hackett and L.C. Eisenlohr [a], The Wistar Institute, Phildelphia, PA; [a] LVD,NIAID, Rockville, MD.

Influenza-specific, class II MHC-restricted T cells recognizing viral structural proteins generally respond to antigens present on non-replicative virions. In contrast, we have obtained BALC/c I-E^d-restricted T hybridomas specific for the neuraminidase (NA) glycoprotein of A/PR8 influenza which recognize infectious, but not non-replicative virus, closely resembling recognition requirements observed for most class I MHC-restricted responses to influenza. Recognition correlated with the de novo synthesis of viral NA within antigen-presenting cells, but did not depend strictly upon the amount of NA present in cultures, since high NA concentrations could be achieved by addition of non-replicative virus without being stimulatory for NA-specific T cells. Recognition of a neo-antigen was ruled out, since, in high concentration, NA isolated from purified virions, even if reduced and alkylated, was recognized by the T hybridoma clone. Isolated NA was recognized when added to pre-fixed APC, suggesting that this form of antigen was able to bypass the usual processing pathway of exogenous proteins. This suggests that endogenously-synthesized antigen may use different pathways to achieve class II-associated presentation.

C 123 ANTI-SENSE RNA-MEDIATED INHIBITION OF CD2 EXPRESSION IN JURKAT CELLS, J. Hambor, L. Schultz, D. Gould, M. Tykocinski and D. Kaplan, Institute of Pathology, Case Western Reserve University, Cleveland Ohio 44106.

T lymphocyte activation is a complex event which is influenced by a variety of distinct cell surface molecules. In order to determine the role of individual molecules in the activation process, we have developed an efficient methodology for generating cell variants in which expression of molecules is selectively inhibited by expression of anti-sense RNA from an Epstein-Barr virus episomal replicon. In a previous study, we reported that marked inhibition of CD8 cell surface expression could be achieved in a human T cell clone using this approach. We have now extended this strategy to another T cell surface molecule, CD2, as a first step towards ascertaining its role in T cell activation. To this end, we synthesized a 55-mer oligonucleotide corresponding to a sequence in the 5' end of the coding region of human CD2 and inserted it in an anti-sense orientation into this replicon. This α-CD2/REP3 construct was electroporated into Jurkat cells. Analysis of stable α-CD2/REP3 transfectants by immunofluorescence staining and flow cytometry demonstrated complete and selective inhibition of CD2 expression. In contrast to the nontransfected parent, this $CD2^-$ variant demonstrated a partial loss in its ability to form conjugates and to secrete interleukin 2 when stimulated with anti-CD2 monoclonal antibodies. However, stimulation of the $CD2^-$ variant with A23187 and PMA did result in interleukin 2 secretion.

C 124 FUNCTIONAL DISSECTION OF THE MOUSE CD8 MOLECULES. Arnd Hoeveler, Jean Gabert and Bernard Malissen, Centre d'Immunologie INSERM-CNRS de Marseille-Luminy, Case 906, 13288 Marseille Cedex 9, France.
Several observations suggest that CD8 functions not only as an adhesion molecule recognising MHC class I on the adjacent cells but also potentiate the transducting capacity of the TCR/CD3 complex.
Comparison of the mouse Ly 2 protein sequence with the homolog rat OX8 and human T8 sequences revealed most highly conserved regions in the membrane and cytoplasmic part of the molecule. The conservation of the transmembrane and cytoplasmic sequences in different species may be significant for the function of the CD8 molecule.
In order to initiate the functional dissection of the CD8-molecule we constructed mutations in different parts of the molecule. By transfecting the α and β chain genes donated by a CD8 dependent cytotoxic T cell clone(KB5 C20) into the MHC class II restricted and $CD4^-$ T cell hybridoma DO-11.10 we were able to reconstitute the ability to respond to K^b only if the transfer was done with the Ly 2 molecules (Gabert et al., 1987, Cell, 50, 545-554).
In this system surface expression of mutated and non mutated Ly-2 molecules were checked by FACS-analysis and the molecuar size of the proteins were analysed by immunoprecipitation with the anti-Ly-2 monoclonal antibody 19/178. Finally functional effects of the mutations were investigated in response towards the K^b alloantigen.

C 125 THE USE OF BONE MARROW MONONUCLEAR CELLS (BMM) INFLUENCES THE STRENGTH OF PRIMARY IN VITRO REACTIVITY BETWEEN HLA-IDENTICAL SIBLINGS, T.Hoffmann, M.Theobald, D. Bunjes and W.Heit, Dep.of Internal Medicine III, University of Ulm, OE, 79 Ulm, FRG
We have simulated graft versus host and host versus graft reactivity in vitro by studying primary anti-minor H responses in a limiting dilution culture system. The ability of BMM and peripheral blood mononuclear cells (PBM) to stimulate and respond in this system were compared by estimating the number of proliferating cells. In GvH-direction the combination of donor-BMM (d-BMM) and host-PBM (h-PBM) was 2 to 15 times more effective in stimulating proliferation than any other combination; the same applied to the combination h-PBM/d-BMM in HvG-direction.- Using these combinations the median frequency of proliferating cells in GvH-direction was 1/5300 (range 1/590-1/18100) in 10 pairs, in HvG-direction (7 pairs) 1/2330 (range 1/115-1/6100). 85% of the proliferating cells had the phenotype of mature T-cells.- Using the same combination of responder/stimulator cells we have also estimated the number of cytotoxic cells specific for the HLA-identical target cell. In GvH-direction the median estimate (n=8) was 1/10300 (range 1/660-1/45700), in HvG-direction (n=5) 1/2250 (range 1/400-1/6500). By split well analysis similar or higher frequencies of cytotoxic cells with specificity for NK-targets were detected (GvHR: 1/5750, HvGR: 1/3620). It was however possible to identify a significant number of minor H-specific clones by segregation analysis; their specificity could be confirmed after clonal expansion. The clones had the phenotype of typical cytotoxic T-cells.- The relevance of the two cytotoxic subpopulations described above to clinical events such as GvHD, graft rejection and relapse needs to be clarified.-

C 126 MOLECULAR CLONING OF MURINE ICAM-1, K.J. Horley, B. Baker, and F. Takei, Terry Fox Laboratory, B.C. Cancer Research Centre; Departments of Microbiology and Pathology, University of British Columbia, Vancouver, B.C., Canada.
We have previously reported a novel cell surface antigen expressed on activated and proliferating murine lymphocytes. The antigen, termed MALA-2, is absent or present at low densities on thymocytes, lymph node cells, and fibroblast cell lines, indicating it is not a universal proliferation antigen. Some cells of the spleen and bone marrow express MALA-2 at a high density possibly representing in vivo proliferation in these tissues. MALA-2 has an apparent molecular weight of 95-100 kD under both reducing and nonreducing conditions, and is susceptible to Endo F digestion. The monoclonal antibody YN1/1.7 that reacts with this antigen, profoundly inhibits MLR. A λgt10 cDNA library was constructed from NS-1 cells that express a high level of MALA-2, and screened with synthetic oligonucleotides resulting in the isolation of a full length cDNA clone (~3.2 Kb). The cDNA sequence has high homology with the human ICAM-1 sequence, indicating that MALA-2 may be the murine homologue of this characterized protein. Studies are now underway to examine the adhesion properties of MALA-2, and its involvement in immune responses in vivo.

C 127 ET-5: A TUMOR THAT GENERATES ANTI-MINOR H IMMUNITY IN VIVO BUT FAILS TO PRIME FOR MINOR H-SPECIFIC CTL GENERATION IN VITRO. Lawrence L. Johnson and David L. Hines, Trudeau Institute, Inc., P.O. Box 59, Saranac Lake, NY 12983
A tumor cell line, ET-5, has been derived from an apparent fibrosarcoma that arose in a C57BL/6 male mouse. ET-5 expresses H-Y antigen and at least several other known minor H antigens. Mice that have rejected ET-5 become immune to these minor H antigens, judged by accelerated skin graft rejection, and this immunity can be transferred to immunodeficient mice with lymphoid cells. However, spleen cells from mice that have rejected ET-5 are not primed to generate CTL specific for H-Y or several other minor H antigens upon stimulation with antigen in vitro. This failure to be primed does not appear to be due to the induction of suppressor cells. Thus, ET-5 is immunogenic with respect to generation of anti-graft immunity in vivo, but is nonimmunogenic with respect to priming for CTL generation in vitro. This property suggests that Ly-2^+ CTL may be unnecessary for ET-5 rejection when H-Y is the target antigen, although Ly-2^+ cells are believed to be necessary for rejection of H-Y-incompatible skin grafts. ET-5 may be a useful resource for studying the properties necessary for cells to prime for CTL generation.

C 128 A NOVEL SUBSET OF CD2-CD3+ HUMAN T CELLS. **Dieter Kabelitz , Institute of Immunology, University of Heidelberg, D-6900 Heidelberg, Fed. Republic of Germany.**

According to the widely accepted view, CD2 (T11, sheep erythrocyte receptor) is the first T cell-specific antigen to appear on differentiating thymocytes during ontogeny. It follows that CD2 should be expressed on all immature and mature T cells. Using two-color cytofluorometry I have here identified subsets of CD2-CD3+ T cells both in fetal human thymus or spleen and in adult peripheral blood. CD2-CD3+ T cells constitute 1-25% of fetal thymocytes and 0.1-0.8% of peripheral blood T cells. IL-2-dependent longterm clones of CD2-CD3+ cells do not react with a panel of monoclonal antibodies (mab) directed against the $T11_1$, $T11_2$ or $T11_3$ epitopes of CD2 and do not transcribe CD2 mRNA. Fetal tissue-derived clones react with the TigammaA mab and thus express a functional TCR gamma chain, while CD2-CD3+ clones from peripheral blood are BMA031+ and express a full-length 1.3 kB TCR C_δ mRNA. The clones established here are currently being characterized with respect to functional capacities. I conclude that expression of CD2 is not an absolute prerequisite for the expression of the CD3/TCR molecular complex on human T cells.

C 129 THE MOLECULAR MECHANISM OF HUMAN DENDRITIC CELL INDUCED IMMUNE RESPONSES. David R Katz and Philip King, Department of Pathology, The Bland-Sutton Institute, University College and Middlesex School of Medicine, Riding House Street, LONDON W1P 7AA, United Kingdom.
It is becoming clear that a key determining factor in immunogenicity is the nature of the antigen presenting cell. Several studies have demonstrated that dendritic cells are the most potent antigen presenting cells which can be isolated from lymphoid tissues. Therefore the mechanism of dendritic cell function is of major significance in immunogenicity, but this mechanism is still poorly understood. To study this question we are studying the mechanisms of dendritic cell function using control B cell blasts (isolated from the same human tonsil) with respect to their capacity to interact with autologous T cells in oxidative mitogenesis and clustering assays. Thus, for example, monoclonal antibodies against CD11a and CD18, but not CD11b and CD11c, will inhibit both dendritic cell and B cell induced oxidative mitogenesis responses. These antibodies also inhibit clustering of T cells around presenting cells in the initial stage of the response, and have no effect if they are added after 24 hours. These interactions are bidirectional, since both CD11a and CD18, and their ligand I-Cam 1, are expressed on the presenting cells as well as the T cells. However, all such early adhesion related events are not bidirectional since anti-CD2 and anti-LFA-3, which are expressed differentially on T cells and presenting cells respectively are also effective as inhibitors. In contrast, anti class II MHC antibodies, anti CD4 and anti CD25 antibodies do not inhibit clustering but do inhibit proliferation, and this is seen irrespective of when the antibodies are added into the assay. Our findings suggest that there are two mechanisms involved in dendritic cell - T cell interaction, firstly an immediate cell-cell adhesion step and later a secondary signal transduction process possibly mediated via cytokines. The qualitative differences between dendritic cell and B cell induced immunogenicity may thus lie in either of these two steps.

C 130 CULTURED TISSUE IS CAPABLE OF STIMULATING AN IMMUNE RESPONSE WHEN TRANSPLANTED SYNGENEICALLY, Robert J. Ketchum and Orion D. Hegre, Dept. of Cell Biology and Neuroanatomy, University of Minnesota, Minneapolis MN 55455. Neonatal rat islets derived by culture-isolation have been shown to be free of MHC class II+ cells, and are immunologically silent when transplanted to either syngeneic or allogeneic hosts. Allogeneic transplantation of cultured neonatal non-islet pancreatic tissue, which is known to contain class II+ cells, results in rapid allograft rejection. Unexpectedly, syngeneic transplantation of cultured non-islet ductal tissue also resulted in mononuclear immune cell (MNC) infiltration of the graft in 86% of grafts examined. Highly purified syngeneic islets and ductal elements grafted syngeneically at remote sites display an immune response in the ductal element graft, while the islet graft is free of any immune cell infiltrate. This syngeneic immune response does not result from the use of xenogeneic serum in the medium, since cultures carried out using syngeneic rat serum supplemented medium yielded identical results. Uncultured neonatal pancreatic tissue grafted syngeneically does not result in MNC infiltrate, indicating this response is not to developmental antigen. This immune response to a syngeneic stimulus correlates with the presence of class II+ (antigen presenting) cells. In grafts free of class II+ cells (culture-isolated islet grafts) no immune response to syngeneic stimulus was observed, while a response was present when syngeneic ductal elements, known to include class II+ cells, were grafted. This indicates a need for cells capable of antigen presentation to stimulate this syngeneic response, and suggests that either a modified self antigen or a normally sequestered antigen is being presented. This syngeneic immune response demonstrates many of the same characteristics of, and may be analagous to, the *in vitro* syngeneic, or autologous, mixed lymphocyte reactions.

C 132 THE PRESENCE OF "SELF" MHC CLASS II (HLA-DR) ANTIGENS DETERMINES WHETHER BLOOD TRANSFUSIONS IMMUNISE OR SUPPRESS. EL Lagaaij, A Termijtelen, E Goulmy, & JJ Van Rood, Leiden University Hospital, The Netherlands. Blood transfusions can immunise the recipient, as well as induce prolonged allograft survival. It is not known what makes that some transfusions immunise the recipient whereas others induce immune suppression. We investigated if certain MHC compatibilities or differences between recipient and transfusion donor and organ donor are required to induce the beneficial "transfusion effect" in man. We studied graft survival and blood transfusion induced changes in cellular and humoral immunity in 4 different patient groups. The patients received a single blood transfusion of a randomly choosen donor.
We found in all 4 groups that to induce a beneficial "transfusion effect" compatibility for at least 1 HLA-DR antigen between recipient and transfusion donor is required. If the transfusion and recipient are mismatched for both HLA-DR antigens, the recipient is immunised, resulting in an increased antibody production (P=0.001), an increased cytoxicity (CML) (P=0.005), an increased mixed lymfocyte reaction (MLR) (P=0.008) and a decreased graft survival (P=0.003). After a beneficial (HLA-DR sharing) transfusion, the in vitro test remain unchanged or decrease. Graft survival increases with the number of shared antigens between transfusion donor and organ donor (P=0.02), suggesting that a donor specific suppression is induced.

C 133 RECOMBINANT gp120 WILL INHIBIT THE FUNCTIONAL INTERACTION BETWEEN CD4 AND HUMAN MHC CLASS II ANTIGENS, D. Lamarre, D. Capon* and R.P. Sekaly, Clinical Research Institute of Montreal, Canada; *Genentech, San Francisco, CA, USA. CD4 T lymphocytes interact predominantly with target cells expressing class II MHC antigens. Recent experiments have revealed a direct interaction between the CD4 molecule and HLA-DR antigen. To address the nature of this interaction we have used a xenogeneic system in which a human CD4 cDNA was expressed in the murine CD4- and CD8-negative hybridoma 3DT52.5.8. The TcR of 3DT52.5.8 recognizes the murine class I molecule D^d. A class II expressing D^d-positive cell line was obtained by cotransfection of the human class II cDNAs together with the murine D^d gene into the murine fibroblast line DAP3. Coculture of 3DT52.5.8 and DAP3 expressing DP-D^d resulted in a 20 fold increase in IL-2 production and in rosette formation only when both CD4 and DP were present on the responding T hybridoma and the presenting cell, respectively. We are using this system to map regions of the CD4 molecule that interact with the class II MHC Ag. The CD4 molecule has also been shown to be the receptor for the human immunodeficiency virus (HIV) via the gp120 molecule. Since gp120 and class II both interact with CD4, we have used our functional assay to verify if gp120 exerts an inhibitory function on CD4 class II interaction. Recombinant gp120 inhibits the functional interaction and rosette formation in a concentration dependent fashion with maximal inhibition at about 10 µg/ml. This inhibition is specific since it can be reversed by recombinant soluble CD4. The fact that recombinant gp120 can inhibit the functional interaction between CD4 and its physiological ligand (class II Ags) suggests that the use of gp120 on a vaccine against HIV infection could alter the immune response of such individuals. This work was supported by SRC, MRC and NCI.

C 134 *RESIDUES ON CLASS I CRITICAL FOR RECOGNITION BY ALLOREACTIVE CTL*, Linda C. Lowen and James Forman, Department of Microbiology, University of Texas Southwestern Medical Center at Dallas, Dallas, TX 75235

T lymphocytes discern self from non-self molecules through the interaction of their antigen-specific receptors and proteins encoded by the MHC. Although the nature of this association is not well-defined, a model has been proposed whereby the v-segments of the T cell receptor interact with residues along the 2 alpha helices of the class I antigen (Davis *et al.*; Nature 334:395, 1988). We have recently shown that CTL generated against the class I molecule $Q10^d$ crossreact on several unrelated murine class I antigens containing the shared $Q10^d$ residues at amino acid positions 152, 155, and 156 (Mann *et al.*; JEM 168:307, 1988). These residues contributed by the α-2 domain occur in the alpha helical portion of the class I molecule and amino acids 152 and 155 could interact directly with the T cell receptor. To further characterize the role of these amino acids, we are in the process of determining whether insertion of these 3 residues by site-directed mutagenesis into a human class I molecule will allow for the antigen's recognition by anti-Q10 CTL.

C 135 T CELL RECOGNITION SITES ON CLASS II, I-Aβ^d CHAIN INVOLVED IN SYNGENEIC AND ALLOGENIC RESPONSE, M. Manickasundari, Z. Novak, E. Fraga and B. Singh, Dept. of Immunology, University of Alberta, Edmonton, Alberta Canada T6G 2H7

T cells recognise foreign antigen in association with class II (Ia) molecules on the surface of antigen presenting cells. In MLR, T cells recognise Ia molecules in the absence of nominal antigen. The exact mechanism underlying this phenomenon at the molecular level is not clear. In this study, we have identified the functional sites on class II MHC antigens in MLR by using the synthetic peptides of I-Aβ^d from H-2^d mice. We have carried out in vitro T cell proliferation assay and antigenic competition assays for this study. The results suggest that three regions on the I-Aβ molecule namely 1-14, 21-36 or 29-46 and 62-78 induce in vitro T cell proliferation in secondary MLR and SMLR after in vivo priming with the peptides. The same sequences are found to block the IL-2 secretion from alloreactive and autoreactive T cell hybridoma by antigen competition experiments. These results suggest that the autoreactive and alloreactive T cells recognise multiple sites on I-Aβ^d in MLR and SMLR. These results indicate that the same regions of I-Aβ^d are involved in inducing both alloreactive and autoreactive T cell hybridoma. Results from in vivo studies suggest that the 62-78 region of I-Aβ^d may be the most dominant region for T cell recognition in MLR.

C 136 RECOGNITION OF OLIGONUCLEOTIDE-ENCODED T CELL EPITOPES EXPRESSED IN THE CONTEXT OF A PROTEIN NOT RELATED TO THE ORIGINAL ANTIGEN, Janet L. Maryanski[1], Giovanna Chimini[2], Pietro Pala[1], Josephine Sire[2], and Bertrand R. Jordan[2], 1) Ludwig Institute for Cancer Research, Lausanne branch, 1066 Epalinges, Switzerland, and 2) Centre d'Immunologie de Marseille-Luminy, France. Antigen recognition is currently thought to involve the presentation of degraded protein fragments by class I or class II MHC molecules to the T cell antigen receptor. We have previously shown that H-$2K^d$-restricted CTL specific for HLA-CW3 or HLA-A24 can recognize peptides corresponding to residues 170-182 of the HLA molecules. We have now inserted synthetic oligonucleotides theoretically encoding the CW3 or A24 peptides into an unrelated gene, the influenza nucleoprotein (NP). We found that P815 (H-2^d) cells transfected with the NP-oligo recombinant genes were specifically lysed by HLA-specific CTL. Our results imply that there must be a high degree of flexibility for the expression of T cell epitopes in different molecular contexts.

C 137 MHC-RESTRICTED IMMUNOLOGIC TOLERANCE AND THYMIC ANTIGEN PROCESSING: AGGREGATION CHIMERAS DO NOT REJECT F1 SKIN GRAFTS BEARING "HYBRID" COMBINATIONS OF MHC AND MINOR HISTOCOMPATIBILITY ANTIGENS, Jim McCarrick and Barbara Knowles, The Wistar Institute, 36th & Spruce Sts., Philadelphia, PA 19104

Many of the events critical to the maturation of T lymphocytes occur in the thymus. Here the T cell repertoire becomes restricted, so that foreign antigen can be recognized only when associated with the MHC products of the host, and mature T cells are tolerized to self antigens, a process which also seems to be MHC-restricted. Thus, T cells should be non-reactive to self antigens when they are associated with MHC products present on the tolerance-inducing thymic cells, whereas they may still react to the same self antigens when associated with different MHC products. To examine MHC-restricted tolerance *in vivo*, a model system must have: a) self antigen in the context of one MHC haplotype, and b) tolerance to both that and a second MHC haplotype. Chimeras were prepared by aggregation of preimplantation embryos of two strains of mice, C57BL/6 (B6) and BALB/c. The thymus of such chimeras should be composed of two distinct and completely intermixed populations of cells, one from each parental strain (isozyme analysis indicates no detectable fusion of cells). Thus, T cells maturing in the chimeric thymus should be exposed to and tolerized to minor histocompatibility antigens (mHAs) of one parental strain only in association with the MHC of that strain. For example, mice might be expected to express B6 mHAs only with H-2^b (the B6 MHC).

However, our chimeras were fully tolerant to F1 skin grafts, which have "hybrid" combinations of mHAs and MHC (e.g., B6 mHAs with H-2^d). These results are most consistent with either, a) "wholesale" antigen processing and presentation of all mHAs by the tolerizing thymic cells, and/or, b) functional sharing of MHC products between the parental thymic cell populations.

C 138 MECHANISM OF SUPERIOR ANTIGEN PRESENTATION BY DENDRITIC CELLS (DC). C.J.M. Melief, C.J.P. Boog, J. Boes, A. Voordouw, W.M. Kast. Div. of Immunology, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands.

Under conditions of conventional antigen presentation most $CD8^+$ cytotoxic T-cell (T_C) responses, including those across minor or major histocompatibility differences are greatly augmented by $CD4^+$ T helper (T_h) cells. Certain MHC types do not permit effective antigen presenting cell (APC) - T-cell interactions, resulting in specific T-cell response defects. In the case of T-cell responses against viruses such response defects are associated with a marked increase in disease susceptibility as illustrated by class I MHC controlled susceptibility to lethal pneumonia induction by Sendai virus. Certain class I or class II MHC determined T_C response defects (four out of six tested by us) can be restored by immunization in vivo and/or restimulation in vitro with DC. DC are the most effective APC. Their superior APC capacity is due to 1) a very high absolute number of class I and class II MHC molecules, and 2) a low degree of sialylation of MHC and other surface molecules, reducing negative charge and facilitating access of the T-cell receptor to the MHC groove presenting the antigenic peptide and/or improved clustering with T cells. The more effective antigen presentation by DC allows a more prominent role for a $CD4^+$ T_h cell independent pathway of $CD8^+$ T_C activation. It is postulated that the more effective direct triggering of $CD8^+$ T_C precursors lowers the threshold for IL-2 production by $CD8^+$ cells, reducing the requirement for IL-2 production by the $CD4^+$ cells. Failure of DC to overcome certain MHC-linked specific T_C response defects probably reflects complete failure of any foreign peptide derived from the processed antigen to interact efficiently with the MHC or a true T_C repertoire defect.

C 139 A CLASS OF MUTANT B-LCLS PRESENT PEPTIDE, BUT NOT WHOLE ANTIGEN TO HLA CLASS II RESTRICTED T CELLS, Elizabeth Mellins, Benjamin Arp, Esteban Celis and Donald Pious, Department of Pediatrics, University of Washington, Seattle, WA 98195

MHC class II molecules bind immunogenic peptides derived from soluble antigen and the complex is recognized by specific T cells. We have isolated eight independent mutant B-LCL clones which are altered in their ability to present antigen. In standard proliferation assays using four different soluble protein antigens, the mutants are unable to stimulate the majority of T cell clones restricted to HLA DR or DP. Although unable to present whole Hepatitis B surface antigen (HBsAg), they effectively present a HBsAg peptide to a DP-restricted T cell clone.

The fact that both DR and DP restricted antigen presentation is abnormal in these mutants made it likely that the class II structurual genes are unaltered. This hypothesis is supported by the finding that DNA sequences from the DR genes of one mutant are normal. However, two observations indicate that the mature class II dimers expressed by the mutants are structurally altered. Binding to the mutants with two polymorphic anti-DR antibodies and one anti-DP antibody is reduced, although the level of cell surface class II expression is normal. Second, the class II dimers from the mutants dissociate into monomers under in vitro conditions (SDS-PAGE) which preserve dimers in the progenitor line. Together, these functional and structural data suggest that the mutants are defective in a molecule that either associates with or post-translationally modifies class II molecules and is required for the physiologic formation of an MHC/antigen complex.

C 140 EXPRESSION OF MODIFIED Qa-2 ANTIGENS IN TRANSGENIC MICE, Andrew L. Mellor, Jane Antoniou, Peter Tomlinson, Erin Lovering, Elisabeth Simpson[1], Philip Chandler[1], Peter Robinson[1] and Patricia M. Taylor. Division of Immunology, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, U.K. and [1]Transplantation Biology Section, Clinical Research Centre, Watford Road, Harrow, Middlesex HA1 3UJ, U.K.

Mouse Qa-2 antigens are encoded by MHC class I genes but, in contrast to H-2 antigens, they do not function as restriction elements presenting foreign antigens to T-cells. To investigate the nature of this functional defect we have constructed 3 different recombinant class I genes using DNA segments from the B10 Q9 (Qa-2) or H-2D^b genes. In each case the structural protein encoded by the recombinant genes is derived entirely from the Q9 gene whereas the cis-acting transcriptional regulatory elements or the DNA segment encoding the membrane anchoring domain is derived from the H-2D^b gene. Each DNA construct was introduced into fertilised CBA/Ca embryos by microinjection and transgenic lines were established. To date we have established 13 transgenic lines. We have shown that the Q9 (Qa-2) antigen encoded by the recombinant genes behaves as a major transplantation antigen in skin grafts and provokes strong secondary cytotoxic T-cell responses in grafted animals irrespective of the tissue distribution or mode of membrane anchorage of the Q9 antigen. At present, we are investigating whether the Q9 antigens encoded by the recombinant genes are able to present influenza virus or mouse minor histocompatibility antigens to T-cells during immune responses and hence whether they can function as restriction elements.

C 141 DISTINCTIVE FEATURES OF DENDRITIC CELLS AND ANTI-IMMUNOGLOBULIN ACTIVATED B CELLS AS STIMULATORS OF THE MIXED LEUKOCYTE REACTION, Joshua P. Metlay, Ellen Pure' and Ralph M. Steinman, Department of Cellular Physiology and Immunology, Rockefeller University, New York, NY 10021. Stimulation of the primary mixed leukocyte reaction [MLR] is a distinctive system because it permits an analysis of the activation requirements for antigen specific, resting T cells. Highly enriched populations of B lymphoblasts have been isolated following culture with anti-Ig-sepharose and compared to dendritic cells as stimulators of CD4$^+$ T cells in the MLR. Both cells expressed comparable levels of class II MHC products and independently stimulated the 1° MLR and the production of several T derived lymphokines, including IL-2 and Il-4. However, the relative potencies of dendritic cells and anti-Ig blasts as 1° MLR stimulators varied in a strain dependent fashion. Only anti-Ig blasts could stimulate across an Mls barrier, being at least 100 times more active in stimulating Mls-mismatched, MHC-matched T cells, relative to syngeneic T cells. In contrast, dendritic cells were 10-30 times more potent than the anti-Ig blasts when stimulating across an MHC barrier and were likewise more effective in binding MHC-disparate T cells to form the clusters in which the MLR was generated. Dendritic cell-T cell clustering was resistant to anti-LFA-1 mAb, while B blast-T cell clustering was totally blocked. Thus, anti-Ig B lymphoblasts and dendritic cells, two cell types which differ markedly in phenotype, also differ in efficiency and mechanism for initiating responses in allogeneic T cells.

C 142 IN VITRO ALLOSTIMULATION WITH CHEMICALLY MODIFIED CELLS: REQUIREMENT FOR ANTIGEN PROCESSING BY STIMULATOR CELLS PRIOR TO TREATMENT, Milcho St. Mincheff and Harold T. Meryman, Transplantation Laboratory, American Red Cross, Rockville, MD 20855. Treatment of stimulator cells with 0.1% paraformaldehyde for 1 minute eliminates their ability to elicit T-cell proliferation in a primary mixed lymphocyte reaction (MLR). However, prior incubation of the cells at 37^0 for 18 hours in complete medium results in T-cell proliferation equal to 40-50% of unfixed, control cells despite subsequent chemical modification. The incubation also induces a one log increase in the intensity of fluorescence when the cells are stained with monoclonal antibodies against class II molecules DR and DP as well as the lymphocyte function antigen 3 (LFA-3) and the intracellular adhesion molecule (ICAM). We interpret this as an increase in the membrane expression of these structures following incubation. The increase is blocked by the translation inhibitor, cycloheximide, implying that protein synthesis is involved. Chlororquine and cerulenin, known to inhibit protein degradation and antigen processing and presentation do not influence the upregulation in membrane expression of these class II and adhesion molecules, but do prevent incubation from overriding the effect of paraformaldehyde treatment. We propose that incubation of stimulator cells in the presence of these inhibitory compounds results in the membrane expression of class II molecules without associated peptides. The inability of stimulator cells expressing such nude molecules to elicit T-cell proliferation after chemical modification could be due to easier crosslinking of the allodeterminants by paraformaldehyde when the binding site is empty, but could also mean that "nude" MHC molecules are not per se immunogenic and become so only after acquisition of a peptide.

C 143 DEVELOPMENTAL ACQUISITION OF B CELL ANTIGEN PROCESSING AND PRESENTATION. Jennifer F. Morris and Susan K. Pierce. Dept. of Biochem. Molec. Biol. Cell. Biol., Northwestern University, Evanston, IL 60208. Helper T cell responses to soluble globular proteins require processing of the protein by Ia-expressing antigen presenting cells (APC). Antigen is internalized into acidic vesicles, proteolyzed, and peptides containing T cell antigenic determinants are transported to the APC surface where they are recognized by the antigen-specific T cell in conjunction with Ia. Most Ia-expressing cells are competent APC, however, only B cells have antigen-specific receptors on their surface allowing bound antigen to be processed and presented at 1/1000 the antigen concentration required by nonspecific APC. Little is known about B cell antigen processing function during differentiation, or if Ig-mediated APC function is altered at different maturational stages, thus allowing regulation of B cell-helper T cell interactions. Neonatal acquisition of APC function was examined in mice ages day 1 to day 15. Splenic cells from d1 to d10 mice process and present pigeon cytochrome c, Pc, at 0-20% of adult levels. By d15 neonatal spleen cells acquire the ability to process and present soluble Pc, at 35% of adult levels. The ability to internalize antigen through Ig receptors was determined using an antigen-antibody conjugate, Pc-αF(ab')$_2$. Neonatal spleen cells acquire the ability to process antigen through Ig simultaneously with the ability to process soluble antigen. Lack of processing by neonatal spleen cells prior to d10 is not attributable to insufficient levels of surface Ia, since d3 neonate spleen cells are able to activate T cell hybrids to 50% of adult levels when provided with Pc 81-104, containing the T cell determinant. D10-d15 neonate presentation of Pc81-104 is indistinguishable from adult levels. B cell maturation into memory B cells was identified by the loss of the J11d differentiation marker. Splenic J11d^{lo} B cells increase from 5-30% following immunization and return to nonimmune levels after 4 weeks. During antigen-induced B cell maturation, J11d^{lo} B cells are indistinguishable from splenic B cells in the ability to present antigen introduced into the processing pathway either pinocytotically or via surface Ig. Pc-antibody conjugates specific for mouse F(ab')$_2$ IgM, IgD, or IgG are presented equally well by both splenic and J11d^{lo} B cells. Thus, acquisition of B cell processing function appears to be developmentally regulated and may play an important role in B cell tolerance mechanisms. Once B cells have acquired the ability to process antigen this function is maintained and is not regulated during maturation into memory B cells. We are currently investigating the role of Ig isotype during neonatal acquisition of antigen processing. (Supported by NIH grants AI-18939, AI-12001, and AI-2317)

C 144 TYPE 3 PNEUMOCOCCAL POLYSACCHARIDE (S3)-SPECIFIC T CONTRASUPPRESSOR (Tcs) AND T SUPPRESSOR (T_S) CELLS ARE ACTIVATED BY ANTIGEN PRESENTED BY DISTINCT CELL TYPES. Helen Braley-Mullen, University of Missouri, Columbia, MO 65212.
Previous studies have shown that S3 coupled to syngeneic spleen cells (S3-SC) can activate both T_S and Tcs. Activation of T_S by S3-SC requires I-J identity between carrier SC and T_S donors whereas Tcs activation requires I-A identity between the SC and Tcs donors. These observations suggested that the carrier SC were functioning to present S3 to T_S and Tcs since they were not being re-presented by antigen presenting cells (APC) present in the T cell donors. As the preliminary studies suggested that carrier SC (APC) for T_S vs. Tcs activation might be distinct, studies were done to directly address this possibility by assessing the ability of S3 coupled to various cell populations to activate T_S and Tcs. The results indicated that T_S activation required that S3 be coupled to plastic adherent cells which bear both I-A and I-J determinants. These cells are nonadherent to anti-Ig and nonfunctional in cyclophosphamide (Cy) treated mice. In contrast activation of Tcs required coupling of S3 to plastic non-adherent and anti-Ig adherent cells. These cells are functional in Cy treated mice and bear the B cell markers J11d and I-A but not I-J. Thus S3-specific T_S are activated by I-A+ I-J+ adherent cells (presumably macrophages) whereas Tcs are activated when antigen is presented by B cells. (NIH Grant CA25054.)

C 145 BIOCHEMICAL AND CELL BIOLOGICAL PARAMETERS OF PEPTIDE ANTIGEN UPTAKE AND RETENTION[1]. Roderick Nairn, Maxine J. Solvay & David W. Thomas[2], Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, MI 48109-0620. Recent evidence supports the hypothesis that peptide antigens associate directly with MHC molecules. However, there may be other membrane structures involved in peptide antigen uptake and retention. Our studies have identified a class of membrane-bound proteins which form stable associations with a number of peptide antigens. These membrane-bound proteins, which we have termed specific antigen retention structures, have been shown to be important for T cell responses. They are found on a variety of cell types, suggesting cellular heterogeneity, and form complexes with a variety of peptides, suggesting molecular heterogeneity. In some cases a covalent bond is formed between the retention structure and the peptide antigen. The existence of a free unmodified COOH terminal group appears to be critical for this covalent bond formation. Recent information, derived from both biochemical and EM autoradiographic studies, suggests that these antigen-retention structures are rapidly turned over in cells and may transport peptide antigens into various subcellular compartments, perhaps thereby facilitating their interaction with MHC molecules. [1]Supported by grants from the NIH and ACS. [2]Present address for Dr. Thomas is Biogen, Cambridge, MA 02142.

C 146 IDENTIFICATION OF TUMOR MEMBRANE PROTEINS INVOLVED IN INTERLEUKIN-2 ACTIVATED KILLER CELL-TARGET RECOGNITION. Allen J. Norin, Victor A. Hatcher, Departments of Medicine, SUNY Health Science Center, Brooklyn, NY 11203, Albert Einstein College of Medicine, Montefiore Med. Ctr., Bronx, NY. Interleukin-2 activated killer (IAK) lymphocytes (also known as LAK cells) which destroy a broader spectrum of tumors invitro than NK cells have been used sucessfully in an adoptive immuno-therapy protocol for the treatment of patients with a variety of advanced cancers. The cell suface molecule(s) on tumor cells that are involved in specific binding to IAK cells and in programming IAK cells for cytolysis (IAK acceptor molecules) have not been characterized. Inorder to identify such acceptor molecules a crude membrane digest of the lung carcinoma cell line A549 was biotinylated and adsorbed to IAK cells or to unstimulated human peripheral blood lymphocytes (UPBL) (each from the same person). Proteins from the washed solubilized cells were separated by PAGE, Western Blotted and probed with streptavidin-alkaline phosphatase. Several experiments demonstrated that different tumor membrane proteins bound to IAK cells compared to UPBL. The unstimulated cells bound one tumor membrance protein (about 40KD) not found on the IAK-adsorded blot. The IAK cells bound three tumor proteins (approximately 30,46 & 50KD) not found on the UPBL-adsorded blot. Three other proteins (about 35,44 & 55KD) were found to adhere equally well to IAK cells and UPBL. Utilizing a streptavidin affinity column, solubilized tumor membrane proteins that bound to IAK cells could be separated from solubilized IAK membrane proteins. The isolated tumor membrane proteins that adsorded to IAK cells inhibited IAK mediated lysis of A549 tumor cells by >85%. These studies suggest that specific cellular adsorption techniques may be useful in isolating and characterizing tumor membrane proteins involved in interactions unique to cytolytic lymphocyte-tumor cell target binding and lysis.

C 147 INHIBITION OF THE CD2 MEDIATED ACTIVATION OF HUMAN T CELLS BY ANTI-CD4 MONOCLONAL ANTIBODIES. Chantal Cerdan, Jacques Nunès, Marc Lopez, Anne Pierrès, Daniel Olive and Claude Mawas. INSERM U.119, 27, Bd Leï Roure, 13009 Marseille, France.
Activation of human T lymphocytes occurs via the T cell receptor-CD3 complex but can also be induced through the non-antigen-specific CD2 molecule. Selected combinations of mAbs or the soluble CD2 ligand, namely LFA-3 and a unique anti-CD2 mAb (CD2.1) induce human T cell activation. CD4 is an accessory molecule implicated in the activation of human T lymphocytes. This molecule may exert this function by increasing intercellular avidity through binding to MHC class II molecules and/or by transmitting intracellular signals. We have investigated the action of mAbs directed against different epitopes on the CD4 molecules in the activation of human T cells via the CD2 pathway. We show that anti-CD4 mAbs inhibit CD2 induced T cell proliferation in an epitope-dependent fashion. This inhibition does not appear to be linked to the lower CD2 mediated [Ca^{2+}] response induced by anti-CD4 mAbs, since [Ca^{2+}] response is equally affected by anti-CD4 mAbs whether or not they inhibited T cell proliferation. In conclusion, the partial inhibition of the CD2 induced [Ca^{2+}] response of T cells by various anti-CD4 mAbs suggest that : 1) this inhibition does not totally account for the inhibitory effect of anti-CD4 mAbs, 2) and the proliferation induced by anti-CD2 mAbs may not be completely ascribed to the [Ca^{2+}] response of T cells.

C 148 ANTIGEN PRESENTATION TO T_H CLONES BY SUBPOPULATIONS OF PULMONARY FIBROBLASTS, Richard P. Phipps, D. Penney, P. Keng, H. Quill, A. Paxhia, S. Derdak and M. Felch, Cancer Center and Depts. of Microbiology & Immunology, Radiation Oncology, Medicine and Pathology and Laboratory Medicine, University of Rochester School of Medicine, Rochester, NY 14642

Excessive collagen production by pulmonary fibroblasts, which is associated with tissue injury and chronic inflammation, may result from the expansion or altered function of one or more subpopulations of fibroblasts. We have investigated whether murine lung fibroblast subpopulations could be identified using surface markers. Stable lines of morphologically distinct lung fibroblasts have been generated based on the presence or absence of Thy-1. These lines secrete collagen and lack the surface markers of dendritric cells, macrophages, B cells and T cells (with the exception of Thy-1). Interestingly, only the Thy-1 negative subpopulation was found to display Class II MHC in response to gamma-interferon. After treatment with interferon and pulsing with antigen, the Thy-1 negative population induced T_H clones to proliferate and secrete lymphokines. These observations indicate that subpopulations of lung fibroblasts can be identified and separated based on surface marker expression. Furthermore, the Thy-1 negative subpopulation, with its ability to present antigen and stimulate T cells, may play a key role in promoting pulmonary inflammation and fibrosis. Supported by HL-39949, CA-42739, 5-P30-CA-11198 and 5-T32-HL07216.

C 149 A SINGLE AMINO ACID SUBSTITUTION IN THE α3 DOMAIN OF AN H-2 CLASS I MOLECULE ABROGATES REACTIVITY WITH CD8 DEPENDENT, BUT NOT CD8 INDEPENDENT, CTL. Terry A. Potter*, and Jeffrey A. Bluestone+, *Division of Immunology, Department of Medicine, National Jewish Hospital, Denver CO 80206, and the +Department of Pathology, University of Chicago, ILL 60637. We isolated by immunoselection, a somatic cell mutant which expressed a D^d molecule that failed to express a serological determinant (34.2.12) which had been mapped to the α3 domain while retaining all of the serologically defined α1 and α2 determinants. This somatic cell mutant was not killed by anti D^d reactive CTL. We have now determined that exon 4 of the mutant D^d gene has undergone a single mutation resulting in a glutamic acid to lysine substitution at residue 227 in the α3 domain. Using oligonucleotide directed mutagenesis, we have reproduced this mutation in the cloned H-$2D^d$ gene. Cells transfected with this gene are not recognized by alloreactive anti D^d CTL generated in an in-vitro primary response. A hybrid gene consisting of the exons encoding the α1/α2 domains of H-$2K^b$ together with the α3 domain of the mutant H-$2D^d$ gene was constructed and transfected into recipient cells. Cells expressing the product of this mutant gene were not killed by anti H-$2K^b$ CTL generated in a primary in-vitro response. Anti H-$2K^b$ CTL that were not inhibitable with anti CD8 antibody were generated from the spleens of mice primed in vivo and restimulated with H-$2K^b$ in vitro in the presence of CD8 antibody. These CTL populations, in contrast to the CTL generated in the primary in vitro response, readily killed transfectants expressing the product of the hybrid H-$2K^b$(α1\α2)\mutant H-$2D^d$ gene.

These observations raise the possibilty that residues in the α3 domain of H-2 class I molecules contribute to the determinant recognized by the CD8 molecule of alloreactive CTL.

C 150 INDUCTION OF SUPPRESSOR CELLS FOLLOWING IN VIVO EXPOSURE TO Qa-1 ALLOANTIGEN IN MICE. Michael A. Rees, Amy S. Rosenberg, and Alfred Singer, Experimental Immunology Branch, NCI, NIH, Bethesda, MD 20892. We have devised a model to study the in vivo generation of suppressor cells by using mice congenic at Qa-1, a class I-like molecule encoded to the right of H-2D. We found that B6 mice did not reject Qa-1 disparate tail skin grafts (TSG) unless a second graft with additional helper determinants was also present. However, animals engrafted with a Qa-1 graft alone, without any source of additional help, failed to reject their Qa-1 graft, and were unable to reject them even upon the subsequent addition of exogenous help. Thus, exposure to Qa-1 disparate grafts, in the absence of additional help, either led to Qa-1 specific tolerance or suppression. Adoptive transfer studies with spleen cells from mice failing to reject Qa-1 allografts revealed the presence of Qa-1 specific suppressor cells that inhibited the in vivo activation of antigen specific effector cells capable of rejecting Qa-1 bearing allografts. Additional adoptive transfer experiments using T cell subpopulations should allow for further characterization of these Qa-1 specific suppressor cells.

C 151 CULTURE OF MURINE THYMUS EPITHELIAL CELLS IN DEFINED SERUM-FREE MEDIUM, Carsten Ropke, Ole W. Pedersen and Bo van Deurs, Department of Anatomy A, The Panum Institute, University of Copenhagen, DK-2200 Copenhagen N, Denmark. In the thymus, the T-cell receptor genes are rearranged, the T cells learn to recognize their own major histocompatibility complex (MHC), and they learn to respond to foreing MHC. These events seem to be linked to the interaction between T-cell precursors and the stromal cells of the thymus. Thus increasing evidence points to an essential role for the thymus epithelial cells (TE cells) in development of at least MHC class II recognition by the T cells. To be able to study the importance of TE cells in T cell maturation, we have developed a method for growing murine T cells in serum-free medium with well defined constituents. The medium allows for growth of TE cells without concomitant growth of bone marrow derived cells as macrophages and fibroblasts. Data obtained by EM and immunocytochemistry showing the epithelial nature of the cultured cells, as well as autoradiographic data on the growth pattern, and characterization of TE cell supernatants will be provided in addition to results obtained from co-culture of TE cells and T-cell precursors ($CD4^-CD8^-$thymocytes).

C 152 REJECTION MECHANISM OF Ia DISPARATE SKIN ALLOGRAFTS BY $CD4^+$ T CELLS, Rosenberg, A.S., Katz, S.I., and A. Singer, Experimental Immunology Branch and Dermatology Branch, National Cancer Institute, Bethesda, MD 20892

The effector limb mediating skin allograft rejection is highly antigen specific, rejecting cells that express allogeneic MHC antigens while sparing those which fail to express allogeneic MHC determinants. Paradoxically, Ia disparate skin grafts are completely rejected in spite of the fact that only a small percentage of the cells within the graft express Ia antigens. Thus, it is possible that MHC class II disparate grafts are rejected by a mechanism that does not assess the expression of MHC determinants on each cell. We assessed the specificity of the rejection of Ia disparate grafts by using allophenic skin grafts in an adoptive transfer system and concluded that skin graft rejection across an MHC class II disparity required recognition of allo-Ia determinants expressed by every cell in the graft. Therefore, we reasoned that MHC class II antigens must be induced on these Ia negative populations. Indeed, injection of mice with gamma interferon dramatically induced Ia antigens on previously negative keratinocytes. We next tested whether the induction of allogeneic Ia determinants on keratinocytes was necessary for graft rejection by engrafting parental strain mice with skin from F_1->parent bone marrow chimeras. Such grafts failed to be rejected, in spite of the specific rejection of the allogeneic Langerhans cells, indicating that the failure of keratinocytes to express allogeneic class II determinants leads to graft preservation. In conclusion, MHC class II disparate skin allografts are rejected in a highly antigen specific fashion, secondary to the induction of MHC class II antigens on skin cells that fail to constitutively express them.

C 153 HLA-Aw68.1 DOES NOT BIND CD8 DUE TO A UNIQUE SUBSTITUTION AT POSITION 245. Russell D. Salter*, Ann M. Norment+, Carol Clayberger#, Alan M. Krensky#, Dan R. Littman+, and Peter Parham*, Departments of Cell Biology* and Pediatrics#, Stanford Medical School, Stanford, CA, 94305, and Department of Microbiology and Immunology+, UCSF Medical School, San Francisco, CA, 94143. A cell-cell adhesion assay was recently developed in Dan Littman's laboratory which directly demonstrated binding of CD8 alpha chain to HLA class I molecules. To address whether the extensive polymorphism characteristic of class I molecules influences CD8 binding, we have screened a panel of transfectants expressing individual class I MHC alleles. Of 18 alleles tested, only Aw68.1 did not bind. All other molecules did bind, including A2.1 and Aw69, which differ by 13 and 7 amino acids respectively from Aw68.1. Position 245 in the alpha 3 domain was identified by site-directed mutagenesis as the critical residue differing between A2.1 and Aw68.1 which determines binding. A mutant Aw68.1 molecule containing alanine at position 245 bound CD8, while a mutant of A2.1 with valine at 245 did not. Alanine is found at position 245 of all human and murine class I molecules sequenced to date except Aw68.1 and Aw68.2 , which have valine at that position. Bulk cultures of A2-allospecific CTL were also sensitive to this substitution, and preferentially recognized both molecules with alanine at 245. This study shows that Aw68.1 differs from other class I molecules in its capacity to bind CD8, and raises the possibility that Aw68.1 may not function as a restriction element as effectively as other class I alleles.

C 154 B CELL-LIKE ANTIGEN PRESENTING CELLS CANNOT TRIGGER HEPATITIS B SPECIFIC T CELL HYBRIDOMAS, Jean-Pierre Y. Scheerlinck†, Lea Brys†, Guy Burssens†¶, Pierre Hauser¶ and Patrick De Baetselier†, †Department of General Biology, Free University of Brussels, Paardenstraat 65, 1640 St Genesius Rode, ¶Biological division, Smith-Kline RIT, rue de l'Institut 89, 1330 Rixensart, Belgium.

T cell hybridomas were generated against Hepatitis B SpreS2 particles via cell fusion of the BW5147 T cell lymphoma with sensitized lymphnode cells (LNC) (BALB/c, H-2^d and CBA, H-2^k). T cell hybridomas derived from H-2^d LNC recognised S and preS2 antigens in a I-A^d restricted way, while T cell hybridomas from H-2^k LNC manifested a specificity for either preS2 in association with I-A^k or for S in association with I-E^k. The activation of these hybridomas by antigen and antigen presenting cells (APC), as measured by IL-2 secretion, was found to be sensitive to prostaglandines and could be completely inhibited by anti-LFA-1 monoclonal antibodies. Different APC populations were tested for their capacity to present SpreS2 particles to these T cell hybridomas. Various macrophage-like populations such as resident, Con A induced, Thioglycolate induced peritoneal exudate cells as well as splenic adherent cells were found to present efficiently the SpreS2 antigen. In contrast B cells and Ia+ B cell lines (TA3, M12.4) could not function as accessory cells in the SpreS2 specific stimulation of these T cell hybridomas. The inability of these cells to present this antigen was not due to inhibitory effects since these cells did not inhibit the presentation capacity of other potent APC's. Furthermore the addition of APC's of a different haplotype could not complement for the defective presentation of SpreS2 by B cells and B cell lines indicating that MHC independent accessory factors are not implicated in this process. Hence it is clear that macrophage-like APC's and B cells differ in their capacity to process and present SpreS2 antigens. Since SpreS2 is a very stable particle composed of lipids and proteins it is conceivable that such antigen requires a strong degradation and such processing might occur in certain macrophage-like APC's but not in B cells.

C 155 PARTICULATE MYCOBACTERIUM LEPRAE ANTIGEN PRESENTATION BY HUMAN B CELLS. M. Selvakumaran and I. Nath, Department of Pathology and Biotechnology, All-India Institute of Medical Sciences, New Delhi, Pin code : 110 029, INDIA.

Various cell types have been shown to present antigens to T cells, provided they express class II antigens on their surface. Using intact M.leprae as an antigen, we have analysed the antigen presenting ability of Epstein Barr Virus transformed B cells to resting and activated T-cells. B cells were unable to present antigen to resting T cells but were able to present antigen efficiently to Con A, PPD and M.leprae activated short term and long term T cell lines. B cells were able to present particulate, naive M.leprae antigens as equal as soluble, sonicate M.leprae antigens to T cells.

C 156 PROCESSING OF BIOSYNTHETICALLY RADIOLABELED HUMAN INSULIN BY MOUSE TA3 ANTIGEN PRESENTING B CELLS. John W. Semple, Janet Ellis and Terry L. Delovitch, Banting and Best Department of Medical Research and Department of Immunology, University of Toronto, Toronto, Ontario, Canada, M5G 1L6

We investigated the pathway of antigen processing in situ in B cell APC. Recombinant human insulin biosynthetically labeled with ^{3}H and ^{35}S at several amino acids was used as an antigen and was exposed for varying lengths of time to TA3 mouse B cell APC. Subcellular fractionation and HPLC chromatography permitted several of the processed peptides distributed throughout the insulin molecule to be monitored. Many insulin peptides localized to both the extracellular (8 peptides) and intracellular (4 peptides) compartments of TA3 cells were detected. The identification of plasma membrane-associated peptides is in progress. Many of the peptides processed by TA3 APC in situ co-elute with those obtained upon digestion in vitro by the insulin-specific insulin degrading enzyme (IDE). These data together with those we previously obtained for the processing of ^{125}I-labelled human insulin suggest that insulin may be processed in B cell APC into immunogenic peptides by an enzyme(s) present on the plasma-membrane, intracellularly and extracellularly. (Supported by MRC and CDA).

C 157 CD8 INTERACTION WITH THE THIRD DOMAIN OF CLASS I MHC MOLECULES IN CYTOTOXIC T CELL RECOGNITION OF TARGET CELLS. Michael J. Irwin, William R. Heath and Linda A. Sherman. Scripps Clinic and Research Foundation, La Jolla, CA 92037. The interaction of the CD8 molecule on a cytotoxic T lymphocyte (CTL) with its target cell ligand has been shown to have an avidity enhancing effect. Recent evidence has implicated the third domain of the class I molecule as containing the CD8 binding site. Using a human/murine chimeric class I molecule comprised of the first two domains of HLA-A2 and the third domain of H-$2K^b$, we provide strong support for this conclusion. Murine CTL clones specific for HLA-A2 were generated with the human cell line JY. Four of five CTL clones were found to lyse $A2K^b$ transfected murine cells more effectively than A2 transfectants. Anti-CD8 specific mAb inhibited the lysis by these four clones, and this inhibition was more pronounced for $A2K^b$ transfectants than A2 transfectants. One clone, which lysed A2 and $A2K^b$ transfectants equivalently, was shown to be insensitive to anti-CD8 antibody inhibition. These findings indicate that A2-specific murine CTL clones possess greater avidity for murine target cells expressing the $A2K^b$ hybrid molecule relative to those expressing the A2 molecule. This implies that a CD8 interaction with the same molecule seen by the T cell receptor is important for target cell recognition.

C 158 ALTERNATIVE SPLICING OF HLA-DQ TRANSCRIPTS AND SECRETION OF HLA-DQ BETA PROTEINS, Linda Shookster, Paola Briata, Susan F. Radka, Silvia Sartoris and Janet S. Lee, Department of Molecular Immunobiology, Memorial Sloan-Kettering Cancer Center, New York, N.Y. 10021.
HLA class II antigens are highly polymorphic cell surface proteins involved in initiation and regulation of the immune response. Allelic sequence variation primarily affects the structure of the first external domains of the α and β component chains. Here we provide evidence for other types of allelic polymorphism for these genes. The sequences of two cDNA clones corresponding to the HLA-DQβ mRNAs from an HLA homozygous cell line exhibit both alternative splicing and readthrough of polyadenylation. Furthermore, the alternative splicing event is associated with only a subset of HLA-DQβ alleles, while the polyadenylation site readthrough is found in a larger subset. This suggests that polymorphic cis acting elements within the HLA-DQβ gene control both processing steps. Proteins, presumably encoded by the alternatively spliced mRNAs lacking transmembrane exons, are immunoprecipitated with a monomorphic monoclonal antibody directed against HLA-DQ. These proteins are found in supernatants of cultured cell lines for which secretion is predicted, but not in those of cell lines which do not contain the alternatively spliced mRNAs. Such secretion class of II allelic products could profoundly affect interactions between effector and target cells in an immune response.

C 159 GENE STRUCTURE AND FUNCTIONAL ANALYSIS OF MURINE ICAM-1; **Gerald Siu, Peter Kuhlman, and Adrienne A. Brian; Departments of Biology and Chemistry and the Cancer Center, Q-063, University of California at San Diego, La Jolla, CA 92093.** We are studying the murine ICAM-1 gene and the effects of ICAM-1 on antigen recognition. Using the human ICAM-1 cDNA, we have isolated cDNA and genomic clones encoding the murine homologue. The murine ICAM-1 gene is a single-copy gene that consists of multiple exons spanning 25kb of DNA and encodes a 2.5kb mRNA that is expressed at high levels in a wide variety of different cell types. Sequence analysis indicates that murine ICAM-1 is 50% and 60% homologous to human ICAM-1 on the protein and DNA levels, respectively, and is a member of the immunoglobulin gene superfamily, consisting of several V-like domains linked tandemly. We are also studying the effects of ICAM-1 on antigen recognition. The T-cell clone I4D transfected with an alpha-beta TCR that recognizes moth cytochrome c 88-103 in the context of I-E^k, I-E^s, and I-E^b (Engel and Hedrick; CELL 54:473-484, 1988). This response is blocked with the anti LFA-1 antibody FD441.8 when antigen is presented by B10.A(2R) spleen cells but not when antigen is presented by DCEK, a fibroblast transfected with I-E^k. We are currently transfecting the murine ICAM-1 cDNA into DCEK in order to determine if we can enhance the I4D response and to determine if the enhancement is LFA-1 dependent.

C 160 CD4-Ia INTERACTIONS CAN OCCUR IN THE ABSENCE OF T CELL RECEPTOR/ANTIGEN-Ia RECOGNITION. **Moncef Slaoui Fabienne Andris, Oberdan Leo, Jacques Urbain.** Laboratory of Animal Physiology, Université Libre de Bruxelles, Belgium.
The T cell differentiation antigen, CD4, is expressed by MHC class II restricted T lymphocytes. $CD4^+CD8^-$ T cells use their T cell receptor to recognize foreign antigens in association with MHC class II products. The association between CD4 expression and restriction by MHC class II products has led to the hypothesis that CD4 may interact with monomorphic determinants of MHC class II molecules.
A large body of experimental evidence suggests that CD4 interaction with MHC class II molecules leads to an increase in the binding avidity of T cell-stimulator cell interactions.
A direct test for a functional CD4-MHC class II interaction in T cell activation requires a separate evaluation of CD4-Ia interactions from TcR-Ag/Ia recognition. However, a separate evaluation proves difficult since the T cell receptor and CD4 may interact with the same MHC class II molecule.
In this report, we use a T cell activation protocol, where TcR-Ag/Ia recognition is replaced by TcR complex- anti-CD3 antibodies interactions. Using this activation protocol, we have analyzed the effects of monoclonal anti-MHC class II antibodies on the activation of a $CD4^+$ T cell hybridoma in the absence of its TcR restricting MHC class II molecule (IE^k) but in the presence of unrelated MHC class II molecules (IE^d,IA^d). The data obtained clearly indicate a functional role for CD4-MHC class II interactions in T cell triggering.

C 161 **SEPARATE PROCESSING FATES OF PROTEIN ANTIGEN TARGETED TO SURFACE IGD OR MHC MOLECULES ON NORMAL SPLENIC B CELLS,**
Denis P. Snider, Ingeborg K. Uppenkamp, Julie A. Titus, and David M. Segal, Exp. Imunuol. Branch, NCI, NIH, Bethesda MD 20892.
We have targeted hen egg lysozyme (HEL) to murine B cells using heterocross-linked antibodies which specifically bind to surface IgD or different MHC molecules. Internalization and presentation of HEL to hybridoma T cells occurred more quickly with targeting to IgD than to MHC structures as assessed by fixation and pronase stripping experiments. HEL was internalized quickly into acidic compartments when targeted to IgD but was detected much later when targeted to MHC molecules, as assessed by shifts of fluorescent signal of internalized FITC-HEL. However, the data indicate that not all endocytosed HEL entered low pH (<5.5) compartments. Degraded HEL was released from B cells following endocytosis of 125-I-HEL. This release was detected earlier with targeting to IgD than to MHC structures. Interestingly, the total amount of internal 125-I-HEL decreased with time after endocytosis via IgD, but the internal 125-I-HEL was almost entirely whole undegraded HEL at all times following endocytosis. These data and those of chloroquine and leupeptin inhibition studies indicate differences in the fate of antigen entering B cells via IgD or MHC structures, and support the notion of a neutral pH storage compartment for antigen endocytosed via surface IgD on normal splenic B cells.

C 162 "INNOCENT BYSTANDER" REACTIONS INDUCED IN VIVO BY CYTOTOXIC T CELL-TARGET CELL MIXTURES, David Steinmuller, Mary Ellen Snider and Roger L. Noble, Surgical Research Laboratories, Department of Surgery, University of Iowa, Iowa City, IA 52242
Target cell lysis by $CD8^+$ CTL is a highly specific phenomenon in vitro, as we have confirmed repeatedly in reverse labelling tests by showing that admixed "third party" target cells are not lysed in the presence of specific CTL-mediated cytolysis. However, when mixtures of CTL and their specific targets are inoculated into the skin of hosts *syngeneic* to the CTL, host cells at the site of inoculation are destroyed, often to an extent that results in grossly observable, full-thickness necrotic lesions. We have evoked these "innocent bystander" reactions in mice with CTL directed against single and multiple non-H-2 antigens and TNP-hapten and influenza A virus-specific antigens. Thus, the ability to trigger bystander tissue destruction appears to be a general characteristic of CTL-target cell interaction in vivo. Our current evidence suggests that *host* inflammatory cells recruited and activated by factors stimulated by CTL-target cell recognition actually mediate the tissue destruction. These CTL-initiated bystander reactions may be the basis of the non-specific tissue destruction that contributes to allograft rejection and that is observed in many serious virus infections and in intense DTH reactions and contact dermatitis.

C 163 T CELL IMMUNITY OR TOLERANCE AS A CONSEQUENCE OF SELF ANTIGEN PRESENTATION. Brigitta Stockinger and Rong Hwa Lin. Basel Institute for Immunology, Basel, Switzerland.

We have investigated the basis for immunity or tolerance to a mouse serum protein - the fifth component of complement (C5). In C5 deficient mice this protein is absent from serum and as a consequence they are not tolerized to C5. C5 deficient mice generate CD4 bearing T cells which recognize C5 in the context of class II. In contrast, C5 sufficient mice in which C5 protein is continuously produced do not mount T cell responses against C5. We have tested if this self protein is processed and presented with class II in normal mice and can be recognized by C5 specific T cells in the absence of exogeneously added antigen. All class II bearing cells from C5 sufficient mice activated C5 specific T cell clones without additional antigen. Presentation was not a consequence of C5 secretion by macrophages in culture but was shown to be derived from endogeneously generated C5/class II complexes. Thus this self protein is efficiently presented in vivo and available for tolerance induction. Although C5 deficient mice cannot secrete C5 they still synthesize a precursor molecule, pro-C5, in their macrophages. However, they are not tolerant to pro-C5 either and can be readily immunized with isolated pro-C5. Furthermore, some T cell clones can recognize endogeneous pro-C5 from C5 deficient macrophages in the absence of exogeneous antigen. Therefore some endogeneous pro-C5 peptides obviously have access to the class II presentation pathway. However, presentation of pro-C5 with class II is not efficient enough in thymus to induce tolerance to pro-C5. An explanation for this could be the very low levels of class II on thymic macrophages which are the only cells that could present endogeneous pro-C5. Thus efficient generation of self antigen peptide/class II complexes has a strong influence on the level of tolerance induction.

C 164 IDENTIFICIATION OF THE SUBPOPULATION OF ANTIGEN PRESENTING CELLS THAT ACTIVATE DELAYED-TYPE HYPERSENSITIVITY T CELLS, Stephen A. Stohlman and Glenn Matsushima, Department of Microbiology, University of Southern California School of Medicine, Los Angeles, CA 90033.

Accumulating evidence from a number of models suggests that unique subsets of antigen-presenting cells are responsible for the induction of specific T cell-mediated responses. We have previously described an age-dependent maturational defect in the ability of the SJL strain of mice to activate DTH-inducer T cells to a wide variety of antigenic stimuli. None of the other 14 strains tested exhibited a similar defect and all other accessory cell dependent responses were unaffected in the DTH unresponsive SJL. We have also shown that the adoptive transfer a macrophage from older DTH responsive SJL or other DTH responsive I-A^s strains can overcome this defect in DTH responsiveness. We have recently found that a subpopulation with the Mac-1^+, Mac-3^+ and Mac-2^- surface phenotype are able to transfer responsiveness. FACS analysis indicate that the Mac-3 phenotype is expressed on less than 20% of macrophages. Titrations of the Mac-3^+ cells isolated by FACS indicate that adoptive transfer of only 100 Mac-3^+ cells can overcome the defect in DTH responsiveness. By contrast, transfer of 10^4 Mac 3^- or Mac 2^+ cells were unable to overcome the defect. Our data suggest that the induction of $CD4^+$ antigen specific cells DTH-inducer T cells is mediated by a phenotypically unique small subset of macrophage accessory cells.

C 165 IN VIVO ADMINISTRATION OF Fab' FRAGMENTS OF ANTI-L3T4 MONOCLONAL ANTIBODY, GK1.5, DEPLETES T HELPER CELLS AND INDUCES LONG-TERM HUMORAL UNRESPONSIVENESS, Koichi Uyemura, Jonathan W. Uhr, and Ellen S. Vitetta, Department of Microbiology, University of Texas Southwestern Medical Center, Dallas, Texas 75235.

In vivo administration of anti-L3T4 monoclonal antibody (MoAb) has been shown to deplete T helper cells (T_H), resulting in suppression of a variety of immune functions. In contrast, administration of $F(ab')_2$ fragments of GK1.5 do not cause significant depletion of $L3T4^+$ cells, but are able to block primary humoral immune responses and induce immune tolerance in vivo. In our studies, we have examined the effect of administering Fab' fragments of anti-L3T4 MoAb (Fab'-GK1.5) on the inhibition of humoral immunity. Treatment of KLH-primed mice with 0.5 mg Fab'-GK1.5 depleted $L3T4^+$ cells from lymph node tissue while leaving other lymphocyte subpopulations intact. After injection of KLH in complete Freund's adjuvant, these T_H-depleted mice were unable to produce anti-KLH antibodies. Long-lasting unresponsiveness against KLH (12 weeks) was observed despite the apparent regeneration of the T_H population of the lymph node. The results obtained using either Fab' or intact GK1.5 antibody were comparable and suggest that a transient depletion of T_H does not account entirely for the long-term humoral unresponsiveness.

C 166 ACTIVATION OF T-CELLS BY HUMAN DENDRITIC CELLS IS BASED ON EFFICIENT STIMULATION OF INTERLEUKIN-2 SECRETION, Jukka Vakkila and Mikko Hurme, Department of Bacteriology and Immunology, University of Helsinki, Finland.

We have studied the mechanisms of action of dendritic cells (DC) in T cell activation. We compared the capacity of human blood-derived DC and monocytes (Mo) to induce IL-2 receptors and IL-2 secretion in autologous and allogeneic MLR. DC were found to stimulate effectively IL-2 secretion when cultured with autologous or allogeneic T cells. This was not entirely specific for DC since also allogeneic Mo were able to induce IL-2 secretion. However, supernatants from cultures stimulated by autologous Mo did not contain detectable amounts of IL-2. Both stimulator cell types induced equally well IL-2 receptors on autologous or allogeneic T cells as studied with anti-IL2R antibody. Also T cell proliferative responses to DC and Mo were equally high if the cultures were supplemented with exogenous IL-2. We conclude that the higher capacity of DC to stimulate T cell proliferation is based on efficient stimulation of IL-2 secretion.

C 167 THE ROLE OF CATHEPSIN D IN ANTIGEN PROCESSING

J.M. van Noort, J. van Nieuwkerk and A.C.M. van der Drift; TNO Medical Biological Laboratory; P.O.Box 45, 2280 AA Rijswijk, The Netherlands.

The aim of this study was to gain a more detailed insight into the molecular aspects of antigen processing during the immune response. As a first approach, endosomal vesicles were isolated from bovine alveolar macrophages and their proteolytic activity with respect to a model protein antigen, sperm whale myoglobin (Mb), was characterized. During the first stage of digestion of Mb by the endosomes, a limited number of fragments were preferentially released from the antigen. We have isolated and identified these fragments.

The digestion of myoglobin is completely prevented by pepstatin, a specific inhibitor of aspartic proteinases, and only marginally by other proteinase inhibitors. When Mb fragments preferentially released upon digestion with purified bovine cathepsin D, an aspartic proteinase abundant in macrophages, were identified, almost all coincided with the fragments released by the endosomes. To define in more detail the selectivity of cathepsin D under the mild conditions applied, other protein antigens were similarly treated with the enzyme and the peptides released were identified.

The location of the preferential cleavage sites - when related to known T-cell epitopes - suggests a dominant role for cathepsin D in the processing of protein antigens to yield fragments for presentation to T-cells. Possibly, the observed selectivity of the enzyme may account for the structural similarities among T-cell epitopes, noted by others.

C 168 INDUCTION OF B CELL UNRESPONSIVENESS TO NONINHERITED MATERNAL HLA ANTIGENS DURING FOETAL LIFE, Jon J. van Rood, Yvonne Gijbels, Jacqueline van der Velden-de Munck and Frans H.J. Claas, Department of Immunohaematology & Blood Bank, University Hospital, 2333 AA Leiden, the Netherlands.

Actively acquired tolerance in mice to the antigens of the MHC (H-2) is induced by exposure of the animals to allogeneic lymphocytes within 24 hours of birth. Actively acquired tolerance to the MHC in humans (HLA) cannot be studied in the same way. However, we have evidence for the existence of actively acquired tolerance in humans in a study of 26 highly sensitized patients waiting for a renal allograft. They had developed complement dependent antibodies to the HLA antigens of almost all unrelated caucasoid donors. The sera of these highly sensitized patients were tested against a panel of lymphocytes that were mismatched for only one HLA class I antigen. We found for these 73 patients HLA class I antigens that, although different from those present in the recipient, did not lead to a positive crossmatch. We called such antigens "permissible mismatches" and show that they often included those HLA antigens of the patient's mother that the patient had not inherited (noninherited maternal antigens; NIMA). In 15 of the 26 patients, the permissible class I mismatches included the NIMAs. The noninherited paternal antigens (NIPAs) were analyzed as a control; only two of the 25 NIPAs tested were acceptable mismatches, which emphasized the preferential nonresponsiveness to NIMA. Recent experiments indicate that what holds true for antibody formation also holds true for T cell activation.

C 169 THE CD8 ANTIGEN IS OF FUNCTIONAL IMPORTANCE IN BOTH CD3 McAb-INDUCED AND CD2 McAb -INDUCED NON-SPECIFIC CYTOTOXICITY OF CLASS I AND CLASS II ALLOSPECIFIC CYTOTOXIC T-CELL CLONES,* G.A. van Seventer, R.A.W. van Lier, K.C. Kuijpers, H.Spits and C.J.M. Melief. Central Lab. of the Netherlands Red Cross Bld. Transf. Serv., Amsterdam, The Netherlands.

We investigated the function of the CD8 moiety in antigen-specific and alternative activation of HLA class I and HLA class II allospecific CD8-positive CTL clones. Monoclonal antibodies (McAb) directed against the CD8 structure were only found to inhibit antigen-specific cytotoxicity of a series of class I allospecific CD8-positive CTL clones and not of a class II allospecific CD8-positive CTL clone. However cytotoxicity induced by CD3 McAb (used at suboptimal concentrations) or CD2 McAbs in both types of CTL clone was blocked by CD8 McAbs. The absence of CD8 McAb blocking of antigen-specific cytotoxicity of the class-II-specific CD8-positive CTL clone may be explained by assuming that it results from a triggering signal which is to strong to be overcome by the down-regulatory signal of the CD8 antigen.

These combined findings clearly suggest a functional involvement of CD8 not only in TCR/CD3 activation, but also in TCR/CD3 controlled alternative activation routes, such as the CD2 activation pathway. Moreover it shows that even an HLA class II allospecific CD8-positive CTL clone expresses a functional active CD8 antigen. The absence of HLA class I expression on the target cells (DAUDI cells) used in the experiments described indicate that the CD8 antigens not act solely in an adhesion-like fashion, but exhibit also a more general regulatory function in T-cell activation. This regulatory role of CD8 may be explained by assuming the induction of a threshold for activation, which is triggered after binding of CD8 McAb or binding to its natural ligand, HLA class I. In our view, CD8-mediated regulation of T-cell activation could therefore prevent non-specific triggering of cytotoxicity by interactions of insufficient affinity. *This study was supported by a grant from the Dutch Kidney Foundation.

C 170 **SIGNAL TRANSDUCTION THROUGH THE CD4 RECEPTOR INVOLVES THE CELLULAR TYROSINE KINASE p56^{lck}.** André Veillette[1], Michael A. Bookman[2] and Joseph B. Bolen[1], Laboratory of Tumor Virus Biology[1] and Medicine Branch[2], National Cancer Institute, Bethesda, MD 20892.

The CD4 T-cell surface antigen is felt to have the dual function of stabilizing the interaction of the T-lymphocyte with the antigen presenting cell (APC) as well as transducing an independent signal that can potentiate the activation related alterations generated through the T-cell receptor. We have found that upon antibody-mediated cross-linking of the CD4 molecules of cloned murine T-lymphocytes there is a time and temperature dependent decrease in the abundance of the lymphocyte-specific tyrosine kinase p56^{lck}. This co-modulation is specific for CD4 and p56^{lck} since cross-linking of other T-cell surface antigens (CD3, T200, Thy1.2) does not result in detectable alterations in the abundance of the lck protein and since CD4 cross-linking does not induce any alteration in the abundance of p60^{fyn}, another src-related tyrosine kinase highly expressed in T-cells. Such data suggest that CD4 and the internal membrane lck protein are in close proximity within the cell. Further analysis has revealed that significant amounts of lck can be immunoprecipitated by anti-CD4 antibodies. In addition, CD4 can be specifically precipitated by anti-lck antibodies. Our data imply that CD4 and p56^{lck} are physically associated in CD4$^+$ T-lymphocytes. The findings that CD4 is associated to the lck protein in either murine or human T-cells and that CD8 is also complexed to p56^{lck} in CD8$^+$ T-cells suggest that the lck tyrosine kinase is involved in the function of the CD4 and CD8 accessory molecules.

C 171 ROLE OF SURFACE IMMUNOGLOBULIN RECEPTORS FOR GENERATION OF EFFICIENT ANTIGEN PRESENTATION ACTIVITY, Motoo Watanabe[1], Tania H. Watts[2], Jean Gariepy[3] and Nobumichi Hozumi[4], [1]Central Research Laboratory, Mitsubishi Petrochemical Co. Ltd., Ibaraki 300-03, Japan, [2]Department of Immunology, University of Toronto, Ontario, Canada M5S 1A8, [3]Ontario Cancer Institute, Toronto, Ontario, Canada M4K 1K9, [4]Mount Sinai Hospital Research Institute, Toronto Ontario Canada M5G 1X5. Several B-lymphoma lines bearing Ia molecules as well as normal B cells have been shown to possess antigen presenting ability. B cells have surface immunoglobulin (sIg) receptors specific for an antigen, and accumulating evidences indicate that these receptors play an important role in the efficient presentation of antigen to helper T (Th) cells. Recently, we have developed the system to analyze antigen specific T cell-B cell interaction using a genetically engineered hapten 2,4,6-trinitrophenyl (Tnp) specific monoclonal B cell, A20-HL. Using this monoclonal B cell and the ovalbumin(Ova) specific Th cell hybridoma, 3DO-54.8, which recognizes a 17 amino acid polypeptide representing residues 323-339 of Ova, we analyzed the effect of epitope density of Tnp-conjugated antigens on the efficient antigen presentation. In order to assess the relationship between the behavior of sIg receptors and epitope density of antigen, flow microfluorometric analysis was carried out. Function and behavior of sIg receptors in specific antigen presentation will be discussed.

C 172 T CELL RECOGNITION OF MLS^a DETERMINANTS, Susan R. Webb and Jonathan Sprent, Department of Immunology, Research Institute of Scripps Clinic, IMM4A, La Jolla CA 92037.

Our studies suggest that Mls^a determinants are expressed in immunogenic form only on cells of the B cell lineage. Thus, typical antigen presenting cells (APC) such as dendritic cells and/or macrophages fail to elicit anti-Mls^a responses by unprimed T cells or T hybridomas. In addition, these APC do not appear to present processed Mls^a determinants. In light of these findings, of apparent interest is the issue of which cells types are responsible for Mls^a-specific T cell tolerance induction. Studies in mice treated from birth with anti-μ antibodies suggest an important but perhaps not exclusive role for B cells in this process. We are currently pursuing the identity of other cell types which may be involved.

C 173 *THE EFFECT OF MEMBRANE BOUND Q10 ON TOLERANCE IN TRANSGENIC* ANIMALS, Kim Wieties, Sharon Jones-Youngblood, Robert Hammer, and James Forman, University of Texas Southwestern Medical Center at Dallas, Dallas, Texas75235 Q10 is a soluble Class I-like major histocompatibility antigen produced specifically by the liver. Previously, it has been shown that mice possessing soluble Q10 can generate anti-Q10 cytotoxic T lymphocytes (CTL), suggesting that this soluble molecule does not function as a tolerogen. We have recently constructed C3H transgenic animals which express an exon shuffled Q10 (α_1, α_2)/L^d (α_3, T_M) molecule. This Q10/L^d molecule is expressed specifically in the liver on hepatocytes but not on nonparenchymal liver cells, spleen, thymus, kidney, or brain. The expression of Q10/L^d in the transgenic hepatocytes is equivalent to L^d expression on BALB/c hepatocytes, suggesting the animals are expressing physiologic levels of the transgene. The presence of membrane bound Q10/L^d in C3H animals has not caused anti-Q10 CTL precursors to be deleted, however, because primary *in vitro* CTL assays show these transgenic animals can specifically lyse Q10/L^d targets. Histopathologic examination of the livers of these animals does not show extensive lymphocytic infiltration or inflammation. In addition, serum levels of alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase are also normal, confirming that these animals do not show overt signs of liver rejection.

C 174 THE ABILITY OF EPIDERMAL KERATINOCYTES TO STIMULATE ALLOGRAFT REJECTION, Yeoman H. & Stanley M.A., Department of Pathology, Tennis Court Road, Cambridge, England, CB2 1QP.

MHC class I +ve, cultured epidermal keratinocytes are not rejected when grafted onto MHC incompatible murine recipients. Whereas uncultured epidermal cells, which contain Langerhans' cells, and cultured grafts reconstituted with MHC compatible dendritic cells are rejected. Thus indicating a pivotal role for antigen presenting cells (APCs) in the initiation of allograft rejection.

We have also investigated keratinocyte allograft survival in rats. Uncultured keratinocytes and IFN -γ pretreated, class II +ve keratinocytes are rejected rapidly by MHC incompatible recipients. However, preliminary data indicates that APC depleted, cultured allografts are also rejected, although there appears to be a significant delay in the rejection response.

C 175 INTRACELLULAR HEMOLYSIN-PRODUCING LISTERIA MONOCYTOGENES INHIBITS ANTIGEN PROCESSING, H. Kirk Ziegler and Christopher W. Cluff, Dept. of Microbiology, Emory University School of Medicine, Atlanta, Ga. 30322. We have found that virulent hemolysin (Hly)-producing Listeria monocytogenes(Hly+) inhibit antigen processing and presentation when added to macrophages in vitro. Neither killed bacteria nor nonpathogenic Hly- bacteria inhibit substantially. Inhibition by Hly was observed with antigens such as heat-killed L. monocytogenes (HKLM), soluble hen-egg lysozyme, and ovalbumin. The Hly produced by intracellular bacteria was responsible for the inhibition. Under conditions which inhibit antigen presentation (1 bacterium/macrophage), macrophages retained normal levels of Ia, maintained normal morphology, and were not permeable when assayed by chromium release. Additionally, the uptake and catabolism of surface-iodinated HKLM or intrinsically ^{35}S-labelled live bacteria was not different for either Hly- or Hly+ bacteria during the initial 2 hr exposure to live bacteria. While Hly+ bacteria could inhibit the presentation of HKLM, the presentation of soluble listerial antigens, which did not require processing, occurred normally. Also, Hly caused greater inhibition of the presentation of intact ovalbumin than of a synthetic ovalbumin peptide (p323-339). The results are compatable with at least two different pathways of antigen handling, a pathway for degradation of antigen, and a "processing" pathway for antigen presentation. Hly+ L. monocytogenes appear to interfere with the processing pathway, either by inhibiting production of antigenic material that can associate with Ia or by inhibiting putative intracellular event(s) involving the binding of Ia to processed antigen and transport of complexes to the cell surface.

Late Abstract

C 176 THE IMMUNOGENICITY AND ANTIGENICITY OF SYNTHETIC MONOMERS AND DIMERS OF T CELL AND B CELL EPITOPES DERIVED FROM THE SEQUENCES OF A STREPTOCOCCAL ANTIGEN, Lehner T, Childerstone A, Walker P, Bergmeier L.A. and J. Haron*. Department of Immunology, United Medical & Dental Schools of Guy's and St Thomas's Hospitals, London Bridge, London SE1 9RT UK. *Biotechnology Centre Inc, P O Box 8284, California.

The immunogenicity and antigenicity of synthetic peptides (SP) derived from the sequences of a streptococcal antigen were investigated in macaque monkeys. Immunization with the free peptides of 17 and 21 residues failed to elicit serum antibodies or T cell responses. However, both serum antibodies and lymphocyte responses were elicited by immunization with the SP linked to tetanus toxoid (TT) as a carrier. Indeed, SP17-TT and SP21-TT elicited serum antibodies and proliferative responses of lymphocytes, not only to the SP but also to the native streptococcal antigen. In vivo recall of SP17-TT or SP21-TT immunized monkeys with suboptimal doses of the native streptococcal antigen resulted in a significant increase in antibodies, both to the SP and native antigen, confirming that the two SP share antigenic epitopes with the native antigen. The B and T cell epitopes were then determined and the B cell epitopes resides in residue 8-13, whereas the T cell epitope overlaps and consists of residue 7-15. The T cell epitope has an amino-terminal leucine and carboxy-terminal glycine and alanine added to residue 8-13 of the B cell epitope. In spite of the B and T cell epitopes being expressed in SP17 (residues 1-15), the monomer failed to induce serum antibodies without a carrier. However, immunization with dimers of peptide-linked or disulphide-linked residues 1-15, without a carrier, elicited both serum antibodies and proliferative responses of lymphocytes. The results suggest that the monomeric SP17 is not immunogenic, whereas the dimeric peptide elicits both antibodies and T cell responses. The minimal T cell-B cell structure required for immunogenicity is now being determined.

Section A
Antigen: MHC Interactions; Regulation of MHC Antigen Expression; The T Cell Receptor Complex; Priming and Function of T Cell Subsets; Immunogenicity in Vaccines

C 200 GENE INTERACTIONS BETWEEN MHC AND TCR IN COLLAGEN INDUCED ARTHRITIS IN MICE. Gary D. Anderson, Subhashis Banerjee, Harvinder S. Luthra, and Chella S. David; Departments of Immunology and Rheumatology, Mayo Clinic, Rochester, MN 55905.
Susceptibility to collagen induced arthritis (CIA) in mice maps to the I-A loci in H-2^q mice. However, SWR (H-2^q) mice are CIA resistant, suggesting a role of non-MHC genes. We have recently shown gene complementation between H-2^q from SWR and TCR V_β genes from several non-susceptible strains. CIA has been induced in B10, C3H.A and A backcrosses with SWR with similar high incidences; 63, 71 and 67% respectively. C57L shares a similar background with B10 and is H-2^b, but has the same V_β TCR mutation as SWR. C57L backcrosses showed a very low incidence (17%) of CIA, and the arthritis observed was of a much milder and transient nature. Crosses to CBA/J and DBA/2 were either low incidence or totally resistant, suggesting a possible role of Mls^a locus in deleting certain T cell receptor subsets, possibly $V_\beta 6$ and $V_\beta 8.1$. Further experiments are now underway to examine the role of specific TCR genes in CIA, and the effect of deletion of V_β cell subsets due to Mls^a.
Supported by grants from NIH (AR-30752) and Minnesota Arthritis Foundation.

C 201 ANALYSIS OF REGULATORY ELEMENTS WITHIN A MURINE TCR β-CHAIN TRANSCRIPTIONAL PROMOTER. Steven J. Anderson, Shinichi Miyake and Dennis Y. Loh, Howard Hughes Medical Institute, Departments of Medicine, Genetics, Microbiology and Immunology, Washington University School of Medicine, St. Louis, MO 63110.

Expression of the genes encoding the α- and β- chains of the TCR is controlled in both a tissue and stage specific manner. We have examined the transcriptional regulation of murine Vβ gene promoters by sequence (Anderson et al, 1988 PNAS 85:3551) and functional analysis. A transient transfection system and S_1 nuclease analysis were employed to quantitate correctly initiated transcripts derived from the Vβ8.3 promoter linked to a functionally rearranged Vβ8.2 gene. Transcripts could be detected at similar levels in both T (YAC-1,BW5147) and B (S194) cells. Promoter deletion constructs reveals an essential cis-acting element located between -85 and -34 relative to the transcription start site. The basal level transcription from this promoter is reduced by >90% upon removal of these 51 base-pairs. This region contains the previously described decamer which is conserved in murine, human and rabbit Vβ genes. DNase I footprint assays show protection of this decamer by nuclear extracts of murine T-cells. Deletion or mutation of this sequence eliminates specific protein binding as revealed by gel-mobility shift assays. The tissue specificity of protein binding to this motif has also been examined.

C 202 THE ROLE OF Vβ IN ANTIGEN:SELF MHC AND NON-SELF MHC RECOGNITION BY CLONED T CELL LINES, Jody Baron, Pilar Portoles and Charles A. Janeway, Jr., Section of Immunobiology, Yale University School of Medicine, New Haven, CT 06510.
Using a cloned T cell line reactive to antigen presented by self MHC and cross-reactive to non-self MHC of five different MHC genotypes, we have asked which epitopes on the receptor are involved in recognition of each ligand. Our analysis suggests that the Vβ region is associated with a high affinity binding site for non-self MHC and a low affinity binding site for self MHC in this system, in that anti-Vβ antibodies inhibit responses to antigen:self MHC more effectively than expected while inhibiting responses to non-self MHC less effectively than expected. This clone uses Vβ8.2 in its receptor. We have prepared a large number of cloned lines expressing the Vβ gene in the receptor and examined their responses to antigen and to non-self MHC. In particular, most of a set of cloned T cell lines specific for myelin basic protein peptide Ac-1-9 presented by $I\text{-}A^u$ express Vβ8.2, but only a fraction show alloreactivity, and the pattern of alloreactivity in each cloned line is different. Thus, alloreactivity may involve Vβ as determined by antibody blocking analysis, but the response to non-self MHC clearly involves other portions of the receptor as well.

C 203 TRANSCRIPTIONAL REGULATION OF THE HLA CLASS II ASSOCIATED INVARIANT CHAIN GENE. Cathy L. Barr and Grady F. Saunders, Department of Biochemistry and Molecular Biology, The University of Texas M. D. Anderson Cancer Center, Houston, TX 77030
The invariant chain associated with HLA class II molecules is a 31-33 kd glycoprotein implicated in antigen processing and assembly and intracellular transport of class II molecules. Class II molecules and invariant chain are expressed primarily by B lymphocytes and antigen-presenting cells such as macrophages and can be induced by interferon-γ in a variety of cell types. To define sequences involved in the human invariant chain gene regulation, 790 bp 5' to the initiation of transcription were subcloned upstream of the CAT gene. Transfection into invariant chain-producing cell lines and non-producing cell lines demonstrated that this 5' region displayed tissue specificity and responsiveness to interferon-γ. Deletion mutants were constructed to ascertain the functional properties of specific regions of the invariant chain upstream regulatory regions. These deletion mutants have led to the identification of 3 putative regulatory regions: 394 to 239, 239 to 216, and 216 to 165 bp 5' to the cap site of the invariant chain gene. Deletion of any one of these 3 regions results in decreased CAT activity. Protein-DNA interactions of these sequences have been characterized by mobility gel shift assay and DNAse I footprinting. Two regions have been identified that exhibit cell type dependent binding of nuclear proteins.

C 204 CHARACTERISATION OF HUMAN LUNG LYMPHOCYTES AND IL-2 INDUCED LUNG T CELL LINES. Susanne Becker, David T. Harris, and Hillel S. Koren. CE Environmental, EPA, Clinical Research Branch, Chapel Hill, NC27599.
Two color flow cytometry was used to characterize the surface phenotypes of human bronchoalveolar lymphocytes (n=32). The CD4/CD8 ratio was highly variable (0.3-6.6, mean=2.1). A high proportion of the T cells expressed HLA-DR (9-38%, mean=21%) indicative of T cell activation. However, detectable levels of the IL-2 receptor were expressed on <3% of the cells. CD45R was absent from $CD4^+$ cells in most preparations (0-10% mean=3%) suggesting that the cells are inducers of Ig synthesis. UCHL1, a marker of memory cells was present on 68-100 % of lung T cells. $UCHL1^+$ $CD45R^-$ lung lymphocytes responded poorly to PHA and ConA but did respond to IL-2 in the presence of accessory cells. Together these data suggest that lung lymphocytes are recently activated memory cells. IL-2 induced lung T cell lines were also characterized for antigen expression and LAK activity. High LAK activity was obtained in preparations containing a high proportion of CD8 cells. These cultures appeared to be suicidal. In contrast, lines with a high proportion of $CD4^+$ had low or absent LAK activity but proliferated in the presence of IL-2 for at least 3 months expressing a $CD2^+$ $CD3^+$ $CD4^+$ TCR 1^+ DR^+ $UCHL1^+$ $CD45R^-$ phenotype.
This abstract is a proposed presentation and does not necessarily reflect EPA policy.

C 205 HLA-DP: STRUCTURAL POLYMORPHISM AND DISEASE ASSOCIATION, Ann B. Begovich, Teodorica L. Bugawan, Barbara S. Nepom*, Gerald T. Nepom*, and Henry A. Erlich, Department of Human Genetics, Cetus Corporation and *The Virginia Mason Research Center, Seattle, WA

The polymorphic second exons of the HLA-DP_α and DP_β genes have been specifically amplified in vitro by the polymerase chain reaction (PCR) method, using the thermostable DNA polymerase of T. aquaticus. Sequence analysis of M13 clones containing the amplified DP sequences from a panel of thirty-four DP typed cell lines revealed only the two previously characterized alleles for DP_α.[1] Fourteen allelic variants were defined for DP_β. Eight of these are associated with the T-cell-defined DPw1-6 types; two subtypes were found for both DPw2 and DPw4. Six additional DP_β alleles which were previously typed in the T cell assay as blanks were also identified. Based on this sequence information, non-isotopic sequence specific oligonucleotide probes have been developed and used to type a panel of controls as well as patients with Juvenile Rheumatoid Arthritis (N = 44) and Adult Rheumatoid Arthritis (N = 32). In addition to the identification of 10 new DP_β alleles, a significant increase in the DP_β allele 2.1 was found in patients with Juvenile Rheumatoid Arthritis over patients with Adult Rheumatoid Arthritis and controls (~55% vs 20%). Sequence comparisons allow predictions to be made on which part of the DP_β 2.1 allele might be involved in disease susceptibility.

[1] Bugawan et al, J. Immunol. (In press)

C 206 USE OF MOUSE CLASS II PEPTIDES TO PRODUCE SITE-SPECIFIC MONOCLONAL ANTIBODIES.
Thomas G. Beito, Christopher J. Krco, Roger G. Little, Jay Zeller, Daniel M. McCormick*, and Chella S. David; Departments of Immunology and Biochemistry*, Mayo Clinic, Rochester, MN 55905.
Six peptides representing polymorphic regions of the α- and β-subunits of mouse class II (Ia) antigens were synthesized: A^b_α (43-61), A^d_α (43-61), A^q_α (43-61), A^b_β (57-78), A^d_β (57-78) and A^q_β (57-74). The peptides were purified by HPLC, conjugated to KLH, emulsified adjuvant and injected into mice. Antiserum (1:200 dilution) to A^d_α (43-61) reacted strongly to free A^d_α peptide on solid phase RIA (11,843 $\pm$ 629) but not to control A^q_β (57-78) peptide (130 $\pm$ 29). There was approximately 44% crossreaction of the antiserum to A^b_α (43-61) and A^q_α (43-61) (cpm's of 5159 $\pm$ 325 and 5139 $\pm$ 227 respectively) which is a reflection of the 95% sequence homology between A^b_α and A^q_α and their 74% homology to A^d_α. Six monoclonal antibodies (mAb) specific for A^d_α were isolated. These probably react with A^d_α unique residues at positions 44, 48, 56, 57, and 59. In addition, two mAb specific for A^b_β residues 75-78 were isolated. Even though these reagents do not react with the native molecules on cells they may interfere in antigen presentation 'in vivo'. The utility of these reagents as immunomodulators, in vivo and in vitro, is currently being assessed and will be presented.
Supported by NIH Grant, AI-14764.

C 207 IL 4 PRODUCTION IN RESPONSE TO PRIMING WITH A CYTOCHROME c PEPTIDE.
Margarita Betz, Dominic Dordai, Brian E. Lacy, and Barbara S. Fox. Department of Medicine, University of Maryland School of Medicine, Baltimore MD 21201. Murine type 2 helper T cells (Th2) secrete interleukin 4 (IL 4) in response to antigen. Despite the likely importance of these cells, little is known about their priming and expansion in vivo. We have demonstrated IL 4 production in response to a cytochrome c peptide following T cell expansion in vitro. This antigen has not previously been shown to induce Th2 cells. B10.A mice were primed with a peptide fragment of pigeon cytochrome c in CFA. Lymph node cells were restimulated in vitro with antigen for 5-7 days, Ficolled and rested for 3 days without antigen. Cells were then tested by limiting dilution for the presence of antigen-specific IL 4 producing cells. IL 4 was detected using the IL 4 sensitive cell line CT4S (provided by Dr. W. E. Paul, NIH). The specificity of the response was confirmed by blocking with the anti-IL 4 antibody 11B11. Following in vitro restimulation of the primed lymphocytes with antigen, IL 4 production was detectable from as few as10^3 cells per well. IL 4 secretion was antigen dependent and required both in vivo priming and restimulation in order to be detected. It is not clear why primed lymph node cells, placed in limiting dilution culture directly after removal from the animal, failed to secrete detectable amounts of IL 4 in response to antigen. Suppression is an unlikely mechanism as fresh primed lymph node cells were unable to inhibit IL 4 production by restimulated cells. We are now investigating the factors that may regulate the development of IL 4 producing T cells.

C 208 A B-CELL DNA BINDING PROTEIN REGULATED BY IL-4 AND BY DIFFERENTIATION,
Mark R. Boothby, Ellen Gravallese, Hsiou-Chi Liou, and Laurie H. Glimcher, Department of Cancer Biology, Harvard School of Public Health, Boston, MA 02115. The class II MHC proteins are expressed in a developmentally regulated pattern, and normally expression is limited to certain cell types such as B cells and macrophages. The differentiation of B cells to plasma cells is accompanied by the loss of class II MHC expression. These genes also respond to external stimuli such as the cytokine IL-4, which increases B cell Ia. A region of the Aα MHC gene activated expression of a CAT reporter gene in a B lymphoma cell line but not in a myeloma cell line. A nuclear protein that bound to two sites within this region was found. This binding activity was present in spleens that lack T cells and in B cell lines, but it was absent from all three myeloma cell lines tested. IL-4 treatment of normal and athymic mouse spleen cells greatly increased the binding of this nuclear protein to its Aα target sites, concomitant with increased Aα transcription. Thus, B cells contain a sequence-specific binding activity regulated both by IL-4 and by differentiation.

C 209 THE αβ T CELL DEPLETED MOUSE - A MODEL FOR γδ T CELL FUNCTION? Willi Born*, Michele Pigeon‡, Rebecca O'Brien* and Ralph Kubo‡, Howard Hughes Medical Institute at Denver*, Department of Medicine‡, National Jewish Center, Denver, CO 80206. This study is based on a new mAb with global specificity for mouse αβ T cell receptors (Kubo et al., submitted). Monoclonal Ab H57-597.2 was isolated from a hamster immunized with purified αβ TCR derived from $V\beta8^+$ mouse T cell hybridoma DO-11.10.H57-597.2 reacts with all mouse αβ TCRs tested so far but not with γδ TCRs. We have now used this antibody to interfere in vivo with the development of the $\alpha\beta^+$ T cell subset. Mice injected from birth with H57-597.2 were found to be depleted of virtually all peripheral $\alpha\beta^+$ T cells as well as αβ TCR^+ medullary thymocytes. Among the remaining cortisone-resistant thymocytes (about 30%), the number of $CD4^-CD8^-$ cells was increased (12 days after birth approx. 5 fold), suggesting that $\gamma\delta^+$ T cells might be unaffected by this treatment or even expand to some extent. Spleen T cells of H57-597.2 treated mice are no longer stimulated by alloantigen in mixed lymphocyte cultures and do not even respond to the T cell mitogen concanavalin A. We are exploring the possibility of using these mice as a model for γδ T cell function in vivo.

C 210 COORDINATED EXPRESSION OF Vγ AND Vδ GENE SEGMENTS IN FUNCTIONAL HUMAN T CELL CLONES Jannie Borst[1], Annemieke Wicherink[1], Evert de Vries[1], Jacques J.M. van Dongen[2] and Peter van den Elsen[3]. [1]Department of Immunology, The Netherlands Cancer Institute, Amsterdam, [2]Department of Immunology, Erasmus University, Rotterdam, [3]Department of Immunohaematology, Academic Hospital, Leiden, The Netherlands. Human TCR γδ occurs in disulphide-linked (Type 1) or non-disulphide-linked (Type 2) forms, dependent on the use of the Cγ1 or Cγ2 gene segment. The Cγ2 gene segment can contain a duplication or triplication of exon 2, which gives rise to different protein forms (Types 2bc or 2abc). It is not known whether functional differences exist between these receptor types. Protein chemical analysis of Type 1 and Type 2bc receptors on functional human T cell clones derived from peripheral blood (PB) has indicated that not only the γ chains, but also the δ chains have a molecular mass and charge which set apart Type 1 and Type 2bc receptors. Two sets of fifteen clones were randomly generated from PB of two normal donors after selection with the anti-TCR γδ-1 mAb, which recognizes all receptor types. DNA rearrangement and mRNA expression analysis of γ and δ genes allowed us to map the specificity of the anti-TCR γδ mAbs δTCS-1 and TiγA to the Vδ1 and Vγ9 gene segments respectively. Subsequently it could be concluded from the analysis of random clones that the majority of Type 1 receptors use Vγ9, while this preference seems absent in Type 2 receptors. The great majority of Type 1 receptors do not use Vδ1, while the majority of Type 2 receptors do. This was confirmed by fluorescence analysis of PBL of a large panel of normal donors. We conclude that Vγ and Vδ gene segments in functional TCR γδ in PB are used in non random combination and that their expression is correlated with rearrangement of the γ gene to Cγ1 or Cγ2.

C 211 TWO PHYSICALLY-SEPARATED REGIONS OF THE Aα CHAIN CONTROL EFFICIENCY OF Ia HETERODIMER EXPRESSION, Ned S. Braunstein, and Ronald N. Germain†. Dept. of Medicine, College of P & S, Columbia Univ., N.Y., N.Y. 10032 and †Laboratory of Immunology, NIAID, NIH, Bethesda, MD 20892

We have previously shown that polymorphic residues in the NH_2-terminal half of the β_1 domain (amino acids 1-48; hypervariable regions 1 and 2 [βHV1 and 2]) determine with which allelic or isotypic α chain a particular β chain can achieve efficient cell surface heterodimer expression. This result might be understood in terms of the current model for Ia structure which predicts that βHV1 would lie adjacent to the first α chain hypervariable region. Therefore, to examine the role of αHV1 residues in controlling heterodimer expression, a mutant $A\alpha^d$ cDNA was created in which the codon for amino acid 11 was mutated to code for the $A\alpha^k$ residue at this position. In addition, recombinant $A\alpha^b$ and $A\alpha^k$ cDNAs, in which the segments encoding the three α hypervariable regions were exchanged between the two alleles, were used to study the contributions of other α chain polymorphisms to this process. Transfection experiments involving the $A\alpha^d$ mutant demonstrate that amino acid 11 in Aα contributes to the control of heterodimer expression although this cannot be the only Aα residue involved as the mutant does not have the complete $A\alpha^k$ phenotype in this assay. Analysis of transfectants using recombinant $A\alpha^b$ and $A\alpha^k$ cDNAs additionally demonstrate an effect of Aα HV2 polymorphisms in controlling heterodimer expression with $A\beta^b$. Interestingly, the polymorphic residues in αHV2 are predicted to lie in a region of the Aα chain α-helix which is adjacent to the βHV4 region of the β chain α-helix. Allelic substitutions in this latter region of Aβ have been shown to similarly affect surface Ia heterodimer expression. Taken together, these results suggest that there are at least two spatially separate areas in which the α and β chains interact and that these interactions are affected by polymorphic residues in both areas, contributing to the efficiency of heterodimer expression and, most likely, Ia quarternary conformation.

C 212 CHARACTERIZATION OF T CELL HYBRIDS WITH MUTATIONS IN GENES INVOLVED IN T CELL RECOGNITION AND ACTIVATION, Hans-Gerhard Burgert, Philippa Marrack and John W. Kappler, Howard Hughes Medical Institute, National Jewish Center for Immunology, Denver, CO 80206.
The aim of this project is to identify contact residues of the T cell receptor (TCR) with antigen and/or MHC class II molecules. As a model system, a Vβ17-containing TCR has been chosen since the majority of $V\beta17^+$ T cell hybrids react with IE molecules of the k,s,d, and b haplotype. T cell hybrids have been made which have a dual reactivity: they are $V\beta17^+$ and recognize IE molecules but also show reactivity towards a known antigen, namely chicken ovalbumin (Ova). One such hybrid has been mutagenized with ethyl methane sulfonate (EMS). Mutants were selected on the basis of their survival after stimulation by either antigen or IE. It was expected that mutations in all different kind of genes involved in T cell recognition and T cell activation would be found. Mutants obtained fall into two major groups: 1) loss variants of TCR α or β chains, T3 or L3T4; 2) mutants with point mutations in one of these genes. We are currently analyzing the mutants biochemically and functionally in order to identify the particular gene affected. Point mutations in the α and β genes of TCR mutants will be localized using the polymerase chain reaction in combination with dideoxy sequencing.

C 213 DIRECT BINDING OF AN INFLUENZA PEPTIDE TO CLASS I HLA MOLECULES, Benjamin P. Chen and Peter Parham, Department of Cell Biology, Stanford University School of Medicine, Stanford, CA 94305

Activation of T lymphocytes requires the intracellular fragmentation of foreign antigens and their presentation by class I or class II Major Histocompatibility Complex glycoproteins. The direct binding of peptides to class II molecules has been shown in a number of experimental systems and its specificity compared to that of T cell activation. In contrast, direct binding of peptides to class I molecules has been difficult to detect; although peptide sensitization experiments and the crystallographic structure of HLA-A2 persuasively argue for its occurence and importance. In this study, we demonstrate specific binding to HLA-A2 of an influenza matrix peptide (FLU-M1 residues 56-68) that has previously been shown to act as a target for certain HLA-A2 restricted influenza-specific cytotoxic T lymphocytes. We estimate that less than 0.3% of the purified HLA-A2 molecules were able to bind the added peptide.

C 214 HUMAN CYTOLYTIC T LYMPHOCYTE RECOGNITION OF PEPTIDES CORRESPONDING TO HLA SEQUENCES, Carol Clayberger, Mark Rosen, Russ Salter, Peter Parham, and Alan M. Krensky, Departments of Pediatrics and Cell Biology, Stanford University, Stanford, CA 94305

We and others have shown that allorecognition by cytolytic T lymphocytes (CTL) is analogous to T cell recognition of foreign antigens in that both can occur via presentation of antigenic peptides by products of the major histocompatibility complex. We have used peptides corresponding to the $alpha_1$ helix of selected HLA molecules to analyze T cell recognition of this polymorphic region. The $alpha_1$ alpha helix of HLA-B44 and -B13 are identical, and show a high degree of homology with those of HLA-Bw58, -B47, and -B27. Peripheral blood lymphocytes from 5 normal donors were stimulated in vitro with targets expressing HLA-B44 to derive allospecific CTL lines and clones. In some individuals, the allospecific response was almost totally directed against the $alpha_1$ helix. The ability of peptides corresponding to the $alpha_1$ helix of these HLA molecules to inhibit and induce lysis as well as to modify other assays of T cell activation will be discussed.

C 215 TISSUE SPECIFIC EXPRESSION OF MOUSE CLASS I GENES, John E. Coligan, Peter A. Burke*, Keiko Ozato* and Diane E. Handy, National Institute of Allergy and Infectious Diseases and *National Institute of Child Health and Human Development, NIH, Bethesda, MD 20892. Classical transplantation antigens are constitutively expressed on cells of all tissues except brain. Transcription is regulated by the interaction of nuclear factors with 5' flanking regions that include the class I regulatory element (CRE). Previously, the CRE has been divided into 3 regions on the basis of nuclear factor binding. Several studies have implicated the nuclear protein (rI) which binds to the inverted repeat (TGGGGATTCCCCA) of region I as necessary for gene transcription. Although region I is identical in all sequenced mouse K, D and L genes, it is not conserved in Qa region genes. A comparison of the CRE from H-2L^d with that of Q10, a Qa region gene expressed only in the liver and fetal yolk sac, shows that there are two changes within the inverted repeat sequence (TGaGGAcTCCCCA). These differences disrupt the dyad symmetry. Another nucleotide difference between H-2L^d and Q10 falls within region II of the CRE. However, Q10 can bind to the nuclear factor (rII) that binds to region II of the H-2L^d CRE, whereas Q10 region I can not bind to the nuclear factor (rI) that binds to the region I inverted repeat. To test whether the differences in region I contribute to the restricted tissue expression of Q10, we have used site-directed *in vitro* mutagenesis to make the inverted repeat of Q10 region I like that of the classical class I genes. A change at either base enhances transcription as measured in a transient transfection system. Either change also allows binding of the nuclear factor that binds to the classical region I sequence. Thus, alterations in the CRE region I contribute to the limited tissue expression of Q10. The presence of disrupted CRE region I in other Qa region genes likely contributes to their tissue restricted expression.

C 216 **Molecular analysis of T cell receptor structure/function in sperm whale myoglobin specific T cell clones.**
Jayne S.Danska, Alexandra M. Livingstone, Toshi Isihara and C. Garrison Fathman, Stanford University Medical School, Stanford, CA.
We have undertaken structural characterization of the T cell receptors (TCR) utilized by a well defined panel of murine DBA/2 T cell clones that recognize epitopes within the 110-120 peptide of sperm whale myoglobin (Sp WMb) presented by I-A^d or I E^d. Only 2 of 14 independent clones show alloreactivity for 10 MHC haplotypes. Using the polymerase chain reaction (PCR) and DNA sequencing of the TCR α and β chains from matched sets of clones bearing either MHC restriction or epitope specificity in common, we are addressing structural relationship between TCR and MHC/antigen for this model system. Among 6 I-E^d restricted T cell clones reactive with SpWMb 110-120, all have highly homologous TCR β chains associated with a minimum of three different TCR α chains, some of which are derived from novel V gene families. To further characterized the specificity of these clones we are generating substituted peptides to identify residues within the epitope important for interaction with TCR or restricting MHC molecule. Functional verification of the relationship between given TCR primary sequences, and recognition capability will be addressed by transfer of the α and/or β chains cDNAs created by PCR amplification into T-cell hybridomas expressing endogenous TCR genes of known sequence and specificity.

C 217 POLYMORPHIC RESIDUES ON THE I-A MOLECULE MODULATE THE PRESENTATION OF PEPTIDE AND NON-PEPTIDE ANTIGENS TO T CELLS C. Davis[1], F. Norton[2], J. Goodman[2], G. Gammon[3], E. Sercarz[3], D. Mitchell[4], L. Steinman[4], P. Jones[1]. [1]Department of Biological Sciences, Stanford University, Stanford, CA 94305; [2]Department of Microbiology and Immunology, University of California, San Francisco, CA 94143; [3]Department of Microbiology, University of California, Los Angeles, CA 90024; [4] Departments of Neurology, Pediatrics, and Genetics, Stanford University, Stanford, CA 94305. Our goal is to determine the contribution of polymorphic residues on the Ia molecule to the interaction of antigenic peptides with the Ia molecule and to the interaction of the peptide-Ia complex with the T cell receptor (TCR). We have isolated a panel of transfected L cells expressing full-length cDNAs encoding $A\beta^k$, $A\beta^u$, or chimeric Aβ polypeptides containing novel combinations of polymorphic residues from the *k* and *u* haplotypes in association with A_α chains of *k* or *u* haplotypes. These transfectants were tested for their ability to stimulate $A_\alpha^k A\beta^k$- or $A_\alpha^u A\beta^u$- restricted T cell clones. The antigens tested were L-tyrosine-p-azobezenearsonate (ABA-tyr), hen egg-white lysozyme (HEL), and myelin basic protein (MBP). Our results indicate that a single substitution within the Aβ chain may have variable effects on the stimulation of different T cell clones or hybridomas, suggesting that one site on the Ia molecule may interact in several different ways with TCRs of distinct specificities. The mutant panel provides a tool for further dissecting the fine specificity of T cells bearing previous characterized receptors, allowing us to correlate TCR junctional region variability with MHC fine specificity. The differential impact of substitutions with the N-terminal and C-terminal portions of the Aβ1 domain is consistent with models of $A_\alpha A\beta$ structure in which the N-terminus interacts with peptide while the C-terminus interacts with both peptide and the TCR.

C 218 EXPRESSION OF THE Q4^{p} GENE, Patricia M. Day, Katherine E. LaPan and Jeffrey A. Frelinger, Department of Microbiology and Immunology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599.
Generally, the transcription of Class I genes from the Qa/Tla region is limited to tissues of hematopoietic origin. Previous work in our lab demonstrated widespread transcription of the Q4 gene in the B10.P mouse, with high levels of mRNA found in liver, lung, lymph node, spleen, testes and thymus. Less RNA was present in muscle and brain tissues. However, it is not known which individual cell types within these tissues are responsible for the transcription of the Q4 gene. We raised polyclonal antisera against a synthetic peptide, derived from the predicted amino acid sequence of the Q4p transmembrane region. We selected this region since it is the most locus specific. This antisera immunoprecipitates a Class I-sized protein. A monoclonal antibody, directed against the same peptide, has also been produced. SV40-transformed H-2^{p} fibroblasts show an abundance of Q4 message. Suprisingly, indirect immunofluorescent staining with the monoclonal antibody reveals a cytoplasmic localization of the protein with a perinuclear concentration. Different patterns have been observed in examination of the H-2^{b} embryonal carcinoma cell lines 402AX and PCC4. Q4-specific antibodies allow us to identify the cell types which express the Q4 gene product. The application of *in situ* hybridization techniques will correlate the cellular site of mRNA synthesis and protein detected by antibodies. Understanding the pattern of expression of the Q4 gene is the first step in determining the so far elusive function of these MHC genes.

C 219 ROLE OF CD45R IN THE DEVELOPMENT OF HUMAN THYMOCYTES, Julie Deans,*, Jennifer Shaw, and Linda Pilarski, Dept. of Immunology, Univ. of Alberta, Edmonton, Canada
It has been proposed that CD45R+ thymocytes, which are a minority (15-30%) of total thymocytes, represent the productive thymic lineage and that CD45p180+ cells are those which are destined for intrathymic death. CD45R is a high molecular weight (p220) form of a series of transmembrane glycoproteins, collectively known as CD45, present in some form on all cells of both myeloid and lymphoid lineages. CD45R and the smallest of the CD45 isoforms, p180, are reciprocally expressed on mature T cells. CD45p180 appears on T cells several days after activation in vitro and is thought to be associated with terminal differentiation. Paradoxically, however, the majority of thymocytes are also p180+. We have used Northern analysis of RNA extracted from CD348- and CD45p180- thymus fractions, both of which are predominantly CD45R+, as well as unfractionated thymocytes, to show that p180- and CD348- fractions are greatly enriched for cells which can be induced to express IL2 and IL2R mRNA after mitogenic stimulation. Antibodies against CD45R, but not against CD45 common determinants, synergise with suboptimal doses of mitogen to induce IL2 and IL2R mRNA expression, suggesting that CD45R molecules are operative in transmembrane signalling in immature thymocytes. There is also an indication from Northerns using CD45 probes that CD45p180 mRNA is not induced in activated CD348- thymocytes as it is in mature T cells. These results support the idea that CD45R+ molecules are essential for generating signals required for cell survival within the productive intrathymic lineage.

C 220 INDUCTION OF ANTIBODY RESPONSES BY Th1 AND Th2 T CELL CLONES: EFFECT OF γ-IRRADIATION ON HELPER ACTIVITY. R. H. DeKruyff, D. T. Umetsu. Stanford University, Stanford, CA 94305.

We examined a panel of Th1 and Th2 T cell clones for the ability to induce antibody synthesis in a Mishell-Dutton culture system under cognate B-T cell conditions. Our findings indicate that both Th1 and Th2 T cells are heterogeneous, i.e., some but not all Th1 and some but not all Th2 clones have the capacity to induce antibody under these conditions.

We examined the effect of γ-irradiation or mitomycin-c pretreatment of our Th1 and Th2 clones on their ability induce antibody synthesis. Asano et al. (J. Immunol 138:667) have reported that Th2 clones are exceedingly sensitive to γ-irradiation, with doses as low as 500 rads abrogating the ability of Th2 clones to induce antibody synthesis. We found that while the helper activity of Th2 clones was very radiation sensitive, helper activity in Th1 clones was very radiation resistant. Th1 clones given 2000 rads of irradiation were as effective as unirradiated clones in inducing anti-TNP plaque forming cells (PFC). Moreover, when used at higher T cell/B cell ratios in culture, irradiated Th1 clones were more effective than unirradiated clones in inducing antibody synthesis. The effect of γ-irradiation on Th2 clones was not simply due to inhibition of proliferation, since mitomycin-c pretreatment of the clones had little effect on helper activity.

C 221 STUDIES ON AN ALTERNATE EXON OF THE MURINE T CELL RECEPTOR β CHAIN GENE: PATTERN OF EXPRESSION AND EVOLUTIONARY CONSERVATION, Alexander L. Dent, Pamela J. Fink and Stephen M. Hedrick, Department of Biology, University of California, San Diego, CA 92093. The β chain gene of the murine T cell receptor has been shown previously to have an alternatively spliced form of message. This message contains a novel exon, termed Cβ0, which is inserted between the VDJ and constant region exons. We have studied expression of the Cβ0 exon at the mRNA level by RNase protection. We have found that about 1% or less of of β messages in normal T cell clones contain the Cβ0 exon, whereas β messages in the thymus contain the exon at 10-20 fold higher levels. To address the importance of the Cβ0 exon in the immune system, we have undertaken a phylogenetic approach. By cloning and sequencing the rat analogue of Cβ0, we have found that while the rat exon is very similar to the mouse exon, both donor and acceptor RNA splice signals are defective in the rat Cβ0 gene. This implies that rat Cβ0 cannot be spliced into rat β messages. Furthermore, we have sequenced the analogous region to mouse Cβ0 in the human β chain locus, and have found no stretch of sequence remotely homologous to Cβ0. Because Cβ0 is not conserved evolutionarily, we believe that the Cβ0 gene element does not serve an important function to the immune system of most vertebrates.

C 222 THE MOLECULAR BASIS OF ALLOREACTIVITY. Dave DiGiusto and Ed Palmer, Divison of Basic Science, Department of Pediatrics, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO 80206. The formation of the molecular complex between a T cell receptor and an allogeneic MHC molecule is currently under investigation in our laboratory. The genes for the α and β chains of an I-A^{bm12} reactive T cell receptor have been isolated and subcloned into the eukaryotic expression vector pTCF/Mo. Site directed mutagenesis will be used to introduce specific amino acid changes in the Vβ8.1 domain of this receptor. Wild type and mutant receptors will be expressed in an α^-/β^- T cell hybridoma that we have isolated. Analysis of the reactivity of a panel of mutant T cell receptors towards I-A^{bm12} should establish which β chain residues are critical for maintaining this allospecificity.

C 223 T CELL RECEPTOR STRUCTURE/FUNCTION ANALYSIS VIA SITE-DIRECTED MUTAGENESIS AND TRANSFECTION, Isaac Engel and Stephen M. Hedrick, Department of Biology and Cancer Center, U.C.S.D., La Jolla, CA 92093. We have reported recently the effects of changes in the VDJ junctional region of the T cell receptor (TCR) β chain on TCR specificity, as determined by the site-directed mutagenesis and transfection of the TCR isolated from the pigeon cytochrome c-specific T cell clone D6 (Cell *54*, 1988). We found that one amino acid substitution at a VDJβ juntional region position found to be highly conserved in pigeon cytochrome c-specific TCR's results in a change in antigen fine specificity, while another change abolishes all detectable responses characteristic of the D6 TCR. We will present the results of mutagenesis/transfection analyses of two other pigeon cytochrome c-specific TCR's.

C 224 USE OF HLA-A2.1 TRANSGENIC MICE TO STUDY THE DEVELOPMENT OF THE T-CELL REPERTOIRE AND T-CELL DEFINED EPITOPES, Victor H. Engelhard[1], Ai-Xuan Le[2], Eric Bernhard[1], Mark Holterman[1], James Barbosa[3], and Elizabeth Lacy[4], Depts. of Microbiology[1] and Surgery[2], University of Virginia, Charlottesville VA; Molecular Diagnostics, Inc.[3], West Haven CT; and Program in Molecular Biology[4], Memorial Sloan-Kettering Cancer Center, New York NY. The murine CTL response to human class I molecules is 1-2 orders of magnitude lower than the response to murine alloantigens, due to structural differences between human and murine homologs. We investigated whether this discrepancy could be overcome by exposure of developing T cells to human class I molecules in transgenic C57BL/6 mice expressing HLA-A2.1. Such mice expressed HLA-A2.1 in spleen, bone marrow and thymus at levels similar to those of endogenous H-2^b molecules. However, the frequency of CTL specific for other human alloantigens remained similar to that of normal mice. The frequency of HLA-A2.1 restricted influenza specific CTL was 1-2 orders of magnitude less than the frequency of H-2 restricted CTL. These results indicate that the poor response of murine CTL to human class I antigens is not determined by selection in the thymus, but by species-specific constraints on the interaction of MHC antigens with T-cell recognition structures. While the mice are tolerant to HLA-A2.1 expressed on murine cells, they still respond to HLA-A2.1 expressed on human cells. The epitopes defined by such clones are present on HLA-A2.1 positive human cells derived from several different tissues. Such epitopes are not dependent upon the species of β_2m associated with the class I molecule, nor upon the structure of the attached carbohydrate. The results suggest that one or more highly conserved normal human proteins contribute to the formation of such epitopes, and provide an explanation for the failure of CTL raised against class I molecules on human cells to recognize the same molecules expressed on murine transfectants. This suggests that normal endogenously expressed molecules may also be important in the formation of epitopes on class I antigens recognized by allospecific CTL.

C 225 T HELPER 2 (Th2) CLONES INDUCE PROLIFERATION OF BOTH CD4+ AND CD8+ T CELLS IN THE PRESENCE OF SOLUBLE ANTI-CD3 mABS, B.D. Evavold, A. Yokoyama, and J. Quintans, Committee of Immunology, University of Chicago, Chicago, IL 60637. To investigate T-T cell interactions, we have used small, resting T cells as the responding population in a costimulatory assay with T helper clones and soluble anti-CD3 mAbs (145-2C11). The T helper clones were classified as Th1 or Th2 following detection of secreted IL-2 or IL-4, respectively. We have observed that T helper 2 clones but not T helper 1 clones would provide costimulatory function. Supernatant from cultures of activated Th2 clones failed to replace the clone, and FC receptor bearing cells were not required to crosslink the soluble mAb. Both freshly, isolated CD4+ and CD8+ splenic T cells (2x105) proliferate in response to irradiated Th2 clones (3x104) and soluble anti-CD3 mAb (1:200 dilution hybridoma culture supernatant). T cell proliferation was completely inhibited (>95%) by anti-LFA-1 mAb, variably inhibited by anti-Ly6A mAb (<50%), and not affected by mAbs to CD4, CD8, MHC Class I, or MHC Class II. In addition using a variant of the costimulatory assay employing cloned populations of responder Th1 or Th2 cells and paraformaldehyde treated Th2 stimulator cells plus soluble anti CD3, we observed proliferation of the Th2 cells only. These findings indicate that Th2 clones possess an accessory function independent of lymphokine secretion which in combination with soluble anti-CD3 mAb induces small, resting T cells as well as Th2 cloned cells to proliferate. (Supported by NIH grant PO1 CA 19266 and T326M-07183.

C 226 CHARACTERIZATION OF SECONDARY Vα-Jα REARRANGEMENTS IN A MURINE T-CELL LINE, Joseph D. Fondell, Jean-Pierre Marolleau*, Daniele Primi* and Kenneth B. Marcu. State University of New York, Stony Brook, N.Y. 11794 and *Institut Pasteur, 75724 Paris Cedex 15 (France).

We previously demonstrated that several subclones derived from a $CD3^+$, $CD4^-/CD8^-$ T-cell line have undergone secondary rearrangements at the T-cell receptor (TCR) α locus while maintaining its original TCRβ and Igh D-J rearrangements (Marolleau et. al., in press). These secondary rearrangements result in the joining of germline Vα and Jα gene segments which replace the pre-existing Vα-Jα complexes of the parental T-cell line.

In an effort to examine the molecular mechanism responsible for these Vα-Jα gene replacements, the structures of TCRα cDNAs prepared from both the parental and subcloned T-cell lines were determined. In addition, Northern blot and Southern blot analyses were performed on both the parental and subcloned T-cell lines using a panel of Vα and Jα probes. Our results indicate that: 1) Secondary rearrangements result in both productive and non-productive Vα-Jα joins, 2) The mechanism whereby secondary rearrangements occur is a deletion event that involves germline Vα genes 5' to the pre-existing Vα-Jα complex joining to Jα segments 3' of the pre-existing complex deleting the region in between, 3) Pre-existing productive Vα-Jα rearrangements do not allelically exclude secondary rearrangements, and 4) Both productively and non-productively rearranged TCRα alleles of the parental cell line can undergo secondary rearrangements. One interpretation of these results is that T-cells have the ability to circumvent allelic exclusion at the TCR α locus early in their ontogeny. This would presumably give T-cells an additional mechanism of generating an antigen receptor repertoire which is not found in B-cells. Studies are underway to determine why the TCRα locus is uniquely involved in these secondary rearrangements which escape allelic exclusion.

C 227 MODIFICATION OF PEPTIDE IMMUNOGENICITY BY THE ADDITION OF HELPER T-CELL EPITOPES

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The immunogenicity of uncoupled peptides is dependent on the chosen sequence containing domains which react with helper T-cell receptors and Ia antigens in addition to defined binding sites for anti-protein antibodies. We have shown previously that the 141-160 sequence from VP1 of foot-and-mouth disease virus is such a peptide i.e. it contains important B and T-cell determinants (Francis et al., 1985, J. gen. Virol., 66, 2347; Francis et al., 1987, Immunol, 61, 1) and that the immune response to this peptide, in congenic mice, is H-2 restricted (Francis et al., 1987, Nature, 330, 168). This latter observation has been used to demonstrate that FMDV peptides containing additional helper T-cell epitopes from ovalbumin or sperm whale myoglobin can overcome non-responsiveness in defined mouse populations. T-cell help for an FMDV antibody response has also been demonstrated using T-cell epitopes from other viruses namely hepatitis B virus and influenza virus. Furthermore, we have shown that helper T-cell epitopes from human rhinovirus can be used to enhance the antibody response to a rhinovirus B-cell determinant. In the course of these studies we have seen that the location of the T-cell site in relation to the B-cell site it is regulating can influence the nature of the antibody response obtained. These studies should lead to a better understanding of T-cell control of antibody responses and to the development of more immunogenic peptides for vaccines of the future.

C 228 SECRETION OF SOLUBLE T CELL RECEPTOR PROTEINS, Nicholas R.J. Gascoigne, Kristina T. Ames and Stephen C. Jameson, Department of Immunology, Research Institute of Scripps Clinic, 10666 N. Torrey Pines Rd., La Jolla CA 92037.

To measure T cell receptor (TCR) affinity and to do detailed analysis of the TCR-MHC-antigen complex, a source of purified, native TCR is needed. We have been trying to produce soluble TCR by truncation of the genes to remove the transmembrane regions. A truncated β-chain construct, using immunoglobulin promotor and enhancer elements, is expressed in both T and B-cell lines after transfection. The β-chain protein is glycosylated and secreted, indicating that truncation of the molecule removes any need for CD3 in the transport and secretion of the TCR. Similar studies on the α-chain, with the aim of making a heterodimer, are in progress and preliminary results indicate that the truncated α-chain is also secreted from the cell.

C 229 A CLONED HUMAN CLASS II MHC DNA-BINDING PROTEIN WITH SPECIFICITY FOR THE DR PROMOTER REGION, Laurie H. Glimcher, Patricia W. Finn, Mark R. Boothby, Nasrin Nabavi, Roger Eddy, Thomas Shows, Hsiou-Chi Liou, Department of Cancer Biology, Harvard School of Public Health, and the Department of Medicine, Harvard Medical School

The class II major histocompatibility complex (MHC) antigens are a family of integral membrane proteins whose expression is tissue-specific and developmentally regulated. A pair of consensus sequences, X and Y, separated by an interspace element, is found upstream to all class II genes. Deletion of each of these sequences eliminates expression of class II genes in vitro or in transgenic mice (1-3). Furthermore, the absence of a specific binding protein for the HLA DRαX box in patients with severe combined immunodeficiency disease whose cells lack class II suggests a critical role for these proteins in class II gene transcription (4). Here we report the cloning of a λgt11 cDNA encoding a DNA binding protein (human X-box binding protein, hXBP-1) which, like the proteins in whole nuclear extract, recognizes both the X box and interspace elements of the human DRα and murine Aα genes. The hXBP-1 cDNA hybridizes to two RNA species, 2.2 kb and 1.8 kb in human, that are expressed in both class II positive and class II negative cells. hXBP-1 does not cross-hybridize to two murine Aα X box binding cDNAs recently isolated in our laboratory which also recognize the DRα and AαX boxes. These observations provide evidence for the existence of multiple X box binding proteins which recognize a common or overlapping motif. Chromosome mapping studies demonstrate that hXBP-1 arises from a multi-gene family two of whose members map to human chromosomes 5 and 22. Taken together, these data suggest a high degree of complexity in the transcriptional control of the class II gene family.

C 230 REGULATION IN THE DEVELOPMENT OF MHC CLASS I - REACTIVE CLONES.
A.Guimezanes, M.Buferne, A-M.Schmitt-Verhulst. Centre d'Immunologie INSERM-CNRS de Marseille-Luminy, Case 906, 13288 Marseille Cedex 9, FRANCE. In an attempt to analyze positive or negative in vivo regulation of clonal expansion of cytolytic T lymphocytes (CTL), we immunized B10.BR mice with the K^b specific CTL clone KB5-C20, and we tested whether T cells obtained from such mice would influence the in vitro development of the CTL clone KB5-C20. A clone-specific helper effect has been observed, which is mediated by $CD4^+$ splenic cells from immunized mice. Control immunizations of B10.BR mice with Ti negative variants of KB5-C20 suggest that this growth regulation involves the recognition of the Ti of KB5-C20. The precise nature of the antigen recognized on the CTL clone, the possible involvement of Ti determinants with or without MHC products are now under investigation.

C 231 CLASS I GENE EXPRESSION IN OVARIAN CANCER; MODULATION IN VITRO.
Stephen Haskill, Karl Olaffson and Wesley C. Fowler, Jr., Department of Obstetrics and Gynecology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599.
Expression of Class I gene products is dramatically down-regulated in many aneuploid ovarian cancers. Our preliminary analysis suggests a close association of Class I gene expression with initial response to therapy. Because of the importance of these proteins in both immune regulation and recognition as well as the possible role these gene products may play in cell growth determination, we have been interested in examining several aspects of regulation of these genes. We have carried out both Northern transfer analysis as well as in vitro culture in the presence and absence of interferons in an attempt to understand the up- and down-regulation of these genes in ovarian tumors. Our results indicate that lower levels of mRNA expression parallel the lower degree of Class I gene products found by flow cytometry. In addition, there was no apparent correlation between loss of Class I expression and presence of the N-myc gene as had been reported in neuroblastoma. We did observe that overnight incubation of tumor samples resulted in marked increases in Class I levels, suggesting an environmental regulation of these genes which might be amenable to therapeutic intervention.

C 232 PHENOTYPIC AND FUNCTIONAL ANALYSIS OF CD3+ CELLS IN MURINE FETAL THYMUS AND SKIN, Wendy L. Havran and James P. Allison, Cancer Research Laboratory, University of California, Berkeley, CA 94720

We have shown that Thy-1+ dendritic cells present in the epidermis of mice (dEC) express CD3 associated $V\gamma3$ and $V\delta1$ gene products. We have produced a monoclonal antibody directed against $V\gamma3$ and found that a wave of cells appearing at the earliest stages of fetal thymic development express $V\gamma3$. Phenotypic and functional analysis of $V\gamma3+$ cells in the early fetal thymus indicates that they have characteristics in common with the $V\gamma3+$ dEC. Both populations express high levels of Ly-1 and are Ly-6C+. Neither express CD4 or CD8. Interestingly, the $V\gamma3+$ fetal cells express elevated levels of IL-2 receptor, indicating that they may have been activated. Functional analysis demonstrated that, unlike other fetal thymocytes, the $V\gamma3+$ cells can be stimulated to produce lymphokines and lyse a panel of target cells which are also lysed by the adult Thy-1+ dEC. These results raise the intriguing possibility that the first receptor-bearing component of the T cell system to appear in ontogeny might give rise to the Thy-1+ dEC.

C 233 MURINE TCR GAMMA GENES: DISTINCT SPATIAL RESTRICTION OF V-GENE SEGMENT USAGE, Adrian Hayday*, Susan Kyes*, Simon Carding#, Charles A. Janeway, Jr.*#, and Laila McVay*, Depts. of Biology* and Pathology#, Yale University, New Haven, CT 06511

Critical to an understanding of the function of cells bearing the gamma-delta T cell receptor will be an understanding of when and where such cells function. In order to investigate this, we have used a variety of techniques (*in situ* hybridisation, cDNA cloning, and PCR) to examine areas of TCR gamma delta gene expression. One conspicuous site of expression is the intestinal epithelium, which is by contrast almost devoid of TCR alpha beta expression. Interestingly, the V gene segment usage in this location is quite specific and is different to the specificity that we have found in the spleen and in the thymus, and that others have found in the skin. This specificity suggests in turn that expression of the restricting elements recognised by gamma delta may also be spatially restricted. Extensive analysis of junctional diversity can provide infomation on the diversity of antigen recognised by gamma delta. The basis for selective expression of V gene segments may in part lie in different requirements of the V-gamma gene promoters. To examine this, the transcriptional capabilities of the various gamma gene promoters in T cells are being compared by linkage to the chloramphenicol acetyl transferase gene.

C 234 AN IMMUNE RESPONSE GENE POLYMORPHISM DEFINED BY STRAIN A SUBLINES, Colleen E. Hayes, Keith D. Hanson, Faye Nashold, and David J. Miller, Department of Biochemistry, University of Wisconsin-Madison, Madison, WI 53706. Murine strain A sublines A/J and A/WySnJ have a genetic polymorphism that regulates serum immunoglobulin responses to several protein antigens. Strain A/J secondary IgG_1 responses to bovine gamma globulin, ovalbumin, hemocyanin and galactosidase were 50-, 10-, 7- and 4-fold greater, respectively, than A/WySnJ responses. The secondary IgG_{2a} responses were also affected. Subline A/HeJ is a low responding strain like A/WySnJ. Analysis of H-2 class I and class II molecules provided no evidence for a breeding error to account for the genetic polymorphism. Instead, an important immune response gene outside H-2 may have been heterozygous when the sublines diverged, and the polymorphism resulted from segregation and differential allele fixation. A mutation subsequent to subline divergence is also a possible source of the polymorphism, but is less likely. The high responder phenotype inheritance pattern in (A/WySnJ X A/J)F1, F2, and backcross mice was consistent with segregation of a single, recessive gene. We named this locus Ir-a for the strain A sublines that define it; strain A/J represents the $Ir\text{-}a^h$ allele and strains A/WySnJ and A/HeJ represent the $Ir\text{-}a^l$ allele.

C 235 MOLECULAR ANALYSIS OF A BACTERIAL ENTEROTOXIN THAT MIMICS MLS. Andrew Herman, Janice White, Joel Boymel, John Freed, Philippa Marrack, and John Kappler. Howard Hughes Medical Institute and the Dept. of Medicine, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO 80206. Staphylococcal enterotoxin B (SEB) is an extremely potent immunogen. Its ability to stimulate murine T helper cells bearing receptors containing certain Vβ elements is reminiscent of the Mls phenomenon. Since SEB is a small protein of known sequence, we have initiated experiments to define the region(s) of the SEB protein that elicit such a strong T cell response in mice. This response requires MHC class II molecules on the antigen presenting cell, yet it is not MHC-restricted, since several I-A and I-E alleles can serve to present SEB to individual T cell hybrids.

Several different proteolytic digests of denatured SEB have been tested for their ability to stimulate T cell hybrids to produce IL-2. A tryptic digest that retains activity has been fractionated by HPLC and the stimulatory component is being analyzed. Examination of the amino acid sequence of SEB and the proteolytic cleavage sites has led us to synthesize several peptides for analysis. These peptides, and their analogues, will be tested for their function in vivo and in vitro.

C 236 **BINDING OF PEPTIDES TO HLA-DR PROTEINS ON CELL SURFACES.**

Julian Hickling, Robert Busch, and Jonathan B. Rothbard. Molecular Immunology Laboratory, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2.

To understand the interactions involved in the formation of peptide-MHC complexes, an assay has been developed to detect DR specific binding of peptide analogues of T cell determinants to cell surfaces. EBV transformed B cell lines (BCLs) were incubated with biotinylated peptide followed by FITC conjugated streptavidin, and then analysed by flow cytometry. A panel of BCLs homozygous for different DR types bound analogues of peptide 307-319 from influenza virus haemagglutinin (previously shown to be a helper T cell determinant restricted through DR1) to varying degrees, whereas no binding was observed to the DR^- BCL RJ225. Binding could be specifically inhibited by the natural unbiotinylated T cell determinant or other DR1 restricted determinants. Competition by a range of peptides revealed quantitative differences in their ability to bind DR1. The assay is currently being used to generate a detailed model of the complex formed between HA 307-319 and DR1.

C 237 DEFINITION OF RESIDUES CONTRIBUTING TO SEROLOGIC AND CTL-DEFINED EPITOPE DIFFERENCES AMONG HLA-A2.1, -A2.2Y, AND -A2.3. Kevin T. Hogan, Carol Clayberger, Peter Parham, Alan M. Krensky and Victor H. Engelhard. Dept. of Microbiology, Univ. of Virginia, Charlottesville, VA 22908 and the Dept. of Pediatrics and Cell Biology, Stanford Univ., Stanford, CA 94305. HLA-A2.2Y differs from HLA-A2.1 by the substitutions of Tyr for Phe_9, Arg for Gln_{43}, Leu for Val_{95}, and Trp for Leu_{156}, and HLA-A2.3 differs from HLA-A2.1 by the substitutions of Thr for Ala_{149}, Glu for Val_{152} and Trp for Leu_{156}. Residues 9 and 95 in the β-sheet of the molecule, and residues 149, 152, and 156 in the α-helix are thought to interact with bound peptide or the TCR. To evaluate the role of these residues on CTL-defined epitopes, two genes were constructed that encoded novel molecules which differ from HLA-A2.1 only at residues 9, 43, and 95, or at residue 156. The effect of α-helix substitutions on serologic and CTL-defined epitopes that varied between HLA-A2.1 and HLA-A2.3 were evaluated by constructing genes that encoded the individual differences at residues 149, 152, and 156, as well as additional non-naturally occuring substitutions at these same positions. HLA-A2.1 specific CTL were found that were: (1) insensitive to substitutions at either residues 9, 43, and 95, or residue 156, but were lost when all four positions were changed; (2) dependent upon the residues 9, 43, 95, but not residue 156; (3) dependent upon residue 156, but not residues 9, 43, and 95; and (4) dependent upon residues 9, 43, 95, and residue 156. Further epitope mapping with the α-helix mutants demonstrated that a substitution at residue 152 often destroys an epitope not affected by substitution at residue 156. Even conservative substitutions at position 152 were more disruptive than nonconservative changes at residue 156. Residue 149, while important in defining an mAb epitope, had no effect on any CTL epitopes. These results indicate that spatially separate residues in the α-helix and β-sheet of the molecule can contribute to the epitope recognized by a given CTL. Furthermore, considerable complexity must exist in the spectrum of T cell receptors utilized to recognize HLA-A2, as 28 CTL clones exhibited 21 distinct fine specificity patterns.

C 238 Mls^a DETERMINANTS: TISSUE DISTRIBUTION AND MOLECULAR CHARACTERIZATION, Brigitte T. Huber, Ignacio J. Molina and Ana Zubiaga, Department of Pathology, Tufts University School of Medicine, Boston MA 02111.

We have re-examined the controversial question of tissue distribution of Mls^a determinants. For this purpose we have generated from bone marrow stem cells of Mls^a genotype lines of macrophages that are 100% Ia^+, Mac-1^+, Mac-2^+, Ig^-. In addition, we have transfected I-Aα^d and I-Aβ^d cDNAs into a panel of phenotypically different T cell thymomas of Mls^a genotype. Transfectants expressing high levels of surface Ia were selected for our analysis. While all these Ia^+ cell types were able to elicit a specific allo-response, none of them were recognized by two independently derived Mls^a specific T cell hybrids or MHC-matched T cells of Mls^b genotype, even in the presence of interleukin-2. These results strongly suggest that Mls^a is a B cell specific product. In order to characterize Mls^a determinants at the molecular level, we have constructed a cDNA eukaryotic expression library in the pCDM8 vector, using RNA from a Mls^a + B cell lymphoma. Transfection of pools of cDNA clones into an Mls^b B cell lymphoma leads to the functional detection of an Mls^a activity. We are in the process of identifying the cDNA clone coding for Mls^a.

C 239 CLONING AND ANALYSIS OF RAT CLASS I MAJOR HISTOCOMPATIBILITY COMPLEX GENES, Stephen C. Jameson*, Austin G. Diamond, Jonathan C. Howard, Dept. Immunology AFRC Institute of Animal Physiology and Genetic Research, Babraham, Cambridge, England. * Present address, Dept. Immunology, Research Institute of the Scripps Clinic, 10666, N.Torrey Pines Rd., La Jolla, CA 92037. The class I MHC genes belong to a large multigene family - in the mouse three types of class I antigen have been defined - classical H-2 antigens and the nonclassical Qa and Tla molecules. We have cloned class I genes from the rat MHC (RT1), in order to follow the evolution of these class I types, to discern the chief selective pressures on its members and thus indicate the probable functional properties of the antigens. A cosmid library was screened for class I genes. 91 clones were mapped and could be grouped into 17 clusters of contiguous DNA spanning 1,264 Kb. By hybridisation studies, 61 class I genes/ gene fragments could be distinguished. Transfection analysis revealed that 10 genes could be expressed as cell surface antigens: two genes, in a block of duplicated DNA encoded serologically defined RT1.C products, the other 8 genes gave rise to novel class I antigens detected by the xeno-antibody OX18. Using region specific probes, we could detect clear rat homologues of the mouse Qa and H-2 genes, however there were only two rat genes with limited homology to the mouse Tla genes. The analysis showed extensive remodelling of the class I region in the evolutionary gap between rat and mouse.

C 240 T CELL RECEPTOR γδ CELLS ARE INVOLVED IN THE IMMUNE RESPONSE TO A COMPLEX ANTIGEN, Eric M. Janis, Drew M. Pardoll, and Ronald H. Schwartz, Laboratory of Cellular and Molecular Immunology, NIAID, NIH, Bethesda, MD 20892

While the immunological role of T cells bearing the αβ T cell receptor (TCR) has been well characterized, much less is known about the function of T cells bearing the γδ TCR. We investigated the role of TCR γδ cells in the immune response to Complete Freund's Adjuvant (CFA). After immunizing mice with CFA, we observed a greater than 26-fold increase in the number of TCR γδ cells present in lymph nodes draining the sites of immunization, compared to a 3-4-fold increase in the number of TCR αβ cells. There were at least three different species of γδ TCR's expressed on these cells in the draining lymph nodes, including two protein products derived from the rearrangements of Cγ1 and Cγ2, and one product derived from Cγ4. 37% of TCR γδ cells from immunized lymph nodes expressed the IL-2 receptor in vivo, and these cells constituted roughly 50% of the proliferative response of total lymph node T cells to IL-2. Tse, et al. (J.Immun.,Vol.125, p.491,1980) have demonstrated that at least three cell types are involved in the T cell proliferative response to antigen, including an antigen specific-T cell, an antigen-presenting cell, and a T cell that is found in unprimed lymph nodes or spleen, which has been termed the recruitable cell. We have utilized their approach of analyzing the slope of log cell number-log response curves to examine whether TCR γδ cells can function as "recruitable" cells. We found that TCR γδ cells as well as TCR αβ cells can function as recruitable cells in this system. These data suggest that TCR γδ cells can participate in the immune response without being specific for the antigen.

C 241 A NOVEL PLASMA MEMBRANE-ASSOCIATED PHOSPHOPROTEIN IN RESTING MURINE B LYMPHOCYTES CULTURED WITH IL-4, Anna M. Jaques, Gail M. McGarvie and William Cushley, Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ, Scotland, U.K.

There is a wealth of information regarding the immunobiological properties of IL-4. However, there is a relative paucity of information with respect to the biochemical mechanisms which underlie its action on B lymphocytes and other IL-4 receptor bearing cells. The low number of cell surface IL-4 receptors have contributed to this problem. However, one of the consequences of culture of B cells with IL-4 is an up-regulation of the receptors for the lymphokine.

Analysis of the membrane associated phosphoprotein profiles of B cells harvested from cultures of resting cells exposed to IL-4 for 18-24 hrs reveals the presence of phosphoprotein with an Mr in the range 75-80,000. Exposure of the gels to alkali fails to destroy the autoradiographic signal from this phosphoprotein suggesting that it is phosphorylated upon tyrosine residues. Culture alone is insufficient to promote the appearance of this molecule, and LPS also apparently fails to result in the presence of a 75Kd structure in the phosphoprotein profiles. Furthermore, inclusion of the neutralising anti-IL-4 antibody, 11B11, in the cultures prevents the appearance of the 75Kd phosphoprotein. The possible identities of this phosphoprotein will be discussed.

C 242 MOLECULAR CONTROL OF mRNA EXPRESSION AT THE MURINE TNF LOCUS, C. Victor Jongeneel, A.N. Shakhov*, S.A. Nedospasov*, and J.-C. Cerottini, Ludwig Institute for Cancer Research, Lausanne Branch, 1066 Epalinges, Switzerland, and *Institute of Molecular Biology, USSR Academy of Sciences, Moscow, USSR.
The genes for TNF-α and TNF-β are tandemly arranged on mouse chromosome 17, with only 1.1 kb separating the 3' end of the TNF-β mRNA from the 5' end of the TNF-α mRNA. Yet, the two genes are independently regulated. In vitro transcription and nuclear run-on experiments indicate that the two genes are transcribed from independent promoters. In macrophages, which express TNF-α but not TNF-β, only the TNF-α promoter is active. In T lymphocytes, which can synthesize both proteins, both promoters are active. Activation of either cell type results in a moderate (up to 10-fold) increase in the level of transcription, while mRNA levels increase more than 100-fold under the same conditions. Interestingly, the TNF-β gene is transcribed 10-fold less than the TNF-α gene in T lymphocytes, although the corresponding mRNA is more abundant. These results indicate that the accumulation of both TNF-α and TNF-β mRNA after cell activation and their relative steady state levels are controlled mostly at a post-transcriptional step. Actinomycin D chase experiments reveal that TNF-α mRNA stability in macrophages is not significantly altered after activation by LPS, and therefore that stabilization alone cannot account for the observed accumulation of TNF-α mRNA. In order to examine more closely which elements are required for the regulation of TNF-α and TNF-β mRNA abundance, we constructed hybrid genes combining putative control regions of TNF-α and TNF-β with known constitutive control elements. Results obtained from the transfection of these hybrid genes into various cell types indicate that elements located both 5' and 3' of the coding sequence are required for the proper regulation of TNF-α and TNF-β mRNA abundance.

C 243 MOLECULAR ANALYSIS OF THE HLA CLASS II D-REGION HAPLOTYPE ASSOCIATED WITH CELIAC DISEASE. Martin F. Kagnoff and Julia Harwood, Dept. of Medicine, UCSD School of Medicine, La Jolla, CA 92093. Celiac disease is characterized by small intestinal mucosal injury and malabsorption. Disease is activated when a genetically susceptible host ingests wheat gliadin or similar proteins (i.e., prolamins) in rye and barley. Disease susceptibility is strongly associated with the HLA class II D region specificities -DR3 and -DQW2. We recently determined that the HLA class II D-region haplotype associated with celiac disease is extended and also includes genes in the HLA-DP subregion. The joint segregation of a DPβ chain gene with those encoding DR3 and DQW2 may indicate that the HLA haplotype associated with celiac disease exhibits an unusual degree of linkage disequilibrium or, alternatively, that disease susceptibility involves the gene products of more than one HLA locus. To characterize possible HLA structural variants unique to celiac disease, the polymorphic second exons of the expressed DR, DQ and DP genes were amplified from genomic DNA of celiac disease patients, and their nucleotide sequences determined. Our studies indicate the presence of a unique constellation of D region genes associated with the celiac haplotype, and exclude the presence of a disease specific DR, DQ or DP structural gene variant in this disease.

C 244 TRANSFER OF ANTIGEN SPECIFICITY, ALLOGENEIC MHC SPECIFICITY, AND Mls^c SPECIFICTY BY A SINGLE T CELL RECEPTOR α- AND β-CHAIN GENE PAIR, Jonathan Kaye and Stephen M. Hedrick, Department of Biology and Cancer Center, University of California San Diego, La Jolla, CA 92093. T cells bearing αβ antigen receptors are biased towards recognition of antigenic protein fragments bound to self major histocompatibility complex (MHC) encoded glycoproteins. This same population of T cells contains a high frequency (>1%) of cells which will respond to a given allogeneic MHC protein, or to differences at two other genetic loci termed Mls, in conjunction with MHC. We have transfered the α and β chain genes from a pigeon cytochrome c/E^k specific, alloreactive, and Mls^c specific murine T cell clone into an unrelated host T cell. We demonstrate that the genes encoding a single αβ receptor chain pair can transfer the recogntion of self MHC molecules complexed with fragments of antigen, allogeneic MHC molecules, and an Mls^c (Mls-2) encoded determinant. In this case the transfer of antigen specificity and alloreactivity requires a specific αβ receptor chain combination, whereas Mls^c reactivity can be transfered with the β chain alone into a recipient expressing a randomly selected α chain. Site directed mutagenesis of the Jα region has also been performed in an attempt to identify sites involved in the alloreactivity of this T cell clone. In addition, we demonstrate that a single amino acid change in the V-J junction of the αβ receptor can alter MHC restriction as well as antigen fine specificity.

C 245 LOCUS-SPECIFIC CIS-ACTING TRANSCRIPTIONAL REGULATION OF K^d, D^d AND L^d CLASS I GENES IN THE BALB/C S49 LYMPHOMA SUBLINES, Jill B. Keeney and Ted H. Hansen, Department of Genetics, Washington University School of Medicine, St. Louis, MO 63110.
The S49 tumor sublines are variants isolated from a single parent BALB/c tumor which demonstrate locus-specific shut-off of their K^d, D^d and L^d genes. Four phenotypically different sublines were characterized at the DNA and RNA level. Southern blot analysis indicated that no major chromosomal deletions have occurred, and treatment of the sublines with 5-azacytidine had no effect on Class I expression. Thus, methylation differences between loci are unlikely. None of the repressed Class I antigens could be induced with interferon even though the expressed antigens were fully inducible. Northern blot analysis revealed message only for the expressed antigens, showing that the repression mechanism is acting at the transcriptional level. Rnase protection analysis confirmed this result and demonstrated that the transcriptional repression is exquisitely specific for the K^d, D^d and L^d genes as other "class I-like" messages are present in all the cell lines. Fusion of the S49 lines with Class I positive tumors resulted in hybrid cell lines expressing Class I antigens from both fusion partners, but the negative Class I antigens originating from the S49 partner were not expressed. These findings are best explained by the occurrance of multiple independent mutations among the Class I genes in their cis-acting elements of transcriptional regulation.

C 246 CO-EXPRESSION OF GM-CSF, INTERFERON-γ, IL-3 AND IL-4 IS RANDOM IN MURINE ALLOREACTIVE T LYMPHOCYTE CLONES, Anne Kelso and Nicholas M. Gough, The Walter and Eliza Hall Institute for Medical Research, P.O. Royal Melbourne Hospital, Victoria 3050, Australia.
Lymphokine gene expression was examined in a panel of 116 short-term murine T lymphocyte clones derived by single-cell micromanipulation from allogeneic mixed leukocyte cultures. About 30% of clonable T cells, including both $CD4^+CD8^-$ and $CD4^-CD8^+$ cells, could be expanded for assay at an average of 22 days after cloning. Following stimulation with concanavalin A or anti-CD3 antibody, all clones secreted detectable granulocyte-macrophage colony stimulating factor (GM-CSF), interleukin-2 (IL-2) and IL-3, but $CD4^+$ clones on average secreted higher levels of each lymphokine than $CD8^+$ clones. By Northern analysis, most clones (85%-96%) expressed detectable GM-CSF, interferon-γ and IL-3 mRNA and 11% expressed IL-4 mRNA. When the frequencies of co-expression of any pair of lymphokine mRNAs were determined, all were found to correspond to the values predicted for random assortment of the individual frequencies. For example, among 13 IL-4-positive clones, 11 also transcribed interferon-γ, giving the frequency of double-positive clones expected for random association (9.6% versus 10.8%). Expression of the four lymphokine genes therefore segregated independently among the clones and did not allow the division of T cells into subsets with distinct patterns of lymphokine synthesis.

C 247 REGULATION OF THE I_A ASSOCIATED INVARIANT CHAIN GENE EXPRESSION BY γ-INTERFERON IN MURINE CELLS. Daniel P. Kolk and Georgia Floyd-Smith, Department of Zoology, Arizona State University, Tempe, AZ 85287. The invariant chain (Ii) is a glycoprotein which forms a transient association with the MHC Class II antigens as they are being transported to the cell surface. We have examined the effect of γ-interferon (IFN-γ) on the expression of the Ii gene in mouse macrophage cell line IC-21, mouse L-cells, mouse embryonic liver cell line BNL CL.2 and mouse adult liver cell line BNL 1MEA.7.R.1. Our results shown that treatment with IFN-γ induces Ii mRNA levels greater than 10-fold in the embryonic liver cell line, greater than 20-fold in the adult liver cell line, 2 to 3 fold in the macrophage cell line and just slightly in L-cells. We have subcloned the region 5' to the Ii gene which contains sequences that may be important to regulating expression of the Ii gene. This region includes a 15-mer (CCTAGAAACAAGTGA) which occurs 5' to many IFN-γ regulated genes. Current research has been directed towards identifying and comparing proteins from nuclear extracts prepared from control and IFN-γ treated cells which bind to this region (-260 to -11). This data indicates the Ii molecule may be expressed in cells not known to be directly involved in the immune response.

C 248 CYTOTOXIC T LYMPHOCYTES SPECIFIC FOR AUTOLOGOUS TUMOR IMMUNOGLOBULIN EXPRESS THE DELTA CHAIN OF THE T CELL RECEPTOR, Alan M. Krensky, Ann Wright, Jeffrey Lee, Michael Link, Stephen Smith, William Carroll, Ronald Levy, and Carol Clayberger, Departments of Pediatrics, Medicine, and Surgery, Stanford University, Stanford, CA 94305

Although there has been considerable interest in the recently identified gamma, delta T cell receptor, relatively little is known as to its function. During our studies of the human immune response to autologous B cell lymphomas, we generated cytotoxic T lymphocytes (CTL) specific for tumor idiotype. These CTL lysed only autologous tumor cells and none of a large panel of other autologous and allogeneic cells. Tumor lysis was inhibitable by anti-idiotypic and anti-immunoglobulin antibodies but not by a panel of classical anti-MHC antibodies. Phenotypic analyses showed that these CTL were CD3+, CD4-, CD8-, and express the delta, and presumably gamma, T cell receptor. Such CTL can be used to gain new insights into the function of the gamma, delta T cell receptor and T cell recognition of immunoglobulin, and may prove clinically useful in adoptive immunotherapy.

C 249 AUTOLOGOUS RESPONSE TO LYMPHOBLASTOID B-CELL LINES WHICH LACK EXPRESSION OF HLA CLASS I OR CLASS II SUGGESTS A ROLE FOR RESTRICTION DETERMINANTS OTHER THAN HLA-A, B, C OR DR, DQ, AND DP, Valery Lam, Benjamin P. Chen, Robert DeMars, Jacquelyn A. Hank and Paul M. Sondel, Departments of Genetics, Pediatrics, and Human Oncology. University of Wisconsin, Madison, WI 53792.

We have used mutagenized, immunoselected Epstein-Barr virus transformed lymphoblastoid B cell lines (LCL) to study the role of specific HLA loci in autologous LCL-specific CTL responses. LCL-.180, which lacks expression of all known class II alpha and beta chains, and which has reduced cell surface class I expression, is still capable of stimulating highly purified autologous T cells. Primed cultures were tested for LCL-specific cytotoxic responses, using as targets a panel of autologous variant LCL, each of which expresses a different set of HLA determinants. Lysis of variant LCLs lacking expression of various class I determinants confirmed the presence of HLA -A, -B, and -C restricted CTL specific for EBV-induced antigens. Furthermore, LCL variant .221, which does not express any HLA -A, -B, or -C determinants, is killed by cultures primed to LCL-.180. Antibody blocking experiments suggested that this killing was mediated by T cells, and was not restricted by known class I antigens. Depletion of leu 19 positive cells from the effector population did not eliminate cytotoxicity on LCL-.221. Cold-target blocking studies further suggested that the class II-nonexpressing LCL-.180 and the class I-nonexpressing LCL .221 share residual determinant(s) other than HLA class I or class II that can restrict cytotoxic T cell responses to EBV-induced antigens.

C 250 ANTI-A INDUCED B-CELL ACTIVATION, John C. Cambier and Kathrin R. Lehmann. National Jewish Center for Immunology and Respiratory Medicine, Denver, CO 80206

One of our primary research interests is the signaling requirements for the activation of small B-lymphocytes in the absence of T-cells. We have devised an in vitro culture system in which anti-Ia antibodies can be shown to induce resting B-cells to proliferate and eventually secrete immunoglobulins. The principle of the culture system relies on the priming of small B-cells with non-mitogenic anti-immunoglobulin antibodies and IL4 prior stimulation with antibodies specific for class 2 MHC gene products. This combination signal promotes entry of virtually all B-cells into cell cycle. The further addition of soluble T-cell factors drive B-cells to secrete immunoglobulin. These studies provide the first direct evidence that a proliferative signal can be provided by ligand (presumably T cell) interaction with B cell Ia.

C 251 RESTRICTED PRODUCTION OF IL4 COMPARED TO IFNγ BY HUMAN T CELLS DURING POSTNATAL DEVELOPMENT, David B. Lewis, Michael Weaver, Kathryn Prickett*, and Christopher B. Wilson, Dept. of Pediatrics, Univ. of Washington, Seattle, WA 98105, and the *Immunex Corp., Seattle, WA 98102

It is uncertain to what extent lymphokines can be differentially produced by activated primary T cell populations. To determine if IL4 and IFNγ were differentially regulated in uncloned human T cells from adults (Ad) and neonates (Nt), these mRNAs were measured by in situ hybridization after maximal stimulation by ionomycin and PMA. IL4 mRNA was detected in 1-2% of total (TL), 3-5% of $CD4^+$, 10% of $CD4^+$ $CD45R^-$, and 0-1% of $CD8^+$ Ad T cells, but in none of the TL, $CD4^+$, or $CD8^+$ Nt T cell populations (virtually all Nt T cells were $CD45R^+$). In contrast, IFNγ mRNA was found in 39-42% of TL, 34-36% of $CD4^+$, 51% of $CD4^+$ $CD45R^-$, and 58% of $CD8^+$ Ad T cells, but only 2-3% of TL, 2% of $CD4^+$, and 4% of $CD8^+$ Nt T cells. These results agreed with other estimates of IL4 and IFNγ production based on RIA of cell culture supernatants, RNA blotting, and gene transcription assays. In contrast to IL4 and IFNγ, IL2 was expressed in similar amounts by Ad and Nt T cell fractions, as well as the Ad $CD4^+$ $CD45R^-$ and $CD45R^+$ subsets. Thus, the capacity for increased IL4 and IFNγ production by Ad T cells appears attributable, in large part, to the postnatal acquisition of the $CD45R^-$ subset (putative memory T cell population). However, additional mechanisms exist which act transcriptionally to limit IL4 production by both neonatal and adult T cells. Such selective expression may be important for restricting the potentially pleiotropic effects of certain lymphokines to appropriate responder cells.

C 252 INHIBITION OF ANTIGEN PRESENTATION BY ANTI-I-A PEPTIDE MONOCLONAL ANTIBODIES. Roger G. Little, Christopher J. Krco, Thomas G. Beito, Jay C. Zeller, Lori A. Ebertowski, and Chella S. David; Dept. of Immunology, Mayo Clinic, Rochester, MN 55905.

A panel of monoclonal antibodies (mAb) against I-A α or β chain peptides was screened for the ability to interfere with class II restricted antigen presentation to a T cell hybridoma. The ovalbumin (OVA) $I\text{-}A^d$ restricted T cell hybridoma, DO11.10 was used to measure the presentation of whole OVA and its peptide, OVA 323-339, by the B cell transfectant, B1D.β. B1D.β is a transfected M12.C3 B lymphoma cell line from the laboratory of Dr. D.J. McKean, which expresses an $I\text{-}A_\beta$ chain composed of a β_1 domain of the d haplotype and β_2 and β_3 of the k haplotype paired with the entire A^d_α chain.

We observed significant inhibition (>70% at 800 ng/ml) of the presentation of wOVA and of OVA 323-339 by the anti-A_β 57-78 peptide mAb's. The anti-$I\text{-}A^d$ mAb, MKD6, also exhibited significant inhibition. Much less inhibition was observed with the anti-A_α 43-61 peptide mAb's. Experiments with glutaraldehyde fixation of the B1D.β cells before or after incubation with antigen +/- mAb, indicate that the inhibition occurs at the level of antigen presentation. Inhibition of $I\text{-}A^d$ allorecognition by the T cell hybridoma, DG11, was also observed for the anti-β chain peptide mAb's and to a lesser extent by the anti-α chain peptide mAb's. These results indicate that mAb's generated against class II peptide sequences are capable of interfering with antigen presentation, in vitro.

Supported by NIH grant, AI-14764.

C 253 STUDIES ON THE MECHANISM OF POLYCLONAL B-CELL STIMULATION BY L. MAJOR-SPECIFIC T-HELPER CELLS OF TYPE 2. Michael Lohoff, Meike Dirks, Peter Rohwer* and Martin Röllinghoff, Institute for Clinical Microbiology, University of Erlangen-Nürnberg, 8520 Erlangen, F.R.G. and the *Institute for Clinical Immunology and Rheumatology, University of Erlangen-Nürnberg, 8520 Erlangen, F.R.G.

Recently we have shown that cloned L. major-specific L1/1 T-helper cells of type 2 (T_h2-cells), when stimulated with antigen, are able to induce polyclonal B-cell proliferation (1). We here present evidence demonstrating that this process is dependent on a direct cell-cell interaction between T- and B-cells, which in the effector phase, i.e. during stimulation of the B-cells by activated T-cells, can be mediated by a mechanism other than cognate interaction. This conclusion is derived from experiments, in which highly purified resting B-cells were polyclonally stimulated by L1/1 T-cells triggered by an anti-T3 monoclonal antibody, in the absence of antigen. The triggering process was independent of the presence of the Fc part of the antibody and occurred in cultures devoid of macrophages. Thus, the well established cognate recognition does not appear to be the only way of B-cell induction by T-helper cells of type 2.

(1) M. Lohoff, Ch. Matzner and M. Röllinghoff. (1988). Infect. Immun. 56 : 2120-2124.

C 254 $CD4^-CD8^-$ T CELLS EXPRESSING THE αβ T CELL RECEPTOR RESPONSIVE TO IL-2, IL-3 AND IL-4. Marco Londei, Adrienne Verhoef, Pier de Berardinis, Maija Kissonerghis and Marc Feldmann. Charing Cross Sunley Research Centre, Hammersmith, London W6 8LW, UK.
Studies show that a proportion of the peripheral blood $CD3^+$ T lymphocytes do not express CD4 or CD8 and are called double negative T cells. They normally have a γδ TCR. However, another population of double negative T cells exists that expresses the αβ heterodimer. We have purified and expanded such a population isolated from the peripheral blood of a healthy individual and studied its phenotypical and functional characteristics. The cells are $CD3^+$ $CD4^-$ $CD8^-$, positive for WT31 and negative for the NK markers. They express α and β mRNA but lack γmRNA. From surface iodinated cells were precipitated with monoclonal βF1 two closely running bands (46 & 48 Kd). Functional studies demonstrate that they proliferate to antiCD3 and PHA, this response was blocked by cyclosporin A. There was no NK lysis but antiCD3 induced lysis of target cells. The cells responded to IL-2 and IL-4 as previously shown for other T cells, but also to IL-3, a lymphokine thought to affect mainly stem cells and not previously shown to stimulate growth of mature cells. Long term growth of these cells was also maintained by these cytokines .

C 255 EXPRESSION OF THE T-CELL RECEPTOR DELTA GENE IN CD3-NEGATIVE CELLS, Roberto Biassoni[1], Silvano Ferrini[2], Rafick P. Sekaly[1], and Eric O. Long[1], Laboratory of Immunogenetics, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892, and Istituto Nazionale per la Ricerca sul Cancro[2], 16132 Genova, Italy.
$CD3^-$ cells grown in vitro in the presence of IL-2 acquire the ability to lyse a wide variety of tumor cells in an MHC-unrestricted manner. We have previously shown that $CD3^-16^+$ clones expressed the CD3 epsilon gene but no functional transcript from CD3 gamma, CD3 delta, TCR alpha, TCR beta and TCR gamma genes. This result suggested that these $CD3^-16^+$ cells represented an early stage in T cell differentiation. To test for expression of the TCR delta gene in these cells, RNA from a panel of $CD3^-16^+$ clones and from three highly enriched populations was hybridized with several DNA fragments of the delta locus. Abundant transcripts were detected with a C delta probe and a J delta 1 probe in 6 out of 8 clones and in all three populations. At least four different transcripts were present with sizes similar to those found in $CD3^+$ TCR gamma-$delta^+$ cells. However, the TCR delta transcripts in $CD3^-16^+$ cells are most likely derived from unrearranged genes because no rearrangement could be detected in DNA from an enriched population using a J delta 1 probe, and because these transcripts hybridized to a DNA fragment corresponding to the unrearranged genomic sequence 5'-upstream of J delta 1. Expression of unrearranged TCR delta genes in $CD3^-$ cells provides further evidence that these cells belong to the T cell lineage.

C 256 NORMAL CD4+ T CELL SUBSETS DISTINGUISED BY PHENOTYPE AND DIFFERENTIAL CYTOKINE PRODUCTION. Mohammad Luqman , Laurence A. Greenbaum , Simon R. Carding , Jeffrey West , Theresa Pasqualini and Kim Bottomly , Section of Immunobiology , Yale University School of Medicine , New Haven, CT 06510. CD4+ clones can be separated into two groups with distinctions in their functional capabilities and by differential release of either IL2 or IL4 upon activation. We have produced a new monoclonal antibody to CD45 which has allowed us to separate normal murine CD4+ cells into two populations based on the density of expression of CD45 epitope. The separated populations seem to be analogous of subsets found in cloned T cell lines. CD4+ T cells with high density of cell surface CD45 after polyclonal activation produce IL2 and mRNA encoding IFN and IL2. It does not produce IL4 or IL4 mRNA. CD45 low density population on the other hand transcribes mRNA for IL4 and secretes IL4 protein. Data will be presented to demonstrate that the two subsets of normal CD4+ cells also differ in their proliferative response to mitogenic stimuli and to exogenously added growth factors.

C 257 POLYACRYLAMIDE STREPTAVIDIN: A NOVEL REAGENT FOR SIMPLIFIED "OFF THE SHELF" CONSTRUCTION OF SOLUBLE MULTIVALENT MACROMOLECULAR CONJUGATES. Michael G. Mage, Bernardetta Nardelli, and Louise McHugh, Lab. of Biochemistry, Division of Cancer Biology and Diagnosis, National Cancer Institute, Bethesda MD 20892. We have developed a general method for the simplified preparation of soluble multivalent macromolecular conjugates, by binding of biotinylated proteins to soluble polyacrylamide-streptavidin (PASA). The method may be potentially useful for construction of such reagents as complex immunogens, imaging reagents, and immunotoxins. We have used this method to produce soluble "polyMHC" molecules for low affinity binding measurements, and heteroconjugates that specifically label tumor cells with allogeneic MHC Class I antigens. Soluble linear polyacrylamide, of approximately one million kD, is partially deamidated by controlled alkaline hydrolysis to introduce carboxyl groups. These are activated by carbodiimide at pH 3. The reactive intermediate is separated from excess carbodiimide by gel filtration at 0 degrees, and reacted with streptavidin at pH 8.5. The resulting conjugate, with approximately 20 streptavidin molecules per molecule of polyacrylamide, can in turn bind biotinylated proteins to produce soluble multivalent conjugates of known composition. The number of activated carboxyl groups per polyacrylamide molecule can be controlled by monitoring their hydrolysis at 0 degrees prior to conjugation with streptavidin.

C 258 PRODUCTION OF TRANSGENIC MICE WITH A^k_α AND MUTANT A^k_β. Javier F. Martin, Bing-Yuan Wei, Suresh Savarirayan, Jean-Marie Buerstedde, and Chella S. David; Department of Immunology, Mayo Clinic, Rochester, MN 55905.
A^k_α genomic DNA was obtained from cos H-2^k 8.1 Hind III fragment (>9 kb) including 5' and 3' flanking sequence which contained promoter and polyadenylation sites. A^k_β mutant (from cos 1.1 Hind III/Hpa I fragment 12 kb) was constructed by site-directed mutagenesis and has amino acid substitutions characteristic of the A^d_β polypeptide at positions 63 and 65-67 in the β1 domain of the A^k_β molecule.

These two genes were co-injected into the pronucleous of 858 embryos of (SJL x SWR)F_2 hybrid mice. The number of eggs implanted in the oviduct of pseudopregnant mothers was 423, resulting in 42 live offspring. These mice were tested for the integration of A^k_α and A^k_β (MB) by Southern blot analysis using A^k_α (cDNA) probe and A^k_β (cDNA) probe and foreign bands were detected in six mice. All of these six "positive" mice showed the integration of both A^k_α and A^k_β (MB) genes. These mice were bred and their progeny inherited the injected gene and showed Mendelian segregation. The segregation pattern indicated that both the A^k_α and A^k_β (MB) genes are integrated at the same site on the chromsome. The transgenic mice are currently being tested for the expression of the Ia genes by FACS analysis and Northern blotting.
Supported by NIH Grant AI-14764.

C 259 DIFFERENTIAL EFFECTS OF AMINO ACID SUBSTITUTIONS IN THE BETA SHEET FLOOR AND ALPHA TWO HELIX OF HLA-A2 ON ALLOREACTIVE VERSUS VIRAL PEPTIDE-SPECIFIC CTL, David H. Mattson, Naoki Shimojo, Elliot P. Cowan, John E. Coligan, W. Lee Maloy, and William E. Biddison, NIH, Bethesda, MD 20892
A CTL-defined variant of HLA-A2, M7-A2.2, differs from the common A2.1 molecule by three amino acids: a L to W substitution at position 156 in the alpha 2 helix; a V to L substitution at position 95 in the beta sheet floor; and a Q to R substitution at position 43 in a loop outside the peptide binding site. The M7-A2.2 molecule fails to present foreign antigens to A2.1-specific CTL and can be discriminated from A2.1 by allospecific CTL. Using site-directed mutagenesis we have investigated which of these individual amino acid substitutions in M7-A2.2 affects recognition by panels of A2.1 allospecific CTL and A2.1-restricted influenza viral matrix peptide 55-73-specific CTL. The substitution of V to L at 95 was the only change that could be discriminated by 2 of 9 allospecific CTL lines, suggesting that those 2 CTL lines recognize A2.1 plus a peptide whose presentation and/or binding is affected by the V to L substitution in the floor of the peptide binding site. In contrast, the L to W substitution at 156 (but not the other 2 substitutions) abolished the ability of the A2 molecule to present the viral peptide to 24 out of 25 peptide-specific A2.1-restricted CTL lines, suggesting that this substitution alters the presentation of the influenza matrix peptide but does not inhibit the ability of the peptide to bind to the A2 molecule.

C 260 DIVERSITY OF γδT-CELL RECEPTORS EXPRESSED BY MURINE DENDRITIC EPIDERMAL T CELLS (DETC), Thomas J. McConnell*, Wayne M. Yokoyama+, Gary E. Kikuchi*, Angel Ezquerra*, Georg Stingl, Ethan M. Shevach+, and John E. Coligan*, *Biological Resources Branch and +Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892 and the Department of Dermatology I, University of Vienna, Vienna, Austria. Although γδTCRs have a great potential for diversity, it remains to be determined whether this potential is realized in terms of expressed γδTCRs. Preliminary studies in several laboratories have indicated that γδTCRs expressed in early thymocytes and adult epithelial tissues are more restricted in diversity compared to adult γδTCR expressing thymocytes. We have derived a panel of cloned dendritic epidermal T cells (DETC) lines and hybridomas that express at least three types of γδ receptors - Cγ1δ, Cγ2δ and Cγ4δ. Immunoprecipitation, Northern and Southern blot analyses, and sequence analyses of λ gt 10 cloned cDNA or polymerase chain reaction (PCR) amplified cDNA segments have been used to analyze in detail the extent of diversity in the expressed γ and δ chains and whether restricted pairing of γ and δ chains occurs. Our results indicate that for this panel of cloned cell lines γ and δ pairing is nonrandom and that variability in certain types of receptors appears to be restricted. However, we have observed significant δ chain diversity in these cells that is obtained by the use of multiple V-regions, and N-region and junctional diversity. We are investigating whether the observed γ and δ chain pairing, and pattern of δ chain diversity are present in other γδTCR bearing cells or whether they are only characteristic of DETC.

C 261 IDENTIFICATION OF CLASS II RESIDUES INVOLVED IN BINDING ALLOREACTIVE T CELL RECEPTORS, D. J. McKean, J.-M. Buerstedde, M. Bell, A. Nilson, C. Chase, B. N. Beck and L. R. Pease, Department of Immunology, Mayo Clinic, Rochester, MN 55905.
A panel of mutant A^k_β-expressing cell lines have been produced by site-directed mutagenesis and DNA-mediated gene transfer into M12.C3 cells. One or more d-allele residues have been introduced into the β1 domain polymorphic positions of the A^k_β polypeptide. A serologic analysis of this panel of mutant Ia-bearing cell lines has identified an immunodominant region near the β chain residues 63 to 67 that determines the binding site for most of the available A^k_β- and A^d_β-reactive mAbs. This panel of cells also was tested with a panel of A^k or A^d alloreactive T cell hybridomas to identify T cell antigen receptor binding sites. In contrast to the antibody binding results, the T cell antigen receptor binding sites can be localized to polymorphic residues in many different parts of the β1 domain. Some of these sites are predicted to be located on the exterior of the molecule while others are predicted to be located at the bottom of the putative antigen binding site. We will discuss the structural implications of these results with regard to the molecular nature of an alloantigen.

C 262 LYMPHOKINE REQUIREMENTS FOR THE GENERATION OF ALLO SPECIFIC CYTOTOXIC T CELLS (CTL), Martha Merrow and Brigitte T. Huber, Department of Pathology, Tufts University School of Medicine, Boston, MA 02111.

Activation of CTL precursors from murine unprimed spleen cells with rIL-2 or rIL-4 results in distinct lytic spectra, depending on which lymphokine is present. We have used allo-stimulation in limiting dilution analysis with subsequent testing on an allo-specific target (A_{20}) and an MHC-deficient, non-specific target (R1E). In the presence of rIL-4 exclusively allo-specific CTL are generated, while rIL-2 supports approximately equal numbers of precursors that kill A_{20} and RIE targets. Dose response analysis of rIL-2-supported killing activity indicates that the lytic spectrum is independent of the amount of rIL-2 used, and therefore this IL-2 effect is intrinsic in its activity on unprimed spleen cells. Mixing experiments indicate that rIL-4 can partially override the effect of IL-2 on the generation of non-specific killer cells. Split well analysis and cold target inhibition experiments are in progress to ascertain the actual proportion of specific killer cells which can be generated with rIL-2. We are also testing the ability of cofactors, such as IL-1 and IL-6, to optimize the response of IL-4 generated CTL. We conclude that IL-4, not IL-2, must be used when CTL are generated from unprimed spleen cells in mice.

C 263 OVERLAPPING T CELL RECEPTOR Vδ AND Vα USAGE IN $CD4^-8^-$ THYMOCYTES AND MATURE T CELLS, Guido C. Miescher, Nan Shih Liao*, Rosemary K. Lees, David H. Raulet* and H. Robson MacDonald, Ludwig Institute for Cancer Research, Lausanne Branch, Epalinges, Switzerland, *Dept. Biology and Center for Cancer Research, MIT, Cambridge, USA.

We have shown previously that a subpopulation of double negative (DN) $CD4^-CD8^-$ adult murine thymocytes (TH) characterized by absence of the B2A2 antigen accounts for most α/β TCR expression by DN TH and utilizes preferentially Vβ8.2 segments. To a much lesser extent $B2A2^-$ DN TH also express γ/δ TCR, as evidenced by biochemical and Northern blot analysis. We have now been able to exclude the possibility that these cells may co-express both types of TCR: $B2A2^-$ DN TH depleted of $V\beta8^+$ cells expressed 1.8 kb TCR δ mRNA whereas the $V\beta8^+$ cells did not. Northern blot analysis indicated that the relative expression of Vδ6 segments compared to total TCR δ mRNA was considerably higher in $B2A2^-$ DN TH than in other γ/δ TCR^+ populations such as $B2A2^+$ DN TH or DN peripheral lymph node cells. RNase protection experiments distinguished up to three crosshybridizing Vδ6 transcripts in γ/δ TCR^+ populations. Interestingly, these same V genes, as well as a further crosshybridizing V gene previously designated Vα7.2, are expressed by peripheral α/β TCR^+ cells as 1.6kb TCRα transcripts. These data suggest that $B2A2^-$ DN TH represent a developmentally unique subset in which both Vδ and Vβ segments are non-randomly expressed. Furthermore they indicate that there is considerable overlap between the Vα and Vδ gene repertoires.

Section B
Antigen: MHC Interactions; Regulation of MHC Antigen Expression; The T Cell Receptor Complex; Priming and Function of T Cell Subsets; Immunogenicity in Vaccines

C 300 IN VIVO LYMPHOKINE PROFILES FOLLOWING 1^0 AND 2^0 SENSITIZATION, Kendall M. Mohler, Neal Roehm, Pamela Riedl and Larry Butler, Dept. of Immunology, Lilly Research Laboratories, Indianapolis, IN 46285 In order to detect the small amounts of lymphokines generated in vivo following antigen stimulation, we developed a co-culture system which allows for detection of IL-2/4, IL-3/CSF and TNF from LN cells stimulated in vivo with picryl chloride (PCL). Utilizing this system in combination with FACS analysis and receptor binding studies, we examined the production of these lymphokines in primary and secondary immune responses. During a primary immune response, the production of IL-2 was not readily detectable on d1, peaked on d3 and was gone by d5. At no time were we able to demonstrate the presence of IL-4. Alternatively, the presence of IL-3/CSF and TNF was easily detected on d1, but also peaked on d3. In comparison to primary responses, secondary immunization lead to at least two alterations. (1) Peak production of all lymphokines shifted towards d1. (2) Although most lymphokines did not demonstrate increases in the amount produced/10^6 cells, the amount of lymphokine generated/LN was vastly increased due to an increased number of cells. Utilizing single and dual color FACS analysis we also examined the LN cells for alterations in T cell subpopulations. During the course of the primary response: (1) the percentage of Thy 1+ and L3T4+ cells decreased until d3 and then began to recover, (2) the percentage of Thy-1+, T4-,T8- cells peaked at the time of greatest lymphokine production (i.e.-d3) and (3) the IL-2 receptor was expressed solely on Thy-1+ cells, was detected on both T4+ and T8+ subsets and peaked on d3. Most of these alterations also occurred during the secondary response, but their timecourse was shifted so that maximal effects occurred earlier (e.g., d1). Finally, the maximal binding of radiolabeled IL-2 by the LN cells following both primary and secondary sensitization correlated with the expression of the IL-2r as detected by FACS analysis. In addition, binding of radiolabeled IL-4 demonstrated similar patterns except for the detection of significant binding on d1. These results demonstrate that (1) an ordered timecourse of lymphokine production occurs in vivo following exposure to antigen and (2) the secondary immune response to PCL is characterized by an accelerated tempo of lymphokine production, rather than an increased level of lymphokine production/10^6 cells.

C 301 Isolation and characterization of a B-lymphocyte mutant with altered signal transduction through its antigen receptor, John G. Monroe, Vicki L. Seyfert, and Charles S. Owen, Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.
A receptor immunoglobulin (sIg) signalling variant of WEHI-231 was constructed to investigate components and linkages between various signalling events associated with signal transduction through sIg. Unlike the wildtype, crosslinking of sIgM on VS2.12-c1.2 did not result in downregulation of proliferation. Similarly, receptor crosslinking was uncoupled from inositol phospholipid (PI) hydrolysis and upregulation of c-fos expression in the variant. The signaling defect in VS2.12-c1.2 appears to be proximal to phospholipase C activation as direct G-protein activation by AlF_4^- triggers PI-hydrolysis and bypassing PI-hydrolysis using phorbol diester stimulation of PKC restores the inhibitable phenotype and the ability to upregulate c-fos. Even more interesting, sIg-linked Ca^{2+} responses by VS2.12-c1.2 are equivalent to those observed in the wildtype WEHI-231. These latter results suggest that contrary to current thought, sIg-generated signals may not be coupled to Ca^{2+} fluxes via inositol phospholipid hydrolysis. Thus, VS2.12-c1.2 is a new and powerful tool with which to analyze signalling through sIg at the molecular level.

C 302 ANALYSIS OF T CELL RECEPTOR γ CHAINS FROM ADULT $CD4^-$,$CD8^-$ THYMOCYTES
Mark W. Moore, I. Nicholas Crispe and Michael J. Bevan, Department of Immunology, Research Institute of Scripps Clinic, La Jolla, CA 92037.

The role of TCR γ genes in T cell development has not been determined. To extend out understanding of the repertoire of TCR γ expression, we prepared a cDNA library from $CD4^-$,$CD8^-$ adult BALB/c thymocytes and cloned and sequenced 15 TCRγ genes from this cDNA library. We found that 2 clones were transcripts of the unrearranged $C\gamma_2$ gene and that 3 clones terminated in the $J\gamma_2$ region. Nine of the remaining clones were $V\gamma_{1.2}$-$J\gamma_2C\gamma_2$ genes and five of these were in frame. Only one clone corresponded to $C\gamma 1$ and was $V\gamma_2$-$J\gamma_1C\gamma_1$-joined in frame. SDS-PAGE analysis of the γ-chain proteins from the surface of both BALB/c and C57BL/6 adult $CD4^-$,$CD8^-$ thymocytes did not detect the 32,000 MW $V\gamma_{1.2}C\gamma_2$ protein, but did detect the 35,000 MW $V\gamma C\gamma_1$ protein. These results suggest that despite the abundance of full-length, functionally joined, $V\gamma_{1.2}C\gamma_2$ transcripts in the thymocyte subset, the protein product is not expressed on the cell surface as the predicted 32,000 MW γ protein. Finally, our analysis of the V-J jointing of the γ genes reveals both flexibility at the V-J junction and extensive N-region nucleotide addition that lead to diversity of the predicted protein sequence.

C 303 INDUCTION OF MURINE IL4 SYNTHESIS BY FRESHLY ISOLATED T CELLS STIMULATED BY ANTI-CD3 ANTIBODIES.
M.Moser, V.Flamand, D.Abramowicz*, M.Goldman, J.Urbain and O.Leo, Laboratory of Animal Physiology and *Department of Nephrology, ULB, Brussels, BELGIUM. It is now well established that helper T cells play a central role in the initiation of a specific immune response. The analysis of pattern of lymphokine secretion has recently allowed the functional identification of discrete subsets of T helper cells designated Th1 and Th2. Th1 cells produce IL2, IFNγ and LT, whereas Th2 cells secrete IL4 and IL5 in response to the same stimuli. The identification of these two subsets of $CD4^+$ helper cells is mostly based on studies performed with long-term cultured T cell lines and it is not clear whether these two subsets exist in vivo and represent distinct lineages of T cells. In particular, the frequency, tissue distribution and ontogeny of cells capable of secreting IL4 in vivo is not known. These studies have been hampered by the fact that freshly isolated T cells from unprimed animals failed to secrete detectable amounts of IL4 and IL5 when stimulated in vitro by lectins or alloantigens, whereas IL2 is readily detectable in these same cultures. Data presented here indicate that freshly isolated T cells from unprimed animals can be induced to produce IL4 in a receptor-dependent, antigen-independent manner upon stimulation by anti-CD3 antibodies. Our results also show that only $CD4^+$ and not $CD8^+$ cells can be induced to secrete IL4 and that cross-linking of the receptor is required for optimal activity. We believe that this approach will be useful in identifying in vivo cells precomitted to the Th2 pathway and study their ontogeny, activation requirements and tissue distribution.

C 304 RECOGNITION OF A CLASS II MAJOR HISTOCOMPATIBLITY COMPLEX PRODUCT BY ALLOERACTIVE AND ANTIGEN-SPECIFIC T CELLS IS ASSOCIATED WITH EXPRESSION OF THE SAME Vα11 GENES,
Pamela B. Nakajima, Carol J. Betz, and Daniel Hansburg, Department of Pathology, Fox Chase Cancer Center, Philadelphia, PA 19111

We have studied the murine TcR repertoire against the C-terminus of cytochrome c in association with certain alleles of the MHC class II molecule, $E\alpha^kE\beta^k$ (IE^k) and $E\alpha^kE\beta^b$ (IE^b). For mice possessing these alleles, the majority of responsive T cells utilize one member of the variable Vα11 gene family in conjunction with a limited set of Vβ genes. As an extension of these studies, we have examined IE specific, alloreactive hybridomas derived from IE non-expressing (Ea^b) cytochrome c non-responder mice to determine their usage of Vα and Vβ genes. One third of the alloreactive hybridomas expressed Vα11. A ribonuclease protection assay showed that fourteen utilized the same Vα11 gene segment used by the majority of cytochrome c specific, IE restricted T cells and eight utilized a closely related Vα11 gene that also is associated with this antigen response. While expression of the Vβ element most commonly used by cytochrome c-specific T cells was not found among the alloreactive hybridomas tested, Vβ genes less frequently used in the cytochrome response were expressed by seven of the 22 alloreactive hybridomas whose Vα segments were defined by RNase protection. These data indicate an important role for the Vα11 genes in determining recognition of IE molecules both in MHC-restricted, antigen specific immune responses and in alloreactive responses.

C 305 Lymphokine profile of $CD3^+$, $CD4^+$, $CD8^+$ minor-histocompatibility-antigen specific suppresser T cell clones.

Navreet K. Nanda, Mitchell Kronenberg* and Eli Sercarz. Department of Microbiology and department of Microbiology and Immunology*, University of California, Los Angeles.L.A. CA90024.

$CD3^+$, $CD4^+$,$CD8^+$ suppressor T (Ts) cell clones have been established. They are specific for minor histocompatibility antigens and are restricted to class II molecules (manuscript to be submitted). We have examined the expression of lymphokines IL-2, IL-3, IL-4, IL-5, IL-6, γIFN and 'serine esterase' in five of these Ts clones and two cytolytic (CTL) clones by RNA hybridization methods. Cells were stimulated by Con A for 2 hrs, 6 hrs and 24 hrs and total cellular RNA was analysed on Northern blots. We will present results on lymphokine synthesis patterns of our five Ts cell clones in comparison with the patterns seen in the CTL clones we analysed and with the well defined patterns seem in the two T_h populations.

C 306 A SYNTHETIC PEPTIDE OF 16 AMINO ACIDS CONTAINS TWO OVERLAPPING T-CELL STIMULATING SITES AND IS PRESENTED BY IA AND IE CLASS II DETERMINANTS, Judith A. Nicholas, Melissa E. Levely, Mark A. Mitchell and Clark W. Smith, The Upjohn Company, Kalamazoo, MI 49001. A 16 amino acid (parent) synthetic peptide was modeled on the 1A protein of respiratory syncytial virus. The T-helper cells of seven mouse strains, representing 5 Class II haplotypes (IA^s, IA^q, IA^b, IA^kIE^k, IA^dIE^d) were responsive to immunization and restimulation with parent peptide. The IE^d determinant was shown to be a presenting element by monoclonal antibody blocking and by use of L-cell-transfectants as APCs to purified T cells and to T cell hybridomas. A series of overlapping synthetic peptides identified two minimal T-cell sites within the parent peptide: Mice expressing IA and IE responded to a fragment at the N-terminus of the parent peptide (site 1) while mice expressing only IA responded to a distinct but overlapping fragment at the C-terminus (site 2). These minimal sites identified <u>in vitro</u> could be used to immunize mice <u>in vivo</u> in an MHC-restricted manner.

C 307 INTRATHYMIC DELETIONAL RECOMBINATION OF THE DELTA TCR. Gabriel Nunez, Richard Hockett, and Stanley J. Korsmeyer, Howard Hughes Medical Institute, Department of Internal Medicine, Microbiology and Immunology, Washington University School of Medicine, St. Louis, MO 63110.

The human δ TCR Locus is strategically located within the αTCR complex between the cluster of Vα/Vδ region and the Jα segments. A repeated motif, T early alpha (TEA), which can be spliced to Cα in pre T cells, separates δ from the Jα segments. Long range pulse field gel mapping as well as molecular cloning link diversity (D_δ), J_δ, C_δ and TEA within 35 Kb. The human δ TCR locus conserves a 12/23 bp spacer paradigm and considerable δ TCR diversity is generated despite the predominant use of one V_δ and J_δ segment. $D_\delta 1$ and $D_\delta 2$ are 9 and 13 bp long, are frequently recombine as $D_\delta 1/D_\delta 2$, and reveal exonucleolytic trimming with extensive "N" segment addition. Specialized 5' and 3' δ deleting elements, δ Rec and ΨJα, separate the δ locus from the α locus. Cells with δ Rec/Ψ Jα recombinations comprise most δ deletion events although δ Rec recombines with 2 other major acceptor sites in fetal and post-neonatal thymic DNA. The 5' δ deleting element (δ Rec) is evolutionarily conserved in the mouse and functional comparisons are underway. An early decision to effectively rearrange versus delete the δ locus may prove to be the pivotal event establishing separate γδ and αβ lineages.

C 308 STUDIES ON THE $\gamma\delta$ RECEPTORS EXPRESSED IN MURINE FETAL AND NEWBORN THYMUS, Rebecca O'Brien*, Mary Pat Happ‡, Ralph Kubo** Philippa Marrack* John Kappler*‡, and Willi Born* Howard Hughes Medical Institute at Denver*, Department of Medicine**, and Department of Pedicatrics‡, National Jewish Center, Denver, CO 80206. We are exploring the $\gamma\delta$ T cell receptor repertoire in an attempt to gain clues for the possible functions of $\gamma\delta$ expressing T cells. We have begun by identifying the genes that encode the functionally rearranged receptor on $\gamma\delta^+$ hybridomas derived from fetal (day 16) and newborn thymocytes. Thus far, we have discovered that the $\gamma\delta$ receptors used by a panel of four surface positive fetal hybridomas are different, and have assigned some of the V-genes used by these cells. In addition, 48 $\gamma\delta$ receptor expressing newborn thymus hybridomas have now been screened for alloreactivity against 7 different mouse strains, but no alloreactivity by these cells has yet been demonstrated. Surprisingly, a high percentage (~30%) of the newborn $\gamma\delta$ hybridomas were found to produce lymphokines in the absence of any deliberate stimulation. A panel of sixteen $\alpha\beta$ expressing newborn hybridomas which arose in the same fusions as did the $\gamma\delta$ hybridomas did not exhibit this property. We believe that the $\gamma\delta$ cells which constitutively produce lymphokines may be autoreactive via the $\gamma\delta$ receptor because we can inhibit lymphokine production with anti-CD3 and anti-LFA-1 antibodies. We hope to use these cells to characterize a $\gamma\delta$ ligand.

C 309 CONTACT-MEDIATED B CELL ACTIVATION BY HELPER T CELLS: A MODEL FOR CELLULAR EVENTS DURING COGNATE T-B INTERACTION. Trevor Owens, McGill University Centre for the Study of Host Resistance, Montreal General Hospital Research Institute, Montreal, Quebec, CANADA H3G 1A4. The T-dependent induction of antibody responses requires an MHC-restricted (cognate) cellular interaction between B cells and antigen-specific helper T cells (T_h). A role for non-cognate components of T-B interaction has been suggested by the demonstration that anti-T cell receptor (TCR)-activated Type 1 murine T_h induced the polyclonal activation of small resting B cells in the absence of ligands for sIg. This required contact with activated T cells, T cell activation itself was independent of cellular interaction. Resting B cells did not respond to supernatants from T_h, which contained IL2 and IFN-γ (Owens, T., Eur. J. Immunol., 18, 395 (1988)). The mechanism of contact-mediated activation has now been further examined. Cells of the CBA-derived hapten-specific non-alloreactive F23.1$^+$ Type 1 T_h clone E9.D4 were co-cultured with small (percoll-dense) resting C57.B1/6 splenic B cells in plastic wells coated with purified F23.1 anti-TCR Mab. T cells were removed after various times by antibody/complement lysis and the capacity of B cells to respond to T_h-supernatants (T_h-SN) and to rIL2 was assessed by ELISA measurement of Ig secretion in a four hour assay after further culture. Optimal culture times were 2 days with T_h and F23.1, followed by 4 days with lymphokines. Culture with F23.1 plus T_h for 2 days without the addition of T_h-SN or rIL2 after T_h depletion was insufficient to induce Ig secretion. The anti-IL2 Mab S4B6 inhibited responses to T_h-SN but had no effect on T-dependent B cell triggering. Anti-IL4 and anti-IFN-γ Mabs had no effect. Anti-LFA-1 Mabs also inhibited, but had no effect on T-dependent triggering, inhibiting only the response of contact-triggered B cells to T_h-SN. These results suggest that IL2 responsiveness is induced in small resting B cells through contact with activated T_h. Cellular interactions involving LFA-1 play a role in the response to IL2, but not apparently in the interaction between T_h and B cells.

C 310 SELECTIVE EXPRESSION OF CD45 ISOFORMS PLAYS A FUNDAMENTAL ROLE IN THYMIC DEVELOPMENT, L.M. Pilarski, Department of Immunology, University of Alberta, Edmonton, Alberta Canada T6G 2H7

Previous work suggested that human thymocytes expressing CD45R defined the set of thymocytes able to exit and populate the periphery. The majority of thymocytes express CD45 p180 with only 15-29% expressing CD45R. CD45 p180$^+$ cells were extensively depleted and the remaining cells characterized. CD45 p180-negative cells were 70% CD45R$^+$ and were enriched in cells bearing a high density of CDw29 (4B4). Only 15-20% of CD45 p180-depleted cells were CD4$^+$8$^+$. CD4$^+$8$^+$ or CD4$^-$8$^+$ cells comprised 40-60% and CD4$^-$8$^-$ cells 20-40% of the CD45 p180$^-$ population.

To determine clonogenic potential of thymocytes expressing CD45R or CD45 p180, CD45R-depleted or CD45 p180-depleted subpopulations were cultured in a limiting dilution analysis. Unfractionated thymocytes included 1/35 cells able to generate a clone. Depletion of CD45R$^+$ cells removes 90% of clonogenic precursors. Depletion of CD45 p180$^+$ cells gives a 10 fold enrichment for clonogenic precursors. This indicates that only CD45 p180$^-$ cells have the potential to generate a clone in vitro. We propose that expression of high molecular weight CD45 isoforms defines the generative lineage within the thymus that exits the thymus, and that expression of CD45 p180 commits a cell to the pathway leading to intra-thymic death. We further propose that CD45 p180, in contrast to CD45R is unable to transmit sufficient signals to maintain viability of an immature T cell.

C 311 T-CELL TOLERANCE TO Mls^a ENCODED ANTIGENS IN T-Cell RECEPTOR ß-CHAIN TRANSGENIC MICE

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To study the mechanism of T-cell tolerance, transgenic mice were generated that expressed the Mls^a reactive T-cell receptor (TCR) ß-chain $V_{ß8.1}$ on ~90 % of peripheral T-cells. In transgenic mice bearing Mls^a, the numbers of high TCR expressing thymocytes and of Thy 1.2$^+$ peripheral T-cells were reduced. The CD4/CD8 ratio of peripheral T-cells was decreased fourfold compared to negative littermates. Both Mls^a and Mls^b TCR ß-transgenic mice were able to mount a T-cell dependent antibody response against viral antigens whereas the capacity to generate alloreactive and virus-specific cytotoxic T-cells was impaired in TCR ß-transgenic Mls^a, but not in transgenic Mls^b mice. RNA analysis and immunofluorescence with TCR $V_ß$-specific mAb further revealed, that the expression of endogenous TCR ß-genes in these mice was suppressed.

C 312 NEONATALLY CLASS II-TOLERANT MICE APPEAR TO BE PRIMED TO THE TOLERATED ALLOANTIGEN. T. J. Powell and J. Wayne Streilien. Dept of Microbiology and Immunology, University of Miami School of Medicine, Miami, FL 33136.
A large percentage of A strain Class II-tolerant mice retain tolerogen-reactive lymphocytes, as measured in the MLR, in spite of their long-term acceptance of a skin graft bearing the tolerated antigens. Lymphokine production by MLR+ tolerant lymphocytes is different from that of syngeneic normal lymphocytes. Normal lymphocytes produce only IL-2 in primary response to tolerogen, while tolerant lymphocytes produce IL-2 and IL-4. Using limiting dilution analysis, we have to estimated the frequencies of pIL-2 and pIL-4 (precursor) cells in these cultures. After primary in vitro stimulation, normal responders have a low but measurable frequency of pIL-4 cells, while tolerant responders have a much higher pIL-4 frequency. However, following subsequent in vitro restimulations, the pIL-4 frequency of normal responders rises and begins to approach that of the tolerant responders, such that the two populations are indistinguishable based on pIL-4 frequencies following the third round of in vitro stimulation. These data suggest that the high frequency of IL-4 producers (presumably T_{H2} cells) among the tolerant lymphocytes resembles unexpectedly a "primed" state, rather than "unprimed" - as in non-tolerant responders (where T_{H1} dominate the early in vitro response). The existence of "primed" T cells in phenotypically tolerant animals raises the possibility that precocious activation of T_{H2} (by neonatal exposure to tolerogen?) suppresses the later emergence of T_{H1}, which would be expected to contain the cells responsible for graft rejection.

C 313 STRUCTURAL REQUIREMENTS OF I-A^k FOR ANTIGEN PRESENTATION. Ed Rosloniec*, Christophe Benoist$^{\#}$ Diane Mathis$^{\#}$, Dave McKean‡,and John Freed*, Dept. of Med.*, National Jewish Center, Denver, CO 80206, Dept. of Immunol.‡, Mayo Clinic, Rochester, MN 55905 and INSERM$^{\#}$, Strausbourg, France. The structural features of the I-A^k molecule required for the presentation of hen egg lysozyme (HEL) peptides to I-A^k-restricted T-cell hybridomas was investigated through the use of antigen presenting cells (APC) expressing mutant or transfected "chimeric" I-A molecules. Chimeric I-A molecules were produced by systematically replacing the 3 polymorphic regions (PMR) of the $A_\alpha{}^k$ gene with b haplotype sequences or the 4 PMR of the $A_ß{}^k$ gene with d haplotype sequences, and transfecting these genes separately with a complementary wild type gene. Use of these APC revealed a functional hierarchy of the PMR in which the 3rd PMR of the $A_\alpha{}^k$ chain (a.a. 69-76) and the 2nd PMR of the $A_ß{}^k$ chain (a.a. 63-67) exerted the dominant influence. That the residues comprising the 3rd PMR of the Aα chain are likely T-cell receptor interactions sites is supported by two complementary approaches. Direct binding studies with the JE50 mutant I-A^k molecule (2 mutations in the 3rd PMR of the $A_\alpha{}^k$ chain) indicate that this molecule binds the HEL(46-61) peptide although it fails to present it to all HEL(46-61) specific T-cell hybrids examined. Anti-I-A inhibition of antigen presentation and I-A/peptide binding indicates that the Ia.19 serological eptitope, located in the third PMR of the $A_\alpha{}^k$ chain, is not involved with the binding of the HEL(46-61) peptide. We are currently examining the contribution of individual residues of the dominant PMR in antigen presentation and peptide binding.

C 314 CLONAL ANALYSIS OF FUNCTIONALLY DISTINCT HUMAN CD4+ T-CELL SUBSETS, Francien T.M. Rotteveel, Ingrid Kokkelink, Rene van Lier, Bart Kuenen, Anthony Meager, Frank Miedema, Cornelis Lucas, Central Lab.Blood Transf. Service, Lab. Exp. and Clin. Immunology of the University of Amsterdam, Amsterdam, The Netherlands

A large number of CD4+ T-cell clones, obtained from peripheral blood T lymphocytes by direct limiting dilution, allowed us to address the question whether functional heterogeneity exists within the human CD4+ T-cell subset. Six out of 12 CD4+ clones were able to lyse Daudi or P815 cells in the presence of anti-CD3 antibodies. The remaining 6 CD4+ T-cell clones tested did not acquire this cytotoxic capacity during a culture period of 20 weeks. In the absence of anti-CD3 mAb, no lytic activity against Daudi, P815 and K562 target cells was observed under normal culture conditions. These two types of CD4+ T cells showed high reactivity with anti-CDw29 (4B4) MAb and no reactivity with anti-CD45R (2H4) mAb. The CD4+ clones without anti-CD3 mediated cytotoxic activities (TH2) consistently showed a higher expression level of CD28 antigens. TH1 CD4+ clones did produce IL-2, IFN-gamma and TNF-alpha,beta, whereas the TH2 T-cell clones produced minimal amounts of IL-2, IFN-gamma and TNF-alpha, beta in response to anti-CD3 mAb and PMA. Not all CD4+ clones did release IL-4, but there was no correlation with cytotoxic activity. Moreover, as compared to the TH1 CD4+ clones, TH2 CD4+ clones proliferated moderately in response to anti-CD3 mAb. However, anti-CD3 mAb induced proliferation of only the TH2 CD4+ T-cell clones was enhanced by anti-CD28 mAb. Both CD4+ subsets provided help for polyclonal B-cell activation with anti-CD3 mAb. Our data suggest that the human CD4+ subset, in analogy to the murine system, comprises two functionally distinct T-cell subpopulations.

C 315 INTRACELLULAR COMPETITION FOR ASSEMBLY AND/OR TRANSPORT DETERMINES IA CELL SURFACE PHENOTYPE Andrea J. Sant and Ronald N. Germain. Laboratory of Immunology, NIAID, National Institutes of Health, Bethesda, MD. 20892.

In the mouse, when Ia antigens are isolated immunochemically, the predominant species isolated are the isotypic matched pairs, AαAβ and EαEβ. However, when Ia αβ dimer expression is studied using an L cell transfection model, it is found that the isotype-mismatched dimer AβdEα is readily expressed at the cell surface. These results suggest that differences in assembly and/ or transport of different Ia pairs may be most readily visualized in a competitive environment where multiple distinct Ia chains are available. To investigate this possibility, the relative efficiency of inter- and intra- isotypic dimer formation and expression was evaluated using a sequential L cell transfection system. L cells already expressing an αβ dimer on the cell surface ($A\beta^dE\alpha$ or $A\beta^dA\alpha^d$) were supertransfected with a third Ia gene ($A\alpha^d$ or Eα, respectively). Synthesis of this second α or β protein led to competition for the unique partner chain. Individual clones were scored for cell surface expression of the distinct dimers (e.g., $A\beta^dE\alpha$ vs $E\beta^dE\alpha$ or $A\beta^dA\alpha^d$ vs $A\beta^dE\alpha$) using FACS analysis with chain specific monoclonal antibodies. In addition, each species of mRNA was quantitated by Northern blot hybridization using locus specific probes. Our results indicate that in the $H\text{-}2^d$ haplotype, isotype-matched dimers are expressed with 3-4X the efficiency of isotype-mismatched dimers. This result suggests that, regardless of the cell type studied, if each of the four murine Ia genes is expressed at equivalent levels, intraisotypic dimers will be expressed to the virtual exclusion of the interisotypic dimers. However, if chain synthesis asymmetry occurs, the isotype mismatched pairs may be expressed at immunologically relevant levels.

C 316 DIFFERENTIAL REGULATION OF HLA CLASS I ANTIGENS BY INTERFERON. H. Schmidt[1], G. Gekeler[2], H. Haas[2], G. Engler-Blum[1], E.Weiss[3], A. Vallbracht[4], C.A. Müller[1]; [1]Medizinische Klinik, Abt. II; [2]Physiologisch-Chemisches Institut; 4Virologie, University of Tübingen, FRG; [3]Institut für Immunologie; München; FRG

To investigate differences in regulation of HLA-class I antigen expression by interferon depending on their polymorphism, the HLA-class I genes A2, Cw3, B7, Bw64, B38, B39, B27, B51 were integrated in mouse L(tk^-) cells by DNA mediated gene transfer. After exposure to 10^4 IU/ml of mouse interferon for 36 hours surface expression of the HLA-Bw6+ genes, B7 and B14 was 2- to 3-times more increased than expression of the other genes. Selection of specific cells during transfection was excluded by similar differences in interferon stimulation after integration of HLA-B7 and HLA-A2 in a single cell. Differential regulation of HLA-class I alloantigens by interferon was confirmed on normal human fibroblasts carrying the respective antigen. To further analyze molecular mechanisms of HLA-class I alloantigen regulation transfectants with a modified HLA-B7 gene lacking 5′ regulatory elements (ICS, interferon consensus sequence) (Friedman, R.L. and Stark, G.R.: Nature 314, 637) were established and shown to express the respective HLA-class I product on the cell surface. Exposure of the cells to mouse interferon still resulted in a 2-fold enhanced expression of the antigen. These results indicate differences in regulatory mechanisms for HLA-class I alloantigens with possible influence on T-cell recognition in immune responses and suggest specific regulatory elements except the ICS involved in the regulation of certain HLA-class I genes like HLA B7.

C 317 T CELL RECEPTORS ON CD4$^-$CD8$^-$ THYMOCYTES AND THEIR ROLE IN THE GENERATION OF MATURE T CELLS, Roland Scollay, Anne Wilson, Martin Pearse, Li Wu, Mark Egerton and Ken Shortman, The Walter and Eliza Hall Institute of Medical Research, Melbourne, Victoria 3050, Australia.
CD4$^-$CD8$^-$ thymocytes contain the precursors of CD4$^+$CD8$^+$ thymocytes, mature single positive thymocytes and peripheral T cells. A proportion of these CD4$^-$CD8$^-$ cells expresses surface T cell receptor (TcR), some $\alpha\beta$ and some $\gamma\delta$. It has been suggested, based on this fact, that important antigen selection events or antigen driven differentiation occurs among double negative thymocytes. However, in an intrathymic injection system, we have been unable to demonstrate the generation of double or single positive thymocytes or T cells from CD3$^+$ double negatives, while CD3$^-$ double negatives have the potential for at least 10^3-fold expansion and differentiation into double positives and mature single positives. We have identified a series of discrete stages among the CD3$^-$ double negatives which seem to form a sequence, with TcR gene rearrangement and RNA expression gradually progressing, but with potential for expansion and repopulation of irradiated thymuses diminishing along the series. On this pathway CD3 must be expressed late or after the acquisition of CD4 and CD8. Cell cycle analysis shows the highest rates of cell division to be among the HSA$^+$ IL-2R$^-$ Pgp-1$^-$ population which probably precedes the transition to CD4$^+$CD8$^+$ and TcR expression. Thus it seems unlikely that TcR/antigen interactions play a role in cellular events occurring among the double negative cells which lead on to mainstream T-cell development.

C 318 EXPRESSION AND ONTOGENY OF MURINE CD2, Jyoti Sen, Robert J. Arceci, William Jones and Steven J. Burakoff, Division of Pediatric Oncology, Dana-Farber Cancer Institute, 44 Binney Street, Boston, MA 02115, and the Departments of Pathology and Pediatrics, Harvard Medical School, Boston, MA 02115.
CD2 was first defined as the erythrocyte rosetting protein on the surface of human T cells. The rat and murine homologues have been identified by cDNA cloning. Recently, we have generated an antiserum against murine CD2 by immunizing rabbits with a peptide corresponding to a hydrophilic region in the extracellular domain of murine CD2. This antiserum immunoprecipitates a 54-58 kD glycoprotein from the surface of the tumor cell line EL4 which has been shown to express CD2 mRNA. Immunoprecipitation with the antiserum can be inhibited by the immunizing peptide. Using this antiserum we demonstrate that CD2 is expressed on the surface of most adult murine peripheral T cells and thymocytes by indirect immunofluorescence. Furthermore, CD2 appears very early during fetal ontogeny. The level of surface expression increases from day 13 of gestation to day 17, after which the surface density appears to reach a steady state. Thus CD2 is expressed on day 13 thymocytes along with Thy1, Pgp1 and the gamma/delta TCR/CD3 complex. Unexpectedly, we have observed that most Ig expressing peripheral B cells also express CD2 on their cell surface. Studies addressing the developmental expression of CD2 on B cells are underway.

C 319 Differential expression of a zinc finger encoding gene in response to positive versus negative signalling through receptor immunoglobulin in murine B-lymphocytes, Vicki L. Seyfert*, Vikas P. Sukhatme@, and John G. Monroe*, *Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA, 19104 and @Department of Medicine, Howard Hughes Medical Institute, University of Chicago, Chicago, IL 60637. Egr-1 is a murine early growth factor inducible gene which encodes a protein with zinc fingers. Its expression was investigated in murine B-lymphocytes stimulated through their antigen receptor (sIg) with anti-recptor antibodies (anti-Ig). Rapid (by 15 minutes) upregulation of Egr-1 mRNA expression was observed at doses of anti-Ig sufficient to drive the majority of G0 cells into cell cycle. Agonists and inhibitors of protein kinase C (PKC) showed that expression was coupled to the PKC component of receptor immunoglobulin transmembrane signalling. Interestingly, signalling through sIg on the murine B lymphoma WEHI-231 did not upregulate Egr-1 expression even though similar signalling pathways are associated with this receptor in these cells. Southern analysis showed that Egr-1 is not deleted or translocated in this cell line. Importantly, cell growth and proliferation of WEHI-231 is inhibited by anti-Ig stimulation suggesting a relationship for Egr-1 expression and differential processing of receptor Ig signals. This notion is further supported by the finding that murine B lymphomas whose proliferation is not inhibited by anti-Ig showed receptor immunoglobulin coupled Egr-1 expression.

C 320 Regulation of Expression of a class I MHC transgene. Dinah S. Singer, Jean E. Maguire, Rachel Ehrlich. Experimental Immunology Branch, NIH, Bethesda,MD.20892.
Introduction of a heterologous class I MHC gene into the genome of a mouse results in the expression of the transgene product. The patterns of expression of the transgene parallels that observed in situ, indicating that regulatory elements necessary for normal patterns of expression are contained within the injected 9 kb DNA segment, and that trans acting factors involved in its regulation function between species. Included among these elements are those specifying preferential expression in B cells relative to T cells. In vivo treatment of transgenic mice with α/β-interferon results in increased expression of the transgene in a number of tissues. The response parallels that observed for the endogenous H-2Kb, but differs markedly from Qa-2. Analysis of the chromatin structure of the transgene reveals a single constitutive DNase I hypersensitive site present in both spleen and thymus, which is not altered by interferon. Both a novel negative and positive regulatory elements have been identified in the 5'flanking region of the transgene. The negative regulatory element reduced the activity of both the homologous class I promoter and a heterologous viral promoter. In vivo competition experiments indicated that the functions of the positive and negative elements are mediated by distinct cellular trans-acting factors. The negative regulatory element requires the presence of a positive regulatory element to function. This interaction between elements represents a novel mechanism for regulating gene expression.

C 321 THE POLYCLONAL T CELL PROLIFERATIVE RESPONSE TO MYELIN BASIC PROTEIN N-TERMINAL PEPTIDE ANALOGS IN H-2^u MICE, Dawn E. Smilek, David C. Wraith, and Hugh O. McDevitt. Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, CA 94305. Published data show that encephalitogenic H-2^u murine T cell clones with specificity for the N-terminal eleven amino acid peptide of myelin basic protein display a restricted fine specificity when tested on substituted analogs of the native peptide. For example, substitution of alanine at certain positions in the peptide totally abolishes the response of each clone (Acha-Orbea et al., 1988. Cell 54:263). Recent experiments also have shown that the ability of some peptide analogs to bind to H-2^u I-A gene products does not always correlate with their ability to stimulate the T cell clones (see accompanying abstract by David C. Wraith and Hugh O. McDevitt). This suggests that H-2^u mice may lack a T cell repertoire capable of recognizing these peptides complexed to H-2^u I-A gene products. To test this possibility, H-2^u mice were immunized with a panel of peptide analogs, as well as the native peptide. The *in vitro* T cell proliferative response to each of the peptides then was measured. The results show that *in vivo* immunogenicity of the peptide analogs also does not strictly correlate with their capacity to stimulate the T cell clones. In this way, the polyclonal T cell repertoire of H-2^u mice for the myelin basic protein peptide analogs was examined, and could be compared with the I-A binding characteristics of the peptides.

C 322 STRUCTURE-FUNCTION ANALYSES OF T CELL RECEPTOR, ANTIGEN AND MHC INTERACTIONS, Simona B. Sorger, Yvonne Paterson, Louis A. Matis and Stephen M. Hedrick, Department of Biology, University of California, San Diego, La Jolla, CA 92093. The murine T cell response to pigeon cytochrome c was carefully analyzed in terms of antigen and MHC recognition. This response involves a limited repertoire of T cells which crossreact on species variants of the antigen. In addition, T cells specific for the antigen in association with syngeneic MHC can recognize antigen on similar allogeneic MHC molecules. The grouping of clones by functional phenotypes defined by these crossreactivities allowed us to correlate TCR gene usage with either antigen or MHC recognition. Some of the pigeon cytochrome c-specific clones within one functional phenotype use receptors that differ by as few as two amino acid residues. Other clones express very different TCRs but exhibit similarities in antigen/MHC recognition. The effect of these TCR differences on recognition was assessed using a panel of antigen analogs with single amino acid substitutions presented on different MHC molecules. Each clone exhibited a unique pattern of response to the antigen analog panel, even clones with very similar receptors. Also, each residue in the antigenic region of the peptide was critical for interaction with at least one T cell receptor. Therefore, the antigen must either be a linear molecule with each residue available to interact with the TCR or be able to assume several conformations to interact with MHC and the TCR.

C 323 THY-1+ CD3+ LY-5(B220)+ CD4- CD8- TCRγ-δ+ HELPER CELLS. Anne I. Sperling and Henry H. Wortis, Sackler Graduate School of Biomedical Sciences, Tufts University, Boston, MA, 02111. Double negative TCRγ-δ cells have been found in very small amounts in peripheral lymphoid organs. We have found that these cells can be preferentially stimulated to proliferate when cocultured with the B lymphoma, CH12. One to 2% of nylon wool non-adherent, Ia$^-$, J11d$^-$, and CD8$^-$ lymph node cells from normal unimmunized mice have the phenotype Thy-1$^+$, CD3$^+$, CD4$^-$, and CD8$^-$. These cells proliferate when co-cultured with a syngeneic surface Ig$^+$ lymphoma, CH12, even in the absence of any added antigen, mitogen, or fetal calf serum. Prior to stimulation we find that approximately 30% of Thy 1.2$^+$ CD3$^+$ CD4$^-$ CD8$^-$ express the marker Ly-5(B220), however after culture with CH12 the majority of cells with this phenotype express the marker Ly-5(B220). After CH12 dependent proliferation the Ly-5(B220)$^+$ T cells are able to provide help for secretion of Ig by fresh CH12 B cells. Surface labelling and precipitation of T cell receptor molecules reveals that most of the Thy-1$^+$ CD3$^+$ Ly-5(B220)$^+$ CD4$^-$ CD8$^-$ cells express TCR(γ-δ). Furthermore, CD3 precipitation shows that as many as four different γ-δ heterodimers are utilized within the entire responding population. This suggests that a heterogeneous population of double negative TCRγ-δ cells are involved in the response to CH12.

C 324 RECIPROCITY IN T CELL-MACROPHAGE INTERACTIONS: T_H1, BUT NOT T_H2, CLONES ACTIVATE MACROPHAGE EFFECTOR FUNCTION, Robert D. Stout. Department of Microbiology, College of Medicine at East Tennessee State University, Johnson City, TN 37614 Interferon-γ producing (T_H1) and Interleukin 4 (IL4) producing (T_H2) clones were assayed for their ability to directly induce cytostatic activity in macrophages generated from splenic myeloid precursors (Mφ-c). In the presence, but not in the absence, of antigen, T_H1 clones activated the Mφ-c to inhibit the growth of P815 tumor cells in vitro. T_H2 clones were not able to activate such effector activity in the Mφ-c. The Mφ-c did effectively present antigen to the T_H2 clones as evidenced by the proliferation of T_H2 cells cultured with antigen in the presence, but not in the absence, of Mφ-c. Therefore, although both T_H1 and T_H2 were activated by cognate interaction with antigen presenting Mφ-c, only T_H1:Mφ-c interactions displayed reciprocity resulting in activation of the Mφ-c. T_H1-derived lymphokines or rIFN-γ, in the presence of LPS, could activate proteose-peptone elicited Mφ, resident peritoneal Mφ, and Mφ-c whereas neither T_H2-derived lymphokines nor rIL4 could induce detectable activity in any of the 3 Mφ populations. Since Mφ-c consistently required at least two signals for activation, the ability of T_H1-derived lymphokines to synergize with T_H2 cells in Mφ activation was examined. T_H2 could activate the antigen-presenting Mφ-c in the presence of IFN-γ. The ability of added IFN-γ to synergize with T_H2 indicates that the cognate interaction between T_H2 and antigen presenting Mφ-c does result in delivery of at least one of the signals required for Mφ activation.

C 325 EFFECT OF IMMUNIZATION ON PRECURSOR DIFFERENTIATION INTO TH1 AND TH2 HELPER CELLS. N.E. Street, H. Bass, D. Fiorentino, T.A.T. Fong, J. Leverah, J.H. Schumacher, and T.R. Mosmann. DNAX Research Institute, Palo Alto, CA 94304. We have previously identified two distinct types of long-term murine T helper cell clones. TH1 clones secrete IL2, IFNγ, and LT while TH2 clones secrete IL4, IL5 and IL6. Unfractionated spleen cells stimulated with Con A secrete high amounts of IL2 but only low amounts of IFNγ, IL4 and IL5. This suggests either regulation of lymphokine (LK) synthesis in the mixed population of cells or the existence of additional T cell stages or subsets. Experiments were performed to determine whether clones only recently established in vitro exhibit the TH1 and TH2 patterns of LK secretion. C57BL/6 mice were left untreated or immunized with Complete Freund's Adjuvant (CFA), Brucella Abortus (BA) or Nippostrongylus brasiliensis (Nb). Spleen cells from these mice were cloned at limiting dilution with alloantigen stimulation, and every two weeks, LK production in response to Con A was measured. Clones derived from, and stimulated with, cells from unimmunized mice initially tended to secrete low LK levels, with few clearly defined TH1 or TH2 clones. By 56 days after cloning, some clones had acquired TH1 or TH2 patterns. CFA, BA and Nb-immunized mice gave rise to clones that were mostly TH1 or TH2 even at early times. CFA and BA immunizations induced almost exclusively TH1 clones, whereas Nb induced more TH2 clones. These results are consistent with a model in which resting, previously unstimulated T cells produce low amounts of LKs, and progress through stage(s) where they secrete both TH1 and TH2 LKs before finally differentiating into TH1 and TH2 cells. The results with CFA, BA and Nb-primed mice suggest that this process occurs in vivo as well as in vitro.

C 326 INTRODUCTION OF PSEUDOMONAS PSEUDOMALLEI (MELIOIDOSIS) ANTIGEN INTO THE GALE SALMONELLA TYPHI TY21 VACCINE STRAIN:POTENTIAL USE OF THIS BIVALENT VACCINE STRAINS AS CARRIERS OF MELIOIDOSIS ANTIGENS TO THE IMMUNE SYSTEM, Deja Tanphaichitra, Mahidol University, P.O. Box 4-217, Bangkok 10400, Thailand
The attenuated galE mutant, Salmonella typhi strain, Ty21a, served as the recipient in a conjugal DNA transfer experiment. The donor strain was a Pseudomonas pseudomallei MU107. Conjugal DNA transfer was obtained by the mating procedure on an appropriate blood agar medium. The resulting antigen clones were repurified by restreaking on the medium and were examined serologically. One selected strain was found to have the serological characteristics of the recipient S. typhi, Ty21a strain and also expressed the Pseudomonas pseudomallei antigen. In this study it appears that Pseudomonas pseudomallei synthesis in the S. typhi transconjugant strain is due to the presence of the Pseudomonas pseudomallei plasmid. A group of subjects when received four doses of this bivalent vaccine strain developed antibodies against Pseudomonas pseudomallei up to 70%. Since Pseudomonas pseudomallei, an intracellular pathogen, produces a characteristic antigen probably to be plasmid coded, we considered that the GalE Salmonella typhi Ty21a oral vaccine strain, highly effective against typhoid fever, might be modified so as to be protective also against melioidosis due to Pseudomonas pseudomallei.

Supported in part by USAID

C 327 PROTEIN KINASE C REGULATION OF TdT GENE EXPRESSION. Jeffery P. Tillinghast, John Russell, Larry Fields, and Dennis Y. Loh, Howard Hughes Medical Institute, Departments of Medicine, Genetics, Microbiology and Immunology, Washington University School of Medicine, St. Louis, MO 63110.

Terminal deoxynucleotidyl transferase (TdT) is a lymphoid-specific nuclear enzyme present in early lymphocytes. To investigate the regulation of TdT gene expression, pre-B and pre-T cells were treated with phorbol 12-myristate 13-acetate (PMA) or three analogs, and TdT steady-state mRNA levels were determined by Northern blot analysis. Treatment of early lymphocytes with PMA results in a rapid and reversible decline in steady-state TdT mRNA levels within six hours. This rapid decline can be blocked by pretreatment of the cells with a protein kinase C inhibitor, implicating protein kinase C activation in the decline of TdT mRNA. Nuclear run-off studies demonstrate that TdT transcription is rapidly down-regulated within 45 minutes after PMA treatment, indicating that this regulation occurs mainly at the level of transcription. Furthermore, cycloheximide blocks the decline in TdT in RNA showing that new protein synthesis is required for transcriptional inactivation.

C 328 IMMUNOGENICITY OF RECOMBINANT-DERIVED INFLUENZA NUCLEOPROTEIN; PRIMING OF CROSS-REACTIVE T-CELL IMMUNITY AND PROTECTION AGAINST INFLUENZA INFECTION. J.P. Tite, D. O'Callaghan, G. Dougan and F.Y. Liew; Departments of Experimental Immunobiology and Molecular Biology, Wellcome Research Laboratories, Beckenham, Kent, England.
The nucleoprotein gene from the influenza virus A/NT/60/68 was stably cloned into the attenuated aroA-strain of Salmonella typhimurium SL3261. Nucleoprotein purified from pNP_2-3261 was tested for the ability to generate virus-specific immunity. Immunization with recombinant derived nucleoprotein induced immunity to all type A influenza tested but not against type B viruses. $CD4^+$ helper T cells were primed but no evidence was found for priming of class I restricted CTC. Mice immunized with recombinant nucleoprotein were protected against a subsequent challenge of influenza virus. The information obtained from the study of the immunity and protection generated by the purified recombinant protein was then used to design experiments to investigate the possibility of using the attenuated Salmonella vector to deliver the nucleoprotein molecule to the immune system by the parenteral or enteral routes.

C 329 **EXTRACHROMOSOMAL CIRCULAR DNAS EXCISED FROM TCR GENE LOCI AT THE DIFFERENT DEVELOPMENTAL STAGES OF LYMPHOID TISSUE CELLS**

Masaaki Toda[1,2], Sunao Takeshita[1,3], Toshiyasu Hirama[1,4], Hideo Yamagishi[1], [1]Department of Biophysics, Faculty of Science, [2]Chest Desease Res. Inst., [4]1st Div. Dept. of Int. Med., Faculty of Medicine, Kyoto University, Kyoto 606, [3]Pharma Res. Lab., Hoechst Japan Ltd., Saitama 350, Japan

We characterized the extrachromosomal circular DNAs in 19-day-fetal and 4-week-old murine thymocytes and 8-week-old murine splenocytes. A population of circular DNAs was cloned into the λgt11 phage vector. We screened ca. 10^5 DNA clones by plaque hybridizations with all four kinds of TCR gene probes derived from Jα1, Vα10, Dβ1, Dβ2, Jγ1, Jδ1 and Jδ2 loci. Out of 10,000 DNA clones from fetal and 4-week-old thymocytes, 30 hybridized with TCR α-probes and 5 hybridized with TCR β-probes. Positive clones with TCR γ- and δ- probes were 3 to 7 in fetal thymocyte-derived library, but few in 4-week-old thymocyte. Of 7 fetal TCR δ clones analyzed, 6 clones had DD or VD reciprocal joints and 1 clone had VD or DD coding joint. Relative frequencies of circular DNA clones for four different TCR genes are consistent with the order of the expression of the genes during the T cell development. Of 10,000 DNA clones from 8-week-old murine splenocytes, 3 clones hybridized with Jα1 and 0.4 with Dβ1+Dβ2. Positive clones with TCR Jγ1, Jδ1 and Jδ2 were 2 to 6. This suggests the extra-thymic gene rearrangements in peripheral lymphoid tissue cells. (The work with fetal thymocytes was performed in colaboration with S. Tonegawa and N. Nakanishi, MIT)

C 330 EXPRESSION OF MHC CLASS II ANTIGENS IN PATIENTS WITH SEVERE COMBINED IMMUNODEFICIENCY, M. Lambert, M.C.J.A. van Eggermond and P.J. van den Elsen, Department of Immunohaematology & Blood Bank, Academic Hospital Leiden, P.O. Box 9600, 2300 RC Leiden, The Netherlands

We are currently investigating MHC class II gene expression in EBV transformed B cell lines derived from four patients with severe combined immunodeficiency. Three of these patients completely lack MHC class II gene expression as judged by Northern blot analysis.
In the fourth patient however, a low level of at least HLA-DR expression is observed. Attempts to induce MHC class II expression in these patients have led to the conclusion that at least three different groups of MHC class II deficiencies can be distinguished in these patients. We have set up experiments that will allow us to identify the molecular events that are responsible for the observed lack of MHC class II gene expression.

C 331 THE CYTOPLASMIC DOMAIN OF THE A_β^k CHAIN IS CRITICAL FOR I-A^k MEDIATED TRANSLOCATION OF PROTEIN KINASE C (PKC). William F. Wade*, Zheng Zhi Chen‡, John Cambier‡, and John H. Freed*, Department of Medicine*, and Department of Pediatrics‡, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO 80206. Site-directed mutagenesis was used to introduce stop condons in the genomic DNA of the α and β chains of I-A^k. These mutant DNA's were transfected into a Ia-negative B cell lymphoma so that the effect of truncated α and β chains on transmembrane signalling could be studied. Transmembrane signalling was measured by the ability to translocate PKC from the cytoplasm to the nucleus after surface I-A^k was bound by α or β chain specific monoclonal antibody.

Removing either 6 or 12 amino acids from the α chain cytoplasmic (Cy) domain did not affect the ability of those I-A^k molecules to translocate PKC to the nucleus. The removal of 12 amino acids from the β chain's Cy domain altered the kinetics and magnitude of PKC translocation: the peak response was shifted by four minutes and reduced by 30 percent. The removal of all β chain Cy amino acids completely abolished the ability of I-A^k to mediate transmembrane signals that translocate PKC from the cytoplasm to the nucleus.

C 332 MODULATION OF B CELL NON-RESPONSIVENESS BY T CELL DERIVED LYMPHOKINES, Garvin L. Warner, José E. Alés-Martínez and David W. Scott, Immunology Unit, University of Rochester Cancer Center, Rochester, New York 14642

We have been investigating mechanisms whereby B cell differentiation is downregulated by crosslinking surface Ig (sIg). Normal splenic B cells were rendered non-responsive to subsequent challenge with LPS, as measured by a decreased ability to generate antibody forming cells (AFC), by incubation overnight (18-24 hours) with 10 ug/ml anti-Ig. Both intact and $F(ab)'_2$ anti-Ig, as well as monoclonal anti-IgM (BET2 and B-7-6), were able to induce B cell non-responsiveness to subsequent LPS challenge, suggesting that sIg/FcR interactions are not necessary in the induction of LPS non-responsiveness. In contrast, induction of non-responsiveness to subsequent challenge with FITC-Brucella abortus required intact anti-Ig. The ability of mitogenic anti-Ig (Rab $F(ab)'_2$ or B-7-6) to induce B cell proliferation or cell enlargement was inhibited by pretreating the B cells for 2 hrs with 10-100 pg/ml cholera toxin (pertussis toxin did not), however, cholera toxin pretreatment did not affect the induction of LPS non-responsiveness by crosslinking sIg. These data suggest that positive signals (growth promoting) are delivered via a cholera toxin sensitive signalling pathway whereas negative signals (inhibition of LPS induced differentiation) are delivered via a cholera toxin insensitive pathway. IL4 (10 U/ml) was able to overcome/reverse the inhibitory effect of cholera toxin on positive signalling via sIg, however, none of the T cell derived lymphokines examined to date (rIL2, rIL4, purified IL5, rTNF, or (gamma)IFN; up to 10,000 U/ml) were able to reverse the induction of LPS non-responsivenss by crosslinking of sIg. (This work supported by NIH grant CA41363 and ACS grant 1M-495.)

C 333 **REGULATION OF LYMPHOKINE AND IL-2 RECEPTOR SYNTHESIS IN $CD4^+$ SPLEEN CELLS,** Andrew D. Weinberg and Susan Swain, Dept. of Biology Q-063, U. of California San Diego, La Jolla, CA 92093. Lymphokine production in $CD4^+$ enriched spleen populations was investigated for both freshly stimulated and in-vitro primed T cells. Northern blot analysis and bioassay data were used to analyze 9 separate lymphokines as well as the IL-2 receptor (murine Tac). Northern blot comparison of fresh and primedT4 enriched RNA revealed that primed T cells produced 10-fold more lymphokine than the fresh T cells. The only lymphokine that showed equal amounts of mRNA for both fresh and primed T cells was IL-2. A time course of fresh and primed $T4^+$ cell lymphokine production was also analyzed. The primed cells produced a short burst of lymphokine mRNA that peaked between 7.5 and 13 hr after Con A stimulation and declined after 18 hr. The fresh T cells produced a longer burst of lymphokine mRNA that peaked 18-44 hr after stimulation. The IL-2 receptor (IL-2R) mRNA time course from activated primed cells showed different kinetics than lymphokine mRNA. This suggested that molecular regulation of the IL-2R might be different than lymphokine regulation. To further examine molecular regulation in the primed T cells polysome profiles were evaluated for lymphokines, IL-2R, and other cellular genes.

C 334 GENETIC ANALYSIS BY PCR-REACTION OF T CELL RECEPTORS FROM INDIVIDUAL MICROCULTURES. NEW DATA ON THE REPERTOIRE OF TNP-SPECIFIC CYTOTOXIC T CELLS.

Hans U. Weltzien+, Bettina Kempkes+, Jerome Bill* and Ed Palmer*, Max-Planck-Institute for Immunobiology+, Freiburg, F.R.G. and National Jewish Center for Immunology*, Denver, CO.

The recently developed method of gene amplification by the polymerase chain reaction (PCR) has proven to be particularly suited for the analysis of T cell receptor (TCR) genes. We adopted existing methods for the preparation of cytoplasmic RNA from as little as 1000 cells and used this material as template for first strand c-DNA synthesis. PCR amplification of this c-DNA, using V- and C-specific oligonucleotide primers yielded enough material to produce single-stranded DNA in a second PCR which could then be sequenced without cloning. In case of unknown V-usage, the PCR was employed for screening for V-beta elements by sequential reactions with different V-beta specific primers. We have used this method to reinvestigate the $H\text{-}2^b$ restricted cytotoxic T cell response to TNP in C57Bl/6 mice. Beta chain sequences of 26 CTL clones obtained by direct cloning of immune spleen cells were compared to sequences of 11 clones obtained by cloning of individual short-term in vitro CTL lines. It was found that a) in vitro bulk-stimulations reduced the heterogeneity of the beta-chain responses to TNP, b) similarities between different TCR-beta-chains concentrated on the usage of certain Jb-elements (Jb2.6, 2.5, 2.1) rather than V-region or N/D-region sequences, and c) the majority of Jb2.6 containing beta-chains was associated with alpha-chains expressing V-segments of the Va10 family. These similarities may indicate a limited number of immunodominant TNP-determinants on the cell surface.

C 335 DUPLICATION OF Vα GENE SEGMENTS IN THE RAT. Calvin B.Williams, Sunil Khurana, Lan Pham, and George A. Gutman. Dept. of Microbiology and Molecular Genetics, University of California, Irvine CA 92717.

Expression of genes which encode the T cell antigen receptor is central to the generation of the T cell repertoire. Our laboratory has been investigating genes for both the alpha and beta chains of this receptor in inbred strains of *Rattus norvegicus* (the laboratory rat), a species in which several autoimmune disease models have been developed, and which is used extensively in transplantation studies. Using genomic Southern blots and mouse probes specific for five different Vα subfamilies, we have estimated the size of the Vα repertoire in ten inbred strains of rat. Results show a significant increase in the size of one subfamily and suggest increases in two others in all ten strains. The rat Vα1 subfamily has about twice as many members as the mouse, while the Vα2 and Vα5 subfamilies, depending upon the enzyme used, show a similiar duplication. The Vα6 and Vα9 subfamilies have a comparable number of members in both species. These data are most easily explained by a single duplication event in the rat involving at least one and perhaps three subfamilies, but not encompassing the entire Vα locus. This implies that the Vα1 subfamily (perhaps together with Vα2 and Vα5 subfamilies) is regionally clustered and not interspersed with either the Vα6 or Vα9 subfamily. Based on restriction fragment length polymorphisms, we find evidence for six distinct Vα haplotypes in the ten strains tested.

We have also cloned eight unique germline Vα1 gene segments. One of these has been sequenced, and has a coding region 87% identical to the most closely related mouse Vα1 sequence. This degree of relatedness is similiar to rat/mouse Vβ homologues, which share 85-88% nucleotide sequence similarity. We are using these clones to generate single copy probes from flanking regions to further map the Vα1 locus.

C 336 BINDING OF MYELIN BASIC PROTEIN PEPTIDES TO I-A MOLECULES: A BIOCHEMICAL APPROACH. David C. Wraith & Hugh O. McDevitt. Department of Microbiology and Immunology, Stanford University, Stanford, CA 94305.

Current approaches to MHC-peptide binding studies require either large quantities of highly purified MHC protein and/or the use of sophisticated detection apparatus. In order to simplify detection of peptide-MHC interactions we have investigated the use of photosensitive-crosslinkers. Two reagents have been successfully tested. A benzophenone derivative of peptide 1-16 from rat myelin basic protein (RMBP) was only effective after the introduction of a glycine spacer residue between peptide and crosslinker. An azido-nitro-benzoyl derivative of peptide 7.4, a heteroclitic analog of RMBP 1-11 (1), had a high affinity and bound specifically to the peptide binding site. The 7.4 photoaffinity probe has been used to test the binding properties of other analogues of RMBP 1-11 and is currently being used to define (a) the kinetics, (b) pH and (c) temperature dependence of the binding event. This particular photoaffinity conjugate retains both the MHC binding and biological properties of the original peptide and is helping us to define the roles of "determinant" versus "T cell repertoire" selection in the MHC linked autoimmune response to MBP (see accompanying poster by Dawn E. Smilek, David C. Wraith and Hugh O. McDevitt).

(1) Acha-Orbea, H., Mitchell, D.J., Timmermann, L., Wraith, D.C. et al. (1988) Cell 54, 263-273.

C 337 BACTERIAL PROTEINS THAT ARE PRESENTED TO A DEFINED SUBSET OF T CELL RECEPTORS BY CLASS II MHC, Junji Yagi, Jody Baron, Steve Buxser* and Charles A. Janeway, Jr. Section of Immunobiology, Yale University School of Medicine, New Haven, CT 06510, *Division of Cell Biology, The Upjohn Company, Kalamazoo, MI 49001.

We have examined the responses of cloned T cell lines and normal T cells to staphylococcal enterotoxins (SE), A, B, and C_1 (SEA, SEB and SEC_1). SEA, SEB and SEC_1 are very potent mitogens for T cells in the presence of B cells. The minimal activating dose of all these SE's is about 1 ng/ml. By blocking the responses of both normal T cells and cloned T cell lines, SEA requires either the I-A or the I-E molecule on B cells for stimulating T cells, while SEB requires the I-E molecule. The TCR:CD4 complex is also involved in the response to SE. The responses to SEB and SEC_1 are inhibited by anti-$V_\beta 8$ antibody F23.1, while the response to SEA is not affected by this antibody. Anti-CD4 effectively inhibited the responses to all SE's. The involvement of TCR is also confirmed by FACS analysis of blast T cells responding to SE and the responses of a panel of cloned T cell lines, showing all $V_\beta 8^+$ cells respond to SEB and none to SEA. By using fixed B cells, processing is not required for the presentation of SE. Furthermore, antigen pulsing shows that SE binds to both B cells and T cells, and can in one case activate T cells. Thus, SEB binds together $V_\beta 8$ expressing TCR with the I-E molecule, while SEA binds together different V_β expressing TCR with either the I-A or the I-E molecule, showing interesting correlation between SE and $V_\beta 8$ expression.

Section A
Antigen Structure (B and T Cell Determinants, Immunogenicity); Adjuvants and Manipulation of Cellular Immunogenicity; T Cell Activation and Inactivation; Generation of the T Cell Repertoire; Autoimmunity and the Immunogenicity of Self Tissues

C 400 PREFERENTIAL EXPRESSION OF THE T CELL RECEPTOR Vβ3 GENE BY Mls^c REACTIVE T CELLS, Ryo Abe, Melanie Vacchio, Barbara Fox, Louis A. Matis, and Richard J. Hodes, Experimental Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD. 20892, Rheumatology Division, University of Maryland Medical School, Baltimore, MD. 21201, Molecular Immunology Laboratory, Division of Biochemistry and Biophysics, center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, MD 20892
The antigen-specific T cell repertoire is diverse in its ability to recognize a wide universe of foreign antigens. This T cell repertoire is composed of a set of clones each of which is specific for a given foreign antigen. Therefore the precursor frequency of T cells specific for any give foreign antigen is extremely low. However, two prominent exceptions to this general rule exist, and these are the T cells present at high precursor frequency which are specific for foreign MHC products or for the products of the minor lymphocyte stimulatory (Mls) genes in the mouse. The present studies were undertaken in order to examine factors involved in T cell repertoire formation by assessing the relationship between T cell repertoire for conventional foreign antigens and for Mls products. Results from these studies indicate a striking degree of overlap between the set of T cells specific for pigeon cytochrome c and the set of T cells specific for Mls^c gene products. Moreover, we demonstrate that the basis for this overlap lies in the predominant expression of one TCR Vβ gene, Vβ3, by those T cells which recognize Mls^c. These results indicate the critical involvement of specific TCR αβ dimers in recognition of Mls^c and further suggest that T cell reactivity to these gene products may play an important role in establishing the T cell repertoire for foreign antigens.

C 401 IL1 AND IL4 INDUCE ESSENTIAL SURFACE STRUCTURES ON ANTIGEN PRESENTING CELLS. Francesca B. Aiello, Dan L. Longo, Roy Overton and Scott K. Durum. National Cancer Institute, Bldg. 560, Frederick, MD 21701
Fixation of antigen presenting cells (APC) with the cross-linking agent ECDI eliminates their ability to present antigen to T cell clones. Using the D10 T cell clone, we have analyzed the defect in ECDI-fixed APC. We conclude that, rather than destruction of some essential APC structure, ECDI fixation prevents the APC from actively responding during the encounter with the T cell. This results in a failure to express new structures (probably located on the APC plasma membrane) that appear to be essential for stimulating T cell proliferation. These structures are distinct from Ia or IL1. The induction of these structures during T-APC interaction occurs in six hours, requires protein synthesis, and can be elicited by IL1, IL4 or LPS, but not IFN-gamma. In the absence of these induced structures, the APC stimulates a partial T cell response, IL4 release, but the T cells fail to proliferate. These induced structures on the APC may be either adhesion molecules that stabilize the T-APC interaction, or they may provide additional stimuli to the T cell.

C 402 ANTIBODY RESPONSE TO A PROTEIN HAVING A DISORDERED AND FLEXIBLE CONFORMATION, Akio Ametani*, Soon Mi Kim, Shuichi Kaminogawa and Kunio Yamauchi, Department of Agricultural Chemistry, The University of Tokyo, Tokyo, JAPAN (*Present address: Department of Microbiology, UCLA, Los Angeles, CA 90024)
αs1-casein (αs1-CN), which is one of the major proteins in bovine milk, has not a rigid and compact structure. The location of immunodominant regions on αs1-CN was compared among three strains of mice (BALB/c, C3H/He and C57BL/6). Anti-αs1-CN antisera were separated 5 weeks after the first immunization. Thirty-seven kinds of peptides were obtained from αs1-CN by proteolysis, and the segmental 19- to 20-residue peptides overlapping with their adjacent neighbors by 5 residues were synthesized over the entire polypeptide chain of αs1-CN. The ability of the anti-αs1-CN antibody to bind peptides was tested by an enzyme-linked immunosorbent assay, in which the peptides were adsorbed to the solid phase. All the strains responded highly to αs1-CN. The immunodominant antigenic regions of αs1-CN were not common to the three strains of mice (BALB/c, regions 1, 3, 5, 6 and 7; C3H/He, regions 2, 3, 5, 6, 6', 7 and 7'; and C57BL/6, regions 2, 4, 5, 6 and 7). These results show that there were regions with varying degrees of immunodominance of αs1-CN whose structure could not be distinguished between the surface and the interior. Our comparison also indicates that the pattern for the location of immunodominant regions on αs1-CN differed markedly between the strains which have the different gene background.

C 403 ANALYSIS OF THE T-CELL RESPONSE AGAINST TYPE II COLLAGEN IN THE DBA/1 MOUSE.
Mikael Andersson, *Michael A. Cremer and Rikard Holmdahl, Dept. of Medical and Physiological Chemistry, Uppsala University, Uppsala, Sweden. *VA Medical Center, Memphis, TN.
Immunisation with type II collagen (CII) leads to development of arthritis in mice with certain MHC haplotypes and is associated with an immune response against CII. We have been studying the T-cell response in the arthritis susceptible strain DBA/1 (H-2q). Analysing the proliferative response in cultures of lymph node cells from immunised mice as well as T-cell lines and clones established from such cultures it was found that:
1/ The T-cell response after immunisation with heterologous CII was preferentially directed against foreign determinants on the CII molecule with little or no crossreactivity against autologous CII.
2/ Both the primary response and the reactivity of established lines and clones were directed against the CB11 fragment of the CII molecule, using CB11 fragments prepared from chick, bovine or rat CII.
3/ Pepsin present in CII preparations after using pepsin digestion for solubilisation of the collagen is strongly immunogenic even in very small amounts and it was therefore necessary to use CII prepared from lathyritic cartilage without pepsin digestion for immunisation.
In contrast to the pattern in lymph node cultures from immunised mice we found that when culturing spleen cells from unimmunised mice there was a T-cell response against collagen that was preferentially directed against autologus CII. Since we earlier have found that autologus CII may induce an immune response and also arthritis in DBA/1 mice we conclude that there exist T-cells capable of reacting with autologus collagen and inducing an immune response as well as arthritis but that these cells are under regulation so that they not readily can be activated into proliferation but may be induced to perform certain effector functions.

C 404 A CTL CLONE FROM TUMOR INFILTRATING LYMPHOCYTES (TIL) DETECTS INTRATUMOR HETEROGENEITY OF MELANOMA CELLS: CORRELATION WITH DEGREE OF PIGMENTATION OF TUMOR CLONES. Andrea Anichini, Arabella Mazzocchi, Giuseppe Fossati and Giorgio Parmiani. Division of Experimental Oncology D, Istituto Nazionale Tumori, 20133 Milan, Italy.

A $CD3^+$, $CD8^+$, $WT31^+$ CTL clone (8B3), isolated from TIL of a metastatic melanoma patient, reacted preferentially against 3 different autologous tumor lesions (Me665/1, /2 and /R) but did not lyse autologous B cells or allogeneic tumor lines. The lysis of Me665 could be blocked by MAb to HLA class I antigens and to CD3 structure. Modulation of the CD3 complex abolished the recognition of Me665 tumor cells, but did not alter lysis of K562. In a screening of 25 tumor clones from Me665/2, only 4 neoplastic clones were lysed by 8B3 and could compete with each other in cold target competition assays. Analysis of the degree of pigmentation revealed that the 4 clones recognized by 8B3 had lower melanin content/cell than all the other tumor clones. These results suggest that 8B3 cells recognize a target determinant in an HLA-restricted fashion and that this determinant might be preferentially expressed on tumor cells in an early phase of differentiation.
Supported in part by grant N. 86.00696.44 of Progetto Finalizzato Oncologia of CNR (Rome, Italy).

C 405 NZW MHC CONTRIBUTION TO LUPUS-LIKE AUTOIMMUNE DISEASE, Virginia B. Appel, Brian Kotzin, Ed Palmer, Department of Pediatrics, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO 80206

NZW mice have been studied because of their contribution to lupus-like autoimmune disease in (NZW x NZB) F_1 mice. (NZW x NZB) F_1 x NZB backcross studies have shown the NZW contribution to be a single locus. Extensive mapping studies of this backcross indicate the gene controlling autoimmunity is tightly linked to the $H\text{-}2^Z$ locus on chromosome 17. Genomic clones for $I\text{-}A^Z$ and $I\text{-}E^Z$ have been isolated, and are being used to produce transgenic mice. These mice will be used to determine exactly which NZW gene product regulates this lupus-like disease.

C 406 CHARACTERIZATION OF TWO cDNA ENCODING THE T CELL ANTIGEN CD6. Alejandro Aruffo. and Brian Seed. Department of Molecular Biology, Massachusetts General Hospital. Boston. MA 02114. CD6 is a 120 kd glycoprotein expressed on the surface of T cells and a number of B cell leukemias and lymphomas. Recent reports indicate that T cells treated with anti-CD3 and anti-CD6 monoclonal antibodies followed by treatment with anti-mouse immunoglobulin antibodies can be induced to proliferate by adding IL-2. Cross-linking of CD6 with anti-CD6 monoclonal antibodies and anti-mouse immunoglobulin antibodies does not induce IL-2 dependent T cell proliferation but results in an increase in the cytoplasmic calcium concentration. We have isolated two cDNAs encoding the CD6 antigen from a eucaryotic expression library prepared from mRNA isolated from the human T cell tumor line HP-BALL. These two cDNAs differ in the length of their 3′ untranslated regions. COS cells transfected with either one of these CD6 cDNAs direct the expression of a protein with a molecular weight of 90 kd which is recognized by all 8 anti-CD6 monoclonal antibodies tested. The characterization of these two CD6 cDNAs will be presented.

C 407 ANALYSIS OF HLA POLYMORPHISM USING SEQUENCE SPECIFIC OLIGONUCLEOTIDE PROBE HYBRIDIZATION TO AMPLIFIED DNA, Lee Ann Baxter-Lowe, Jay B. Hunter, and Jack Gorski, The Blood Center of Southeastern Wisconsin, Milwaukee, Wisconsin 53233. HLA polymorphism plays a key role in antigen:MHC interaction. The polymorphism of the first domain encoding exon of the HLA-DR β chain has been studied by in vitro DNA amplification and use of sequence specific oligonucleotide probe hybridization (SSOPH) to detect polymorphic sequences. A 230 bp segment of genomic DNA was amplified and hybridized with synthetic oligonucleotide probes (12-19 bases) under conditions that detect single base pair mismatches. Identification of these mismatches can be used to predict micropolymorphism in the protein products, including single amino acid changes. Haplotype specific patterns of oligonucleotide probe hybridization were defined for a panel of homozygous typing cells. Analysis of family data demonstrated the expected inheritance patterns. Most known serological specificities are encoded by multiple allelic forms of DR β chains and SSOPH can identify these differences. This was exemplified by detection of unique SSOPH profiles for subtypes of DR4, DRw6 and DRw52 alleles. This procedure was also used for analysis of HLA-DR polymorphism in large numbers of heterozygous individuals, including an HLA-deficient SCID patient. The SSOPH data were correlated with serological specificities and will be useful for delineation of HLA restriction in allo- and autoimmunity.

C 408 ANALYSIS OF TOLERANCE TO CLASS I AND CLASS II MHC ANTIGENS IN CHIMERIC ORGAN-CULTURED MURINE FETAL THYMUS, B.N.Beck, Department of Immunology, Mayo Clinic, Rochester, MN 55905. Murine chimeric thymus lobes have been created in vitro according to established techniques. Thymic lobes are removed from fetal mice of 14 days gestational age and placed in organ culture in the presence of 2-deoxyguanosine for five days to deplete the lobes of lymphocytes and macrophages. After removal from deoxyguanosine culture, the lobes are repopulated with stem cells provided by co-culture with allogeneic fetal liver, also of 14 days gestational age. Following 2-3 weeks of growth and development in vitro the thymocytes are harvested by mechanical disruption of the lobes and assayed for their ability to respond in mixed lymphocyte cultures by proliferation and the generation of cytotoxic cells. As has been previously reported (Jenkinson, et al, Transplantation 39:331, 1985), A → B (A fetal liver co-cultured with B deoxyguanosine-treated thymic lobes) chimeric thymocytes, while tolerant of A, appear not to be tolerant of B stimulator cells in mixed lymphocyte culture. On the other hand, A → B chimeric thymocytes do not develop cytotoxic cells capable of killing B target cells, even when cultured in the presence of exogenous recombinant IL-2. A → B chimeric thymocytes respond normally to third-party strain C stimulators in both mixed lymphocyte culture and the generation of cytotoxic cells. Thus, it appears that tolerance to class I antigens but not to class II antigens has been acquired in the chimeric thymus organ cultures. Experiments to quantitate, by limiting dilution analysis, the precursor frequencies of cells responding to class I and class II antigens are in progress.

C 409 T CELL CHEMILUMINESCENCE : A NOVEL ASPECT OF T CELL MEMBRANE ACTIVATION STUDIED WITH JURKAT TUMOR CELL LINE. Gilles Benichou* and Gerald Leca.
INSERM U131, 32 rue des Carnets, 92140 Clamart, FRANCE *Present address : Department of microbiology, U.C.L.A., 405 Hilgard Ave, Los Angeles, CA 90024, U.S.A.

Different cell membrane receptors have been shown to be involved in human T lymphocyte activation induced by either monoclonal antibodies or mitogenic lectins. These T cell surface molecules can be divided into two categories : a) the T cell antigen receptor (TcR) associated with the non-polymorphic CD3 antigen b) T cell differentiation molecules not linked to CD3/Ti such as CD2 (T11) and Tp44 (9.3). Monoclonal antibodies directed against these T cell surface structures triggered different T lymphocytes functions : mitogenesis, IL-2 receptor expression, IL-2 secretion. Our knowledge about early events involved in T cell membrane activation is not complete, especially involving the transduction mechanism mediated by GTP-binding proteins ; nevertheless, numerous authors have demonstrated that CD3/Ti complex triggering induces the activation of phospholipase C, leading to the phosphoinositide cascade associated with an increase of free cytoplasmic calcium ions.

In the present report, we show that different activating cell molecules (Con A, PHA and PMA) can trigger oxygen free radical liberation when incubated with the human Jurkat tumor T cell line. Since membrane oxidative metabolism has been shown to be related to the stimulation of the phospholipase A2, and to be the final consequence of a membrane NADPH-oxidase : this could represent a previously undescribed pathway of T lymphocyte activation.

C 410 **PROBING ANTIGEN STRUCTURE BY SITE-DIRECTED MUTAGENESIS.** D.C. Benjamin, C.W. Hershey, E.D. Hershey and Alisa M. Smith. Department of Microbiology, School of Medicine, University of Virginia, Charlottesville, VA 22908.

High affinity monoclonal antibodies (MAb), specific for Staphylococcal nuclease (NASE), were produced and characterized. Competitive inhibition assays were conducted resulting in a series of complementation groups that define eight overlapping epitopes. It is estimated that these epitopes account for 70% or more of the accessible surface of NASE. Mutagenesis of the coding sequences for NASE was carried out to produce a series of variant molecules (each differing from wild-type NASE and from each other by a single amino acid) that will enable mapping of NASE epitopes, determination of residues involved in antibody binding, and the contribution of various physical and chemical factors to affinity and fine specificity. Screening some of these mutants with the panel of MAb enabled us to map several nonoverlapping epitopes and further subdivided some of the MAb complementation groups. Oligonucleotide-directed mismatch mutagenesis has been done on codons encoding the original amino acid residue and other surface residues in its immediate vicinity. Determination of enzyme activity and structural analysis by CD spectropolarimetry of several of the mutant proteins suggests that any structural changes that may occur are local and not global. Supported by grants AI20745, T32CA09109 and SO7RR05431 from the National Institutes of Health.

C 411 POSITIVE SELECTION OF A CLASS II MHC-RESTRICTED T CELL RECEPTOR IN TRANSGENIC MICE, Leslie J. Berg, Department of Microbiology and Immunology, Stanford University, Stanford, CA, 94305, Ann Pullen, Division of Immunology, National Jewish Center, Denver, CO, Barbara Fazekas de St. Groth, and Mark M. Davis, Department of Microbiology and Immunology, Stanford University, Stanford, CA, 94305.
We have analyzed transgenic mice carrying functionally rearranged T cell receptor α and β chain genes. These genes, originally isolated from the 2B4 hybridoma, encode a receptor specific for a peptide of pigeon (or moth) cytochrome c bound to the murine class II molecule, I-E^k. This system allows us to examine the factors influencing development of the T cell repertoire, in particular, the generation of self-MHC restriction. We have examined the frequency of cytochrome c reactive T cells in 2B4 α/β transgenics which have been backcrossed onto a compatible (k) versus an incompatible (b) MHC type. W find on average a ten-fold higher frequency (1:150 versus 1:1500) of cytochrome c reactive cells in unimmunized mice carrying a k allele at the MHC. These results support the hypothesis that T cells carrying receptors capable of recognizing self-MHC in the absence of antigen are positively selected for maturation. In addition, we have observed a severe depletion of mature CD4-positive and CD8-positive T cells in mice expressing high levels of the 2B4β transgene plus a self-antigen, Mls-2^a. This depletion, due to the establishment of self-tolerance, is also an MHC-dependent effect.

C 412 THE ROLE OF PROTEIN KINASE C IN ACTIVATION OF T CELL PROLIFERATION, Nicola Berry, Katsuhiko Ase, Ushio Kikkawa, Akira Kishimoto and Yasutomi Nishizuka, Department of Biochemistry, Kobe University Medical School, Kobe 650, Japan. Activation of T cell proliferation is believed to occur via the hydrolysis of inositol phospholipids, which, through the second messengers inositol-1,4,5-tris phosphate and diacylglycerol (DAG), promotes the elevation of intracellular calcium levels and activation of protein kinase C (PKC), respectively. The role of PKC in T cell activation was investigated by comparing the effects of stimulation by 12-O-tetradecanoyl phorbol acetate (TPA), and the DAG, oleoyl acetyl glycerol (OAG), on a >99% pure population of T cells cultured in RPMI 1640 medium containing 10% autologous serum. Treatment with either TPA or OAG caused down-regulation of the T cell receptor, a consequence of its phosphorylation, but only TPA, in synergy with ionomycin, was capable of stimulating interleukin 2 receptor (IL2-R), expression and, subsequently, proliferation. Immunohistochemical staining with antisera specific for the PKC subspecies α, βI, βII and γ shows that resting T cells express α, βI and βII PKC subspecies which are diffusely distributed throughout the cell. After 20 minutes treatment with either OAG or TPA all three subspecies are redistributed to a focal area within the cell. The redistribution is transient in OAG stimulated cells, where the PKC distribution is similar to that in untreated cells after 1 hour of treatment. In TPA stimulated cells, however, the PKC redistribution is prolonged, becoming more marked until mitosis occurs after 48-72 hours of treatment. These results suggest that transient intracellular redistribution of PKC causes phosphorylation and down-regulation of the T cell receptor, but that prolonged redistribution is required for T cell proliferation.

C 413 I-E IS NECESSARY BUT NOT SUFFICIENT FOR CLONAL DELETION OF MURINE $V\beta11^+$ T CELLS. Jerome Bill*, Osami Kanasawa‡, David Woodland* and Ed Palmer*, Department of Pediactrics* National Jewish Center for Immunology and Respiratory Medicine, Denver, CO 80206 and Lilly Research Laboratories‡, La Jolla CA 92037. We have generated a monoclonal antibody, RR3-15, which recognizes murine T-cell receptors containing the Vβ11 domain. Using this antibody to stain peripheral T-cells, we have demonstrated that Vβ11 bearing T-cells are largely absent from strains of mice which express the Class 2 MHC molecule, I-E. Studies with F1 mice demonstrate that this effect is dominant, consistent with tolerance. The clonal deletion of $V\beta11^+$ T-cells are present in the thymus of I-E bearing mice. While the expression of I-E molecule is a necessary condition for the clonal deletion of Vβ11 bearing T-cells, other non-MHC genes control the clonal deletion process, as well. Paradoxically, only a small fraction of $V\beta11^+$ T-cell hybridomas are I-E reactive.

C 414 POSITIVE THYMIC SELECTION OF $V\beta\ 17^+$ T CELL RECEPTORS. Marcia A. Blackman, John W. Kappler and Philippa Marrack, Howard Hughes Medical Institute at Denver, Department of Medicine, National Jewish Center, Denver, CO 80206. Peripheral levels of $V\beta17^+$ T cells vary with MHC haplotype in mouse strains that lack the strongly tolerizing I-E molecule. This difference is particularly striking in the $CD4^+$ T cell compartment. For example, $H\text{-}2^q$ mice contain 14-19% $CD4^+$ $V\beta17^+$ T cells in their periphery, whereas the number is 3-4% in $H\text{-}2^b$ mice. In (b x q)F_1 animals, expression is intermediate, indicating that selection and not tolerance (which would be dominant) might be the controlling mechanism. This was confirmed experimentally in chimeric mice. First, $F_1 \rightarrow$ parent chimeras reflected the parental level, indicating that expression was controlled by the parental selecting cells (thymic epithelium) rather than the F_1 tolerizing cells (bone-marrow-derived). Second, chimeras grafted with syngeneic or allogeneic thymus reinforced this observation. The peripheral level of the $CD4^+$ $V\beta17^+$ population in these mice reflected control by the MHC haplotype of the selecting cells contributed by the thymus graft. Thus, we have a direct demonstration of positive selection in the thymus in a system that measures the T cell repertoire directly, by a T cell receptor antibody, rather than by T cell reactivity.

C 415 GENETIC ANALYSIS OF SM SPECIFIC T-CELL RECEPTORS
D. Bloom, P.L. Cohen, and S.H. Clarke, Department of Microbiology and Immunology, University of North Carolina-Chapel Hill, Chapel Hill, North Carolina 27514
Sm is a nucleoprotein complex associated with small RNA molecules in eukaryotic cells. The spontaneous generation of anti-Sm antibodies is specific for patients with systemic lupus erythematosus (SLE) and develops in 25% of MRL mice. The response has been shown to be T-cell dependent in MRL/lpr mice. T-cells specific for Sm are found only in MRL (H-2k) mice and mice bearing H-2s and H-2f haplotypes (which do not develop anti-Sm antibodies). We are currently working to define the variable regions of the T-cell receptor genes used in the Sm response. A series of T cell hybridomas from MRL mice has been generated and are being screened for Sm positivity. A technique has been designed to amplify specific alpha and beta chain TcR genes using the polymerase chain reaction allowing for a more rapid sequence analysis. It is also our intention to locate the Sm specific epitopes of the T-cell hybridomas.

C 416 THE PRIOR ACTIVATION HISTORY OF A MURINE T CELL CLONE INFLUENCES ITS RESPONSE TO ANTI-T3 MEDIATED STIMULATION, P.R. Bohjanen and R.J. Hodes, Howard Hughes Medical Institute and Experimental Immunology Branch, NCI, NIH, Bethesda, MD 20892.
To determine whether prior activation history affects T cell receptor mediated activation of T cell clones, the murine type I helper clone AE7 was maintained in tissue culture by stimulation every ten days with either (1) antigen (cytochrome c), irradiated H-2^k spleen cells, and IL-2 or (2) IL-2 alone. AE7 cells grown with antigen and antigen presenting cells (AE7-AG) proliferated and produced T cell growth factor activity (TCGF) in its culture supernatants following stimulation with immobilized anti-T3 antibody. The TCGF activity was shown by bioassay using indicator cell lines and specific blocking antibodies to be almost entirely due to GM-CSF with little or no IL-2 activity detectable. Northern blot analysis showed high GM-CSF mRNA levels with low but detectable IL-2 mRNA levels. AE7 cells grown in culture by stimulation with IL-2 alone (AE7-IL2) displayed substantially greater anti-T3 induced proliferation than did AE7-AG cells. In contrast to AE7-AG cells, AE7-IL2 cells produced large quantities of IL-2 in response to anti-T3 stimulation. Furthermore, one cycle of stimulation of clone AE7-AG with IL-2 in the absence of antigen and irradiated spleen cells was sufficient to cause this clone to produce substantial amounts of IL-2 upon subsequent anti-T3 stimulation. These data suggest that T cell receptor mediated stimulation of T cell clones by specific antigen and antigen presenting cells inhibits subsequent anti-T3 induced IL-2 production.

C 417 SPECIFIC IMMUNE RESPONSES RESTORED BY ALTERATION IN CARBOHYDRATE CHAINS OF SURFACE MOLECULES ON ANTIGEN PRESENTING CELLS, C.J.P. Boog, J.J. Neefjes, J. Boes, H.L. Ploegh, C.J.M. Melief, The Netherlands Cancer Institute, Antoni van Leeuwenhoek Huis, Plesmanlaan 121, 1066 CX Amsterdam, the Netherlands Two class I major histocompatibility (MHC) mutant mouse strains, bm14 and bm6, differ from the strain of origin B6 (H-2)^b in one and two amino acids of the H2D^b and H-2K^b molecule, respectively. The bm14 D^b mutation results in specific failure of female bm14 mice to generate a cytotoxic T-lymphocyte (Tc) response to the male-specific antigen H-Y. The allospecific Tc response of CD8$^+$B6 T-cells against bm6 K^b mutant spleen cells, in contrast to that against other K^b mutants, is absolutely CD4$^+$T-helper cell dependent. Purified CD8$^+$T-cells completely fail to respond. We now report that the inability to mount these specific immune responses is restored by the use of dendritic cells (DC) as antigen presenting cells (APC). Comparison of MHC expression on various types of APC by cytofluorimetry and quantitative immunoprecipitation showed very high expression of class I and class II molecules on DC. Strikingly, examination of class I and class II molecules by isoelectric focusing (IEF) revealed qualitative differences as well. We show that the surface MHC class I molecules of DC are present in greater quantity and carry on average fewer sialic acids than the same molecules isolated from other APC types such as spleen cells, LPS blasts or con A blasts. That sialic acids on cell surface molecules, including MHC, may play a role in antigen presentation is suggested by our finding that removal of sialic acids, by neuraminidase, can restore specific responses to non-responder APC as well.

C 418 ENHANCEMENT OF ACCESSORY ACTIVITY OF DENDRITIC CELLS BY CONDITIONED MEDIUM FROM ACTIVATED MACROPHAGES AND RECOMBINANT IL-1 AND GM-CSF, William E. Bowers, Mary S. Ruhoff and Estelle M. Goodell, Bassett Research Institute, Cooperstown, NY 13326. Low density rat lymph node cells (LD-LNC; 5% of total unfractionated LNC) contain 95% of the accessory activity required for responses of T lymphocytes to mitogens. Overnight exposure of LD-LNC to silica or LPS significantly increases their ability to stimulate T lymphocyte proliferation. Conditioned medium (CM) obtained from LD-LNC incubated with silica or LPS mediates the enhanced activity. Macrophages present in LD-LNC and peritoneal exudates produce CM containing this enhancing activity, but their low accessory activity is not influenced by CM. CM has no effect on mitogen-treated T lymphocytes alone. Dendritic cells (DC) isolated from LD-LNC incubated with CM show greatly enhanced accessory activity. Overnight incubation with CM is required to induce maximum enhancement; once induced, accessory activity of DC is not further modulated by continued incubation in the presence or absence of CM. After gel filtration, the enhancing activity showed a broad molecular weight range from 20 to 55 kD, some of which was shown to be IL-1. Various treatments of macrophages indicated that IL-1 and other enhancing activities were not produced in a coordinated fashion. Recombinant human IL-1β and mouse GM-CSF have enhancing activity; other recombinant factors, including human IL-1α, tumor necrosis factor α, IL-4, and rat IFN-γ, as well as L cell-conditioned medium containing M-CSF, lack enhancing activity. At low concentrations recombinant IL-1β and GM-CSF are synergistic, but the activity does not exceed that of CM. Supported by NIH AI-17887 and the Stephen C. Clark Fund.

C 419 HUMAN AND MURINE IMMUNE RESPONSES TO PEPTIDES REPRESENTING SEQUENCES OF THE HUMAN ACETYLCHOLINE RECEPTOR. S. Brocke[1], M. Dayan[1], C. Brautbar[2], J. Rothbard[3] and E. Mozes[1]. [1]The Weizmann Institute of Science, Rehovot, Israel, [2]Hadassah Medical Center, Jerusalem, Israel, [3]ICRF, London, U.K. Myasthenia gravis (MG) is an immune disorder characterized by lymphocyte autoreactivity to the human acetylcholine receptor (AChR). In the present study, T cell proliferative responses and sera antibody levels of myasthenic patients to several synthetic peptides representing different epitopes of the human AChR were examined. We detected significant differences in the humoral and cellular responses of MG patients compared to healthy controls to peptides of the human AChR alpha-subunit with sequences p195-212, p257-269 and p310-327. Proliferative responses of lymphocytes from myasthenic patients to p195-212 and to p257-269 correlated significantly with HLA-DR5 and with HLA-DR3, respectively. In order to investigate further the immune responsiveness to selected sequences of the human AChR, T cell lines and clones specific for peptides p195-212 and p259-271 were established from lymph nodes of C3H.SW mice. The recognition specificities of these lines were tested by examining crossreactivity to a series of shortened and/or extended peptides of the above sequences. Deletions of amino acids in positions 211 and 212 (211=P, 212=L) resulted in a decrease of the peptides' stimulatory activity on the p195-212 specific T cell line, whereas deletion up to position 200 on the N-terminal end had no effect on the triggering potential of the peptides. Similar results were obtained when deleting residues 270 and 271 (270=V, 271=P) in stimulation assays of the p259-271 specific T cell line. This study should help in determining important T cell epitopes on the human AChR.

C 420 FUNCTIONAL AND PHENOTYPIC CHARACTERISTICS OF BULK CELL LINES AND A CLONE FROM A PATIENT WITH AN OLIGOCLONAL EXPANSION OF DOUBLE NEGATIVE T LYMPHOCYTES. Edward Brooks, Randall Goldblum, Daniel Wirt, Smita Vaidya, and Gary Klimpel, Departments of Microbiology, Pediatrics, and Pathology, Univ. of Tx. Med. Branch, Galveston, Texas, 77550. A five month old male with clinical and histologic features of graft-vs-host disease and combined immunodeficiency developed a massive T lymphocytosis consisting predominantly of a novel phenotype. These T lymphocytes lacked the CD4 and CD8 structures but expressed the mature alpha/beta T cell antigen receptor (CD3+,CD4-,CD8-,TCRa/ß). Southern blot analysis of the TCR indicated oligoclonality. Bulk cell lines were developed from peripheral blood mononuclear cells using interleukin-2 (IL-2) and irradiated feeder cells. Cloning of one of these bulk cultures was performed by limiting dilution. Both the bulk cultures and a clone remain dependent on IL-2 for growth. The predominant phenotype of the cells have remained double negative with no evidence for differentiation into CD4+ or CD8+ populations. A minority of the bulk cell line expressed the gamma/delta TCR. The bulk cell line and clone were found to have a high degree of non-MHC restricted cytotoxicity against natural killer cell sensitive and insensitive targets as well as autologous and allogeneic fibroblasts. When stimulated with mitogen, the cell line produced tumor necrosis factor activity but no interferon or IL-2 activity. The phenotype, IL-2 dependence, and functional activity of these T cells resemble those of immature thymocytes. The characteristics of this unique cell line appear to correlate with in vivo manifestations of immunodeficiency and graft-vs-host-like reactions.

C 421 EVIDENCE THAT A GTP BINDING PROTEIN REGULATES PHOSPHORYLATION OF THE CD3 ANTIGEN IN HUMAN T LYMPHOCYTES, Doreen A. Cantrell, Jonathan D. Graves and Sue Lucas, Lymphocyte Activation Laboratory, Imperial Cancer Research Fund, London, WC2A 3PX, U.K.
The role of guanine nucleotide binding regulatory proteins (G proteins) in the regulation of phosphorylation of the γ subunit of the CD3 antigen has been examined. CD3 γ chain phosphorylation in isolated T cell microsomes or permeablised T cells was stimulated by the G protein activator, guanosine 5'-O thiotriphosphate (GTPγS), but other nucleotides such as cAMP or GDPβS were ineffective. GTPγS effects on CD3 phosphorylation were calcium dependent. These data are consistent with the involvement of a G protein in the signalling mechanisms that regulate the phosphorylation of the CD3 γ chain. The regulatory effects of calcium and GTPγS were compared in normal peripheral blood derived T cells and Jurkat cells. There were differences regarding G protein regulation of CD3 γ chain phosphorylation in normal T cell and Jurkat cells and current models explaining these differences will be described.

C 422 ONTOGENY OF T CELL RECEPTOR GAMMA-DELTA EXPRESSION, Simon R. Carding*, Eric J. Jenkinson#, Kim Bottomly*, John J.T. Owen# and Adrian C. Hayday+, Section of Immunobiology* and the Department of Biology+, Yale University School of Medicine, New Haven, CT 06510, Department of Anatomy#, University School of Medicine, Birmingham B152TJ England
Expression of the gamma-delta T cell receptor has been thought to first occur in a population of thymocytes shortly after their precursors populate the thymus between 11 and 13 days of gestation. In the course of our studies investigating the ontogeny of T cell receptor expression in the mouse embryo we have identified an extrathymic site of gamma-delta expression in a population of cells present at distinct times of gestation. Evidence will be presented demonstrating two periods of activity of the murine gamma locus in the developing embryo. The first occurs at a time in development when precursor cells are colonizing the thymus from the liver and the gene segment useage detected is different to that first expressed in cells of the developing thymus. The second (occurring at around the time of birth) involves the functional rearrangement and expression of a gamma gene segment corresponding to the initial functional rearrangements detected earlier in gestation in the thymus, which can occur independently of thymic influence. demonstrate a new site of gamma-delta receptor expression in the liver of newborn mice that can occur in the absence of any thymic influence.

C 423 *RECOGNITION OF ANTIGEN ON L3T4 CELLS INACTIVATES PRIMED CTL PRECURSORS*, Delanie J. Cassell and James Forman, Department of Microbiology, University of Texas Southwestern Medical Center at Dallas, Dallas, TX 75235
The primary *(in vivo)* response of C57BL/6 animals to the class I antigen Qa-1 is a helper (T_H) dependent event as indicated by the requirement for copriming with a distinct antigen capable of activating helper cells. In contrast, the secondary *(in vitro)* response to Qa-1 demonstrates no need for costimulation with the helper antigen. In attempts to more closely examine the helper requirements for activation of primed CTLp, we have observed that depletion of L3T4 cells from spleens of Qa-1 primed mice abrogates the *in vitro* generation of anti-Qa-1 effectors. The response is restored by the addition of concanavalin A induced supernatant (CAS) or by the addition of syngeneic but not Qa-1 allogeneic L3T4 cells. Indeed, even in the presence of CAS, L3T4 cells expressing the Qa-1 alloantigen specifically suppress the activation of anti-Qa-1 CTL in a manner reminiscent of that seen with Lyt-2 veto cells. Although the mechanism whereby L3T4 cells exert suppression is unclear, we have determined that CTLp are susceptible to veto only within approximately the first 48 hours of culture, after which they resist suppression. Results from further studies of the nature of suppression and the L3T4 veto cell will be presented.

C 424 GROUP I AND GROUP II DUST MITE (DERMATOPHAGOIDES) ALLERGENS: ANALYSIS OF MURINE IgG AND HUMAN IgE EPITOPES, Martin Chapman, Peter Heymann, Manuel Lombardero, & Tom Platts-Mills, Division of Allergy, University of Virginia, Charlottesville, VA 22908. The 24kd Group I and 15kd Group II proteins induce IgE ab responses in ~90% of mite allergic patients. We have compared the B cell epitopes on these allergens using panels of murine mAb and human IgG and IgE ab. The GpI and GpII allergens are antigenically unrelated. However, ag binding RIA on 73 sera showed that human IgG and IgE ab recognize crossreactive epitopes on GpI and GpII allergens from different mite species. In contrast, IgG ab in BALB/c mice immunized with 10ug Der p I in CFA was directed against "species specific" epitopes and <1% was anti-Der f I (a GpI homologue, with ~80% amino acid sequence homology to Der p I). Four non-over lapping epitopes were defined by mAb, with one species specific immunodominant site on each GpI allergen. Only one mAb recognized a cross-reactive GpI epitope and this mAb could inhibit human IgE ab binding by ~40%. The specificity of the murine anti-GpI response was not H-2 restricted, but could be altered by immunizing BALB/c mice with lower ag doses (1ug) in alum or B. pertussis. Using these regimes, up to 52% of the murine IgG ab responses was GpI cross-reactive. Murine ab responses to GpII allergens appear to be strain dependant. BALB/c are completely unresponsive to GpII, however, BALB/b, A/J, CBA, C3H & C57B16 all produce GpII cross reactive IgG ab. Thermal denaturation and reduction and alkylation expts suggest that the anitgenic sites on GpI allergens are conformational, whereas those on GpII may be sequential. Results with the GpI allergens suggest that immunization regimes which are known to affect IgE expression in mice may also affect the epitope specificity of IgG ab.

C 425 SELECTION OF ENCEPHALITOGENIC RAT T LYMPHOCYTE CLONES RECOGNIZING AN IMMUNODOMINANT EPITOPE ON MYELIN BASIC PROTEIN, Y.K. Chou, A.A. Vandenbark, R.E. Jones, G. Hashim and H. Offner, Neuroimmunology Research 151D, Veterans Administration Medical Center, Portland, OR 97201. Using the soft agar-cloning technique, we isolated thirteen T cell clones from guinea pig basic protein (GP-BP)-specific T cell lines derived from Lewis rats. The clonal frequency was approximately 2.5×10^{-5}. Each of these clones had a similar but not identical pattern of response to a battery of synthetic peptides representing overlapping epitopes in the encephalitogenic region for Lewis rats (69-89 sequence). All clones responded to the minimal encephalitogenic sequence (residues 72-84) restricted by I-A but not I-E molecules, and all transferred clinical EAE and delayed type hypersensitivity (DTH) reaction to naive rats. Phenotypically, the clones were $W3/13^+$ (Total T), $W3/25^+$ (T helper), $OX\text{-}22^+$ (DTH associated). This report demonstrates for the first time the applicability of the soft agar-cloning technique for obtaining encephalitogenic T cell clones. The exclusive recovery of 72-84-specific T cell clones after only two rounds of stimulation with GP-BP indicates the immunodominance of this epitope and the power of the line selection technique for obtaining encephalitogenic T cell specificities.

C 426 PREVENTION OF EXPERIMENTAL AUTOIMMUNE MYASTHENIA GRAVIS BY INDUCING NEONATAL TOLERANCE TO ACETYLCHOLINE RECEPTOR, Premkumar Christadoss, Mohan Shenoy, and Steve Keve. Department of Pathology, University of Vermont, Burlington, VT 05405.
As a first step to study the role of pathogenic lymphocyte clones in myasthenia gravis (MG), at the molecular level, we attempted to induce neonatal tolerance to acetylcholine receptors (AChR) in C57BL/6 mice. Twenty-four hour old C57BL/6 mice were injected subcutaneously with 70 ug of Torpedo AChR. At eight weeks both AChR injected and uninjected (neonatally) age, sex, and strain matched mice (control) were immunized with AChR in complete Freund's adjuvant, twice at monthly intervals. None of the animals neonatally tolerized with AChR developed muscle weakness characteristic of MG, while forty percent of control mice did develop muscle weakness. Moreover, neonatal tolerance to AChR suppressed serum autoantibody response to mouse muscle AChR. Neonatal injection of AChR could have eliminated AChR reactive clones and/or generated suppressor cells capable of suppressing an autoimmune response to AChR. Further molecular analysis should identify and characterize AChR reactive autoimmune clones and/or suppressor cells.

C 427 FUNCTIONAL LINKAGE BETWEEN MHC CLASS I ANTIGENS AND THE CD3Ti COMPLEX, Mogens H. Claesson, Peter Brams and Steen Dissing, Lab. Exp. Hemat. Immunol., Dept. Med. Anatomy A, and Dept. General Physiol. Biol., The Panum Institute, University of Copenhagen, Blegdamsvej 3, DK-2200, Copenhagen N, Denmark. The T lymphoma EL4, several beta-2 microglobulin deficient H-2 negative variants hereof, and two allospecific CTL clones were activated by immuno-immobilized anti-CD3 ab and IL-2 production, IL-2 receptor expression, serine esterase activity, and intracellular Ca-release, were examined. The expression of H-2 antigens was found essential for activation for all parameters studied. Furthermore, the presence of immuno-immobilized specific anti-H-2 ab exerted a strong costimulatory effect on anti-CD3 ab induced EL4 and CTL activation. Costimulatory effects were not observed in the presence of immobilized anti-Thy 1 ab or non-specific anti-H-2 ab. Our results are compatible with the existence of a functional linkage between MHC class I antigens and CD3 molecules.

C 428 TNF REGULATES LY-6A/E EXPRESSION AND ENHANCES THE LY-6 PATHWAY OF T CELL ACTIVATION. **Elaine K. Codias and Thomas R. Malek, Department of Microbiology and Immunology, University of Miami School of Medicine, P.O. Box 016960, Miami, FL 33101**
The Ly-6 alloantigens represent a family of phosphatidylinositol anchored proteins that function as accessory molecules in the process of T lymphocyte activation. The expression of these alloantigens is often induced on T and B lymphocytes after activation by mitogens or antigens. Previous studies have shown that the induction of Ly-6 alloantigens in T cells is at least in part due to the action of IFN-α/β or IFN-γ. In the present study, we have demonstrated that IFN-γ also induced Ly-6 molecules on B lymphocytes and bone marrow cells. Furthermore, we now show that TNF also participates in the induction of at least one of the Ly-6 proteins, Ly-6A/E. TNF was found to synergize with IFN-γ to induce Ly-6A/E expression in thymocytes, T lymphocytes, and bone marrow cells, but not B cells. For T lymphocytes, the synergistic induction of Ly-6A/E by TNF was restricted to cells from the Ly-6.1 haplotype whereas IFN-γ was sufficient to fully induce Ly-6A/E expression in cells from the Ly-6.2 haplotype. This result is consistent with the notion that there is more complex regulation of the Ly-6A/E molecules in T cells obtained from the Ly-6.1 haplotype. For T cells from BALB/c (Ly-6.1) mice, Ly-6A/E, but not Ly-6C, molecules were induced by IFN-γ and TNF. Furthermore, when compared to Ly-6A/E, the regulation of MHC class I molecules in these T cells by TNF was minimal. The induction of Ly-6A/E molecules on BALB/c T cells resulted in an enhanced capacity to activate these cells through the Ly-6 T cell activation pathway. One transformed T cell line, 5.1.2, was also identified whose Ly-6A/E molecules were synergistically induced by IFN-γ and TNF. Optimal expression of Ly-6A/E molecules on 5.1.2 cells required continuous culture of this cell line with these two cytokines and resulted in the detection of optimal levels of cytoplasmic Ly-6A/E mRNA by Northern blot analysis. This latter result suggests that IFN-γ and TNF regulate Ly-6A/E at the level of transcription and/or mRNA stabilization.

C 429 DIVERSITY OF EXPRESSED IMMUNOGLOBULIN V GENES IN THE NATURAL B CELL REPERTOIRE SPECIFIC FOR AN ENDOGENOUS ANTIGEN, **R.B. Corley, H.J. Sage, and J.D. Conger, Department of Microbiology and Immunology, Duke Medical Center, Durham, NC 27710**
The pre-immune repertoire is comprised of T and B lymphocytes that react with endogenous and exogenous antigens. We are analyzing the diversity of the B cell repertoire to the natural endogenous "antigen" senescent autologous erythrocytes (BrMRBC). We found that the antibodies of all cells of this specificity bind the trimethyl ammonium (TMA) group, part of the phosphatidyl choline molecule. Most or all of the B cells that secrete antibody of this specificity are members of the Ly-1$^+$ B cell subset. We produced a number of BrMRBC-specific hybridomas from mice of different ages and have begun to characterize their V regions by Northern blot and sequence analysis. Some of the hybridomas express Ig molecules that utilize the novel V_H and/or V_L gene segments described by Reininger *et al.* (J. Immunol. 138:316, 1987). Others (including a BrMRBC-specific Ly-1$^+$ B cell lymphoma) express V gene segments that previously have been associated with immune responses to exogenous antigens, presumably by Ly-1$^-$ B lymphocytes. There may therefore be no differences in the expressed V gene repertoires of these two cell types. All V regions so far sequenced can be ascribed to germ line genes; the only variations are due to junctional diversity and the insertion of N sequences. There is diversity in the choice of D and J gene segments used by these antibodies; however, comparison of widely differing sequences shows conserved Asp residues in CDR3 of V_H and CDR1 of V_L that may be important for TMA binding. The lack of mutations in BrMRBC-specific B cells raises the intriguing possibility that the natural autoimmune repertoire is different from the pathogenic autoimmune repertoire, from which the selection of specific B cells that are undergoing somatic mutational events occurs.

C 430 SELECTION OF THE T CELL RECEPTOR REPERTOIRE SOMETIMES DIFFERENTIALLY AFFECTS $CD4^+$ AND $CD8^+$ T CELLS, Nick Crispe, Division of Immunology, National Institute for Medical Research, Mill Hill, London NW7 1AA, U.K.

Clonal deletion of T cell specificities which encounter antigens expressed in the thymus appears to act on $CD4^+$, $CD8^+$ thymocytes, and to remove families of T cell receptors from both their $CD4^+$, $CD8^-$ and their $CD4^-$, $CD8^+$ differentiation products. Evidence is presented that, in certain circumstances, deletion can act selectively on single-positive thymocytes, arguing that susceptibility to clonal deletion occurs at several stages of T cell differentiation. Positive selection of T cell receptors by MHC molecules on thymus epithelium also acts selectively on one or other single-positive population.

C 431 DEFINITION OF MURINE T HELPER CELL DETERMINANTS IN THE MAJOR CAPSID PROTEIN OF HUMAN PAPILLOMAVIRUS (HPV) USING SYNTHETIC PEPTIDES, D.H. Davies[1], C.M. Hill[2], J.B. Rothbard[2] and B.M. Chain[1], Imperial Cancer Research Fund, [1]Tumour Immunology Unit, University College London, London WC1E 6BT, U.K., [2]Lincoln's Inn Fields, London WC2A 3PX, U.K.

HPVs are aetiologically linked with anogenital tract neoplasias, such as the marked association of HPV 16 with cervical intraepithelial neoplasia. Prevention and treatment of HPV infections by immunological means requires identification of 'protective' epitopes within the viral proteins recognised by T- and B-cells. Since capsid proteins possess candidate determinants owing to the presence of antibodies in patients with HPV infections, we studied the major capsid protein, L1, of HPV 16. We located murine T helper cell determinants to complement a similar study using human peripheral blood cells. The murine system enables responses to defined HPV-derived immunogens to be tested directly by immunisation. Eight peptides containing putative T-cell epitopes were synthesised and screened for lymphoproliferative activity by immunisation in FCA of $H\text{-}2^k$ and $H\text{-}2^d$ mice followed by in vitro assay. Three peptides caused proliferation of primary lymph node cells: 40-63 (restricted by $I\text{-}E^{k+d}$ and $I\text{-}A^{k+d}$), 91-106 and 279-294 (both $I\text{-}E^{k+d}$ and $I\text{-}A^k$). Analogous peptides derived from the L1 proteins of other HPV types were synthesised to determine whether they stimulated the proliferation of cells primed to HPV 16 peptides. No cross-reactivity was observed, indicating a peptide vaccine might be HPV type specific; this specificity may allow stringent diagnostic identification of HPV types present in infections.

C 432 THE INDUCTION OF NONRESPONSIVENESS IN FRESHLY ISOLATED T CELLS BY RAPIDLY MODULATING CD3. Laurie S. Davis, Mary C. Wacholtz, and Peter E. Lipsky, Dept. of Internal Medicine, UT Southwestern Medical Center, Dallas, Texas 75235.

An IgM anti-CD3 mAb (38.1) was found to modulate cell surface CD3 on highly purified human T cells within 5 hours in the absence of a secondary antibody or accessory cells. Modulation was documented both by immunofluorescence and by loss of stimulatory activity. Although 38.1 induced a rapid increase in intracellular free calcium ($[Ca^{2+}]_i$), T cells exhibited no other signs of activation and remained in a resting state as assessed by cell cycle analysis and assessment of activation antigen expression. These cells continued to express substantial amounts of surface CD3, suggesting a rapid rate of turnover of CD3 molecules in resting T cells. After modulation of 38.1 bound CD3, T cells were markedly inhibited in their capacity to respond to PHA. Depressed PHA responsiveness persisted for at least 3 days. Inhibition could be overcome with accessory cells or IL2. The inhibitory effects of 38.1 could be mimicked by briefly pulsing cells with the calcium ionophore, ionomycin. 38.1 or ionomycin pulsed cells were inhibited in their subsequent capacity to respond to PHA even when exposures were carried out in the presence of EGTA to prevent increases in $[Ca^{2+}]_i$ from extracellular sources. Inhibition was not the result of an inability to respond to PHA by increasing $[Ca^{2+}]_i$. Moreover the newly expressed CD3 molecules were capable of generating increases in $[Ca^{2+}]_i$ after reacting the cells with anti-CD3 + a cross-linking secondary antibody. These studies demonstrate that a state of nonresponsiveness in resting T cells can be induced by modulating CD3 with an anti-CD3 mAb in the absence of co-stimulatory signals. A brief increase in $[Ca^{2+}]_i$ resulting from the mobilization of intracellular calcium stores appears to be sufficient to induce this state of T cell nonresponsiveness.

C 433 ANALYSIS OF THE REQUIREMENTS FOR HUMAN T-CELL DIFFERENTIATION, Rolien de Jong, Vivienne Rebel, Gijs van Seventer, Miranda Brouwer, Frank Miedema, René van Lier, Central Lab.Blood Transf.Service, Lab. of Exp. and Clin.Immunology of the Univ. of Amsterdam, Amsterdam, The Netherlands

Monoclonal antibodies (mAb) directed against the human CD3 molecular complex induce a strong proliferation of T cells, when immobilized on microtiter wells. This activation system, that was shown to be independent of accessory cells, accessory-cell derived factors or LFA-1 mediated intercellular adhesion (1), allows one to study the requirements for T-cell proliferation and differentiation in a well defined manner. IL-2 and IFN-gamma but no IL-4 could be detected in culture supernatants of coated anti-CD3 stimulated T cells. The addition of rIL-1 or rIL-2 had only a moderate effect on T-cell proliferation, whereas helper activity for Ig production was strongly enhanced in the presence of these factors. In this system differentiation of precursors to cytotoxic T lymphocytes (CTL), as measured in anti-CD3-mediated cytotoxicity, could be demonstrated within 2 days after initiation of the activation. Allospecificity of the induced effector CTL was demonstrated using a panel of HLA class-I P815-transfectants. In this system the regulatory role of the CD28 molecule in T-cell activation and differentiation was studied. Addition of anti-CD28 mAb to T cells stimulated with coated anti-CD3 mAb enhanced IL-2 production, proliferation as well as Ig production. Interestingly, pCTL differentiation was also enhanced by anti-CD28 mAb. This system seems valuable for the analysis of requirements for differentiation of human T cells subsets.

1. Van Noesel et al., Nature 333, 850-852, 1988

C 434 REGULATION OF TCR-δ AND α REARRANGEMENTS, Jean-Pierre de Villartay and David I. Cohen, Laboratory of Chemical Biology, NIDDK, National Institutes of Health, Bethesda, Md 20892.

The newly described T cell receptor (TCR) δ locus is located inside the TCR α locus between Vα and Jα. Despite this unique situation, a highly efficient regulatory mechanism results in the complete independence of these two loci. We have recently described, in humans, a site specific recombination which joins a 5' deleting element (δRec) to the 5'end of the Jα's (ΨJα) resulting in the deletion of the TCR-δ locus in T lymphocytes expressing the α/β TCR. Rearrangements of the TCR as well as immunoglobulin genes are mediated by a unique recombination machinery and therefore, the specificity of these rearrangements is thought to be the result of a differential accessibility of the DNA involved in the recombination process. As a consequence (and/or cause) of the opening of a segment of DNA, the region involved is first transcribed as a sterile transcript prior to the rearrangement. In that regard, we have found that the 2 Kb of DNA upstream of ΨJα are actively transcribed ("T early α" transcript, TEA) early during fetal development. The presence of the TEA transcript presumably reflects the opening of the TEA sequence prior to the TCR-δ deletional rearrangement. In order to better understand the mechanisms involved in the DNA accessibility model, we started to look for DNA-binding proteins which might play a putative role in the opening or blocking of the TEA sequence. By the technique of "gel shift assay" we found such a negative regulatory protein in the nuclear extract from a non-lymphoid cell. The binding activity appeared to be specific as it was competed out by an excess of unlabeled autologous DNA and not by an excess of irrelevant DNA. Further studies are now in progress to determine first wether the presence of this binding activity can be correlated with a "closed" configuration of the TEA region and second to determine the precise location of the DNA binding region.

C 435 THE ROLE OF T CELLS IN MRL/lpr AUTOIMMUNITY, Jan Erikson, Fox Chase Cancer Center Philadelphia, Pa.19111 Homozygous MRL/MP-lpr/lpr (MRL/lpr) mice spontaneously develop an autoimmune disease that resembles systemic lupus erythematosus. Autoimmunity in MRL/lpr mice coincides with massive lymphadenopathy due to the accumulation of Lyt2-/L3T4- T cells. Several observations indicate that autoimmunity in MRL/lpr mice is T cell dependent. First, the clonal expansion of autoreactive B cells resembles an antigen-driven T cell dependent response. Second,both neonatal thymectomy and treatment with anti-Thy1 Mabs retard lymphoproliferation as well as autoimmunity. Interesting, so does the administration of a Mab to L3T4, thus suggesting that the T helper subset, which is not part of the unusual expanding population, is required for initiating the pathology in these animals. As a means of characterizing the expanding population of abnormal cells as well as the phenotypically mature (L3T4+) cells that may be associated with them, I have generated a series of T cell hybridomas from the enlarged lymph nodes and spleen of MRL/lpr mice. In parallel, I have derived a series of control (non-lpr/lpr) hybridomas from MRL/lpr X Balb/c F1 animals (which show no sign of pathology), and a series from MRL mice (which have a delayed onset of autoimmunity without lymphadenopathy). Very few hybridomas (20-30) were obtained in the non-lpr derived fusions. When I Con A stimulated the lymphocytes from non-lpr mice prior to fusion however, many more hybridomas were obtained.(120-220). This is in contrast to the fusion efficiency obtained from lpr/lpr mice which did not require in vitro lymphocyte stimulation to obtain a comparable number of hybrids. This result suggests that the MRL/lpr lymphocytes are activated in situ. In addition, while less than 15% of the lymphoid mass is comprised of T helper (L3T4+) cells, over 50% of the hybrids are L3T4+. The fact that a disproportionate number of T helper cells are rescued by fusion suggests that the cells activated in situ may be autoreactive T helper cells. Currently I am characterizing these T helper cells for their lymphokine production, T cell receptor gene usage and auto-specificity and will compare them to the hybridomas obtained from non-diseased animals.

C 436 EXPRESSION OF REARRANGED T CELL RECEPTOR TRANSGENES AFFECTS T CELL DIFFERENTIATION PATHWAYS. Barbara Fazekas de St. Groth, L. Berg, F. Ivars, C. Goodnow, S. Gilfillan, H-J. Garchon, J. Erikson and M. Davis. Department of Microbiology and Immunology, Stanford University, Stanford, CA 94305.

We have made transgenic mice bearing gene constructs encoding the T cell receptor α and β chains from a cytochrome C-reactive T cell hybridoma. Despite a lack of tissue-specificity in mRNA expression, cell surface expression of transgene-encoded protein was limited to T cells, presumably because both chains require CD3 proteins in order to assemble on the cell surface. In mice carrying only the α chain construct, the transgene was expressed in the thymus as early as day 15 of fetal life, 1-2 days before endogenous α chain mRNA. The first detectable cell surface expression of α transgene was on 25% of day 15 fetal thymocytes. This vast increase in αβ-bearing cells in fetal thymus was due to pairing of transgenic α chains with endogenous β chains, of which a substantial number are normally rearranged by day 15 of fetal life. The balance between αβ-expressing T cell supopulations was grossly disturbed in these mice, the most marked abnormality being an increase in the number of L3T4$^+$Lyt-2$^-$ cells both in thymus and in peripheral lymphoid organs. It therefore appears that premature expression of surface αβ T cell receptor may disturb T cell differentiation pathways by allowing T cells to leave the thymus without expressing L3T4 or Lyt-2. Mice carrying the β construct showed no increase in surface expression of T cell receptor in fetal life, since endogenous α chain rearrangement was limiting. In mice carrying either the α or β chain transgenes, the number and surface phenotype of T cells expressing γδ T cell receptors was unaffected in early fetal life, suggesting that the αβ and γδ T cell lineages diverge before the rearrangement and expression of the appropriate subset of T cell receptor genes.

C 437 A CLONED THYMIC CORTICAL EPITHELIAL CELL LINE INDUCES PROLIFERATION AND T CELL RECEPTOR GENE REARRANGEMENTS IN STEM CELL POPULATIONS, Susan J. Faas, Yasuhiro Hashimoto and Barbara B. Knowles. The Wistar Institute and the University of Pennsylvania, Philadelphia, Pa. 19104.The thymic stroma plays a major role in initiating the colonization, organization and differentiation of precursor stem cells into functionally mature T cells. A variety of cell types including thymic nurse cells, cortical and medullary epithelial cells, nonepithelial dendritic cells, and macrophages, combine to form the thymic stroma. The differential role of such cells in thymic development is unclear. We have isolated a number of morphologically distinct stromal cell lines from the thymuses of SV40 transgenic mice. Several of the cell lines are of epithelial origin, while others have features consistent with non-epithelial "dendritic" cells. We have focused on one of these cell lines, bearing the phenotype of a cortical epithelial cell, for its ability to support the growth and differentiation of stem cells from the fetal liver and fetal thymus, and cloned pre-T cells obtained from adult mice. The cortical epithelial cell line produces factors that induce the dramatic proliferation of fetal liver and thymic stem cells . In addition, fetal liver cells cocultured with this cell line are induced to rearrange and express their T cell receptor (TCR) genes. A cloned pre-T cell line is also induced to rearrange its TCR genes in response to signals mediated by this cell line.

C 438 ALTERED EXPRESSION OF LFA1 ON PERIPHERAL BLOOD LYMPHOCYTES FROM PATIENTS WITH AUTO-IMMUNE THYROIDITIS, Gilbert C. Faure, Violaine Guérin, Marie C. Béné, Corinne Amiel, Nadia Coniglio, Jacques Leclère, Laboratoire d'Immunologie and Clinique Endocrinologique, CHU de Nancy and Faculté de Médecine, 54500 Vandoeuvre les Nancy, France.

The LFA1 molecule, an adhesin of the LFA family involved in cell-cell interactions, is physiologically expressed on all white blood cells. It is absent in some congenital immune deficiencies ((ID), and is expressed on a decreased number of peripheral blood lymphocytes (PBL) in AIDS. We investigated its presence on PBL from 60 patients with auto-immune disorders of the thyroid. A monoclonal antibody (IOT16, Immunotech) directed to a conformational epitope involving both chains of LFA1 was used in indirect immmunofluorescence on PBL from blood drawn at a similar time in all patients. A calibrated flow cytometer (Epics Profile, Coultronics) was used to measure the percentage and numbers of positive cells, as well as the mean fluorescence (MF) and shape of the fluorescent peak. Data were correlated with clinical information,therapeutic, and other PBL features such as the CD4/CD8 ratio. The percentage of LFA1+ cells was significantly decreased in patients with Graves' disease, hypothyroidism and hyperthyroidism. The MF was lower and the shape of the fluorescent peak seldom displayed the bimodal characteristic noted in controls. These data suggest the participation of the altered expression of LFA1 in the pathogenesis or evolution of auto-immune diseases.

C 439 EXCESS ANTIGEN PRESENTATION TO AUTOREACTIVE T CELLS IS THE CAUSE OF CHRONICITY IN THE COMMON HUMAN AUTOIMMUNE DISEASES. Marc Feldmann, Marco Londei, Maija Kissonerghis, Beatrix Grubeck-Loebenstein, and Pier de Berardinis. Charing Cross Sunley Research Centre, Hammersmith, London W6 8LW, UK.
We have speculated that excess HLA class II expression, commonly found in active human autoimmune diseases maintains the activation of autoreactive T cells which in turn produce mediators which maintain class II expression. This hypothesis has been tested in many ways in thyroiditis. Critically autoreactive T cells are found in thyroid autoimmune tissues which are restimulated by thyroid follicular cells. More recently we have been exploring the specificity of the autoantigen reactive T cells in Hashimoto's thyroiditis where thyroglobulin specific clones have been found, in contrast to Graves' disease, where thyrocyte recognizing clones do not react with thyroglobulin. In rheumatoid arthritis, collagen type II clones have been found, persistently in the activated (IL-2R$^+$) T cell pool over several years in the same patient. To verify that antigen presentation is involved in rheumatoid arthritis (RA) a disease in which, unlike thyroiditis the nature of the major antigen presenting cell (APC) is unknown, the effect of anticlass II antibodies at concentration which block activation of T cells on the synthesis of HLA-DR mRNA was evaluated. The inhibitory effect supports a critical role of an as yet unknown APC in maintaining the chronicity of RA.

C 440 T CELL CLONES RECOGNITION OF PEPTIDES AND MUTANT PROTEINS WITH THE SAME AMINO ACID SUBSTITUTIONS. A.Finnegan and C.Amburgey, Rush Med.Sch., Chicago, IL 60612
Amino acid substitutions in small peptides have been used to identify residues that are critical for T cell recognition. If only the limited peptide region of the overall protein antigen is important for T cell recognition, then amino acid substitutions made in the intact protein and the peptide variants will have the same effect on T cell recognition. I-E^k-restricted, staphyloccocal nuclease-specific T cell clones are limited to the recognition of the peptide 86-100. Using peptide analogues, substitutions have been identified that effect T cell responsiveness. A heterogeneity in T cell clone responsiveness was observed when peptide 86-100 was substituted at residue 88 (gly to asp) suggesting that this residue may contact the T cell receptor. Conversely, all the clones were unable to respond to a substitution at 91 (tyr to asp). Nase mutant proteins were constructed with the same single amino acid substitution and T cell responses to peptides and mutants were compared. Preliminary evidence suggests that the mutant proteins like the peptides, substituted at residue 88 and 91, will not induce T cell clone responsiveness. These data suggest that the overall structure of the protein will not compensate for the lose of a particular amino acid which is necessary for T cell recognition.

C 441 SELECTIVE T CELL PRIMING IN THE ABSENCE OF ADJUVANT, Barbara S. Fox, Dominic Dordai and Brian E. Lacy, Department of Medicine, U. Maryland School of Medicine, Baltimore, MD 21201. To explore the variables important in T cell priming, an adjuvant-free immunization regimen was developed. B10.A mice were primed subcutaneously with syngeneic spleen cells that had been pulsed with high concentrations (100μM) of the peptide 81-104, a CNBr cleavage fragment of pigeon cytochrome c. The T cell response was assessed using a sensitive limiting dilution assay that measures lymphokine production with the CTL-L cell line. The precursor frequency of antigen-specific cells in the draining lymph nodes of mice primed with antigen-pulsed spleen (APS) was 1 in 4000, indistinguishable from the frequency of 1 in 3400 found in mice primed in the footpads with 10 nmol of 81-104 in complete Freund's adjuvant (CFA) (data are given as geometric means, n=5, S.E.M = x/÷ 1.7 and 1.3, respectively). Despite the apparent similarity in the T cell compartment of mice primed using these different regimens, antibody induction was strikingly different. Mice primed with 81-104 in CFA developed serum IgM and IgG responses against the peptide, with antibody detectable in an ELISA assay at a 1:3000 dilution. Mice primed with 81-104/APS, however, produced no detectable anti-peptide antibodies. Maximal T cell clonal expansion therefore appears to be possible in the absence of antigen-specific B cells. These data argue against the hypothesis that antigen-specific B cells play an obligate role in T cell proliferation in vivo. The reasons for the lack of antibody induction are currently under investigation.

C 443 TWO NOVEL PEPTIDES ASSOCIATE WITH THE T-CELL RECEPTOR UPON ACTIVATION James D. Fraser, Mark A. Goldsmith, and Arthur Weiss, Howard Hughes Medical Institute, Departments of Medicine and Microbiology and Immunology, University of California, San Francisco, CA 94143

We have identified two peptides of 35 and 39 kD that conditionally associate with the T cell receptor (TCR) complex of Jurkat cells. The coprecipitation of these peptides with TCR requires treatment with monoclonal antibodies (mAbs) directed against TCR (C305 or R140) prior to cell lysis and immunoprecipitation. Treatment of Jurkat cells with mAbs directed against CD2 (9-1 or 9.6) or HLA (W632) does not not induce the association of these peptides with TCR.

The signal-transduction mutant cell lines, J.CaM1 and J.CaM2, have previously been described (1,2). These cell lines, derived from Jurkat, fail to activate the inositol-phopholipid second-messenger pathway in response to anti-TCR mAbs. Treatment with mAb C305 induces the association of the 35 and 39 kD peptides with TCR in J.CaM2 cells but not in J.CaM1. J.CaM1 modulates TCR normally in response to anti-TCR mAb treatment (1). Hence, these observations suggest that the two peptides are involved in the signal-transduction pathway of the T cell receptor complex rather than receptor internalization.

1. Goldsmith, M.A. and Weiss, A. (1987) Proc. Natl. Acad. Sci. USA 84 , 6879-6883
2. Goldsmith, M.A., Dazin, P.F., and Weiss, A. (1988) Proc. Natl. Acad. Sci. USA In Press

C 444 MOLECULAR IMMUNOGENETICS OF SYSTEMIC LUPUS ERYTHEMATOSUS (SLE), Zdenka Fronek, Luika A. Timmerman and Hugh O. McDevitt, Dept. of Medicine, UCSD School of Medicine, La Jolla, CA 92093 and Dept. of Microbiology and Immunology, Stanford University School of Medicine, Stanford, CA 94305.

SLE is an autoimmune disorder associated with several different HLA class II antigens. We studied a large SLE patient population by sequencing of the PCR amplified first domain of the DQβ and DQα chains and by sequence specific oligonucleotide probes to further define these associations. Shared DQβ sequences at amino acid positions 26=leu, 30=tyr, and 57≠asp may predispose some individuals with HLA DR1,2,4, or 6 to develop SLE. A novel DQβ sequence found in two DRw6 DQw1 SLE patients shares these amino acids in the DQβ hypervariable regions. The association of the DR2 DQw1.AZH gene was greatly increased in the SLE patients with lupus renal disease. The HLA association may be directly due to structural aspects of the HLA genes.

C 445 SELF TOLERANCE ALTERS T CELL RECEPTOR EXPRESSION IN AN ANTIGEN SPECIFIC RESPONSE, Fry A.M. and Matis L.A., Molecular Immunology Lab., Division of Biochemistry and Biophysics Biophysics, Center for Biologics Evaluation and Research, F.D.A., Bethesda, MD 20892 The influence of the MHC gene products on the T cell receptor (TCR) repertoire is well documented. We have examined the influence of non-MHC encoded self antigens on the selection of the TCR repertoire using the pigeon cytochrome c immune response. Most pigeon cytochrome c specific, E^k restricted, T cells from B10.A mice express the V_a11 family gene product in association with a V_b3 gene encoded protien. We, therefore, examined V_a11 and V_b3 expression in pigeon cytochrome c specific T cell lines from various mouse strains with different non-MHC backgrounds. V_b3 was not expressed in some of the antigen specific T cell lines or in peripheral T cells from unimmunized mice due to tolerance induction to Mls^c. In contrast, V_b3 was expressed in thymic rna from V_b3- strains which is consistant with intrathymic clonal deletion. V_a11 was expressed in varying amounts by all the T cell lines. Examination of T cell lines from F1 hybrids between high and low V_a11 expressors showed no evidence of tolerance. Skewing of the TCR repertoire alters the fine specificity of the response to cytochrome c. Several V_b3- lines respond poorly to moth 88-103 in association with the E^b molecule. These results have led us to examine the response of E^b,Mls^c+ mice to cytochrome c peptide 88-103 in an attempt to detect an example of an Ir gene defect resulting from a hole in the TCR repertoire.

C 446 TOLEROGENICITY OF THYMIC EPITHELIUM, Er-kai Gao, Osami Kanagawa and Jonathan Sprent, Department of Immunology, Research Institute of Scripps Clinic, IMM4A, La Jolla, CA 92037.
When parent → F_1 chimeras are prepared with supralethal irradiation (1300 rad + 900 rad), the donor-derived $CD4^+$ cells differentiating in the chimeras show partial tolerance to host-type H-2 determinants, despite the apparent absence of host-type APC. Donor-derived $CD4^+$ cells give only low proliferative responses to host-type APC in primary mixed-lymphocyte reactions (MLR); furthermore, in $I\text{-}E^-$ → $I\text{-}E^+$ combinations, the donor $CD4^+$ cells show ≃ 70% deletion of $CD4^+$ cells expressing I-E-reactive $V_\beta 11$ T cell receptor molecules. Tolerance measured by these two parameters applies not only to lymph node (LN) $CD4^+$ cells but also to $CD4^+$ cells recovered from the thymus. This finding implies that tolerance is induced intrathymically, presumably through contact with a non-marrow-derived component of the thymus, e.g. epithelial cells. In support of this possibility, thymectomized a → (a x b)F_1 chimeras given strain a marrow cells and a strain a thymus graft (irradiated) show no detectable tolerance to host-type strain b determinants: the strain a $CD4^+$ cells differentiating in these chimeras give strong MLR to strain b and do not show deletion of $V_\beta 11^+$ cells.

C 447 AUTOIMMUNE UVEORETINITIS AND PINEALITIS INDUCED BY IMMUNODOMINANT AND NON-DOMINANT PEPTIDES DERIVED FROM THE RETINAL PROTEIN IRBP, I.Gery, H. Sanui, B. Wiggert, T.M. Redmond, L.H. Hu, H. Margalit, J.A. Berzofsky, and G.J. Chader, National Eye Institute and National Cancer Institute, NIH, Bethesda, MD 20892
IRBP (= interphotoreceptor retinoid-binding protein) is a glycoprotein of 1264 residues (bovine) which localizes in the retina and pineal gland and induces inflammatory changes in these organs (EAU and EAP, respectively) in immunized animals. The experimental disease is considered a model for certain uveitic conditions in man. We have recently shown that IRBP-derived synthetic peptides can also induce EAU/EAP in Lewis rats. The present study compared two such peptides, "R4" (residues 1158-1180) and "R14" (1169-1191). Peptide R14 was found to be immunodominant, shown by its being recognized by lymph node cells (LNC) or line cells sensitized against whole IRBP. In contrast, peptide R4 was not recognized by the whole IRBP-specific lymphocytes and is considered non-dominant. In addition, LNC sensitized to R14, but not to R4, responded to intact IRBP. R14 was superior to R4 in producing EAU/EAP and cellular immunity (minimal doses: 0.06 vs 67 μg/rat). On the other hand, the two peptides were comparable in their capacity to stimulate presensitized lymphocytes. Moreover, LNC sensitized against R4 were similar to those sensitized against R14 in their capacity to adoptively transfer EAU/EAP to naive recipients. This study thus provides a unique system in which both immunodominant and non-dominant peptides produce autoimmune disease and can be compared for their immunological features.

C 448 ORIGINAL "ANTIGENIC SINS" RESULT IN THE AGE-RELATED INCREASE IN AUTOIMMUNITY. E.A. Goidl, S.J. Martin McEvoy, A. Kaushik*, C. Bona*, J. Urbain††, J.R. Hiernaux† and G.K. Lewis. Department of Microbiology and Immunology, School of Medicine, University of Maryland at Baltimore, Baltimore, MD 21201, *Department of Microbiology, Mount Sinai School of Medicine, New York, NY 10037, †RIBI, Immuno Chem Research, Inc., Hamilton, MT 59840 and ††Laboratorie de Physiologie Animale, University of Brussels, Brussels, Belgium.

The age-related diminution in immune responsiveness has been shown to result from increased regulatory mechanisms and not from a paucity of immunological recruitment (Aging: Immunology and Infectious Disease 1, 47, 1988). We present evidence based on "libraries" of monoclonal antibodies (MAbs) obtained from young and aged donors that there occurs with aging an increase in autoimmunity which is possibly the result of the accumulation of life-long "original antigenic sins". The resultant increased connectivity of the immune system is represented by MAbs obtained from aged donors which are multiply anti-self cross-reactive. Furthermore increased connectivity is supported by the evidence that anti-2,4,6-trinitrophenyl MAbs are AD8 positive, 2D3 positive as determined by inhibition studies using MAbs anti-idiotypic reagents. Analysis of the V_H and V_K region genes utilized by these MAbs indicate a non-random gene usage. Life long stochastic immunological events lead to a pattern of cross-reactivities and non-random usage of V_H genes. These immunological events lead to the emergence of the patterns which are partially elucidated by the data presented. These patterns mimick those seen early in ontogeny, but indicate a possible convergence to an ever-increasing connectance of the idiotypic repertoire expression. In other words, life-long immunological experiences contribute to a down-regulation resulting in both paucity of primary immune responses and an increase in autoimmunity which are both the earmarks of immunity in aging.
(Supported by USPHS grants AG-04042 to EAG and AI 18316 to CAB)

C 449 ALLOREACTIVE T CELL RESPONSE: ROLE OF MHC "EPITOPES" VS ENDOGENOUS MHC-BOUND PEPTIDES. Jack Gorski and David D. Eckels, Immunogenetics Research, The Blood Center of Southeastern Wisconsin, Milwaukee, WI 53233
T cell alloreactivity has often been viewed as response by T cells to MHC restricted epitopes. Since alloreactivity is independent of added exogenous antigen, this has led to models where the MHC molecule itself provides the restricted epitope. With our current picture of MHC-antigen-TcR interactions in antigen presentation the possible role of endogenous antigenic peptide cannot be ignored. This has been strengthened by our recent results that peptides can up or down regulate allo T cell response (Eckels et al PNAS in press) and by the tissue specific allo T cell response (Eckels et al Human Immunol 1988; Marrack & Kappler, Nature 1988).

Further evidence for the role of endogenous peptide comes from our investigation of public alloreactive T cell responses which are restricted by more than one MHC molecule. A panel of 45 T cell clones raised against HLA-DR1 was stimulated by 100 well defined homozygous typing cells. Direct sequence analysis of the MHC molecules involved in restricting such responses revealed that certain public restriction patterns could not be explained by epitopes shared between HLA-DR1 and other stimulating DR molecules. Because these clones are restricted by multiple HLA-DR alleles, endogenous antigen(s) binding only to the restricting DR alleles are the probable recognition elements of these alloreactive T cells.

C 450 INOSITOL PHOSPHATE GENERATION IN JURKAT IS LIMITED BY RECEPTOR NUMBER Martha Graber and Arthur Weiss, Howard Hughes Medical Institute, Departments of Medicine and of Microbiology and Immunology, University of California, San Francisco, CA 94143

We have developed and characterised a series of reconstituted transfectants of the Jurkat mutant J.RT3-T3.5, which lacks functional Tiβ transcripts and therefore fails to express CD3/Ti on the cell surface. These cell lines express varying levels of surface CD3/Ti which can be quantitated by FACS calibrated with microbeads to which known numbers of fluorescein molecules are covalently bound. The number of receptors per cell is constant for each cell line with a range from $7.2x10^2$ to $8.1x10^3$ with the wild type expressing $1.5\text{-}3.5x10^4$ receptors per cell. Stimulation of these cell lines in suspension with saturating levels of mAb OKT3 produces total and fractional inositol phosphate accumulation linearly related to receptor number, ($r > 0.9$). This technique also allows an approximation of the minimal number of receptors which must be engaged for second messenger generation in this system, which we estimate as $6.5x10^2$ receptors per cell. These studies with a series of Jurkat derivatives varying in CD3/Ti β chain expression demonstrate that receptor number quantitatively limits phosphotidylinositol breakdown.

C 451 MOLECULAR CLONING OF THE MURINE HOMOLOGUE OF THE T LYMPHOCYTE CD28 ANTIGEN. Jane A. Gross and James P. Allison. Depart. Microbiology and Immunology, University of California at Berkeley, Berkeley, CA. 94720

CD28 (Tp44) is a homodimeric glycoprotein which has been identified on the surface of human T cells. Stimulating this molecule with the monoclonal antibody 9.3 (Mab 9.3) produces a signal which acts synergistically with other stimuli to restrict, modulate, maintain, or terminate T cell activation. Since this molecule plays an important role in human T cell development we sought to identify the murine homologue of CD28 in order to determine its expression on murine T cell and its role in activation. We have used a human CD28 cDNA clone to isolate a full length cDNA encoding the murine equivalent of CD28 from an EL4 T cell lymphoma library. This clone shows similar domain organization and a high degree of homology to the human CD28 molecule. The murine cDNA clone has been used to examine mRNA expression of CD28 in normal and activated murine T cells, and in various T cell tumors. Peptides generated from the translated sequence will be used to produce antisera to correlate the surface expression of CD28 with mRNA expression, and to biochemically and functionally characterize this molecule.

C 452 DEFINITION OF THE REPERTOIRE OF EXPRESSED Vα AND Vβ GENE SEGMENTS IN A PRE-SELECTED, PRE-TOLERIZED T CELL POPULATION, Mary Pat Happ and Ed Palmer, Basic Sciences Division, Dept. of Pediatrics, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO 80206. We have attempted to determine the frequency of rearrangement and expression of the individual α and β chain V gene segments that make up an unselected, untolerized T cell repertoire. In order to do this we generated over 500 T cell hybridomas from freshly-isolated thymocytes of newborn C57Bl/10 mice and subjected RNA from these hybrids to Northern dot blot analysis using 11 Vα, 16 Vβ, Cγ and Cδ probes. Comparison of the expressed repertoire of Vβ gene segments in this newborn thymocyte population with similar data previously generated from an adult peripheral T cell population reveals two Vβ genes, Vβ12 and Vβ15, whose expression is decreased in the periphery, possibly due to the effects of tolerance. Two additional Vβ gene segments were expressed more frequently in the peripheral population than in the newborn thymus, Vβ5 (4.5 times higher in the periphery) and Vβ10 (2 times higher). It is possible that these represent two instances of positive selection of T cells which is determined primarily by the receptor's Vβ gene segment. Vα gene segments were expressed in only 15% of newborn thymocyte hybridomas (compared to 58% expressing Vβ) and determination of Vα rearrangement frequencies was complicated by the unexpectedly large number (67%) of hybrids expressing Cδ mRNA. Further examination revealed that several Vα gene probes were actually detecting rearrangements to Cδ. The most notable of these was Vα7, which accounted for approximately 34% of the expressed Vα repertoire but was rearranged exclusively to Cδ.

C 453 T CELL CLONES SPECIFIC FOR PANCREATIC ISLET CELL ANTIGEN. Kathryn Haskins, Barbara Bergman, Brenda Bradley, Kevin Lafferty and Mary Portas. Barbara Davis Center for Childhood Diabetes, U. Colo. Health Sci. Ctr., Denver, CO 80262.
We have produced a panel of islet-specific T cell clones by culturing lymphoid cells obtained from non-obese diabetic (NOD) mice in the presence of NOD islet cell antigen and antigen-presenting cells (APC). These clones were selected to the panel on the basis of (a) their antigen-specific reactivity to islet cells and APC in an *in vitro* proliferation assay and (b) their ability to mediate islet graft rejection *in vivo* in a tissue-specific manner. We have further characterized these lines for cell surface phenotype, IL-2 production, and proliferative response to non-NOD islet antigen. All of the clones tested to date are of the CD4 phenotype and make IL-2 in response to islet antigen and NOD APC. Nearly all of the clones we have tested also make good proliferative responses to islet cell antigen obtained from mouse strains other than the NOD or to a mouse beta cell tumor line. Preliminary results indicate that at least one of these clones can lead to islet cell damage in a disease transfer experiment in which the cloned T cells are injected into a non-diabetic NOD F_1 recipient. We are currently carrying out tests to further characterize lymphokine production by these cloned cell lines, to analyze differences in antigen recognition and MHC restriction requirements among the clones, and to determine their effectiveness in mediating the disease process in nondiabetic animals.

C 454 CTL INHIBITION BY SYNTHETIC PEPTIDES: USEFULNESS FOR EPITOPE MAPPING. William R. Heath, Antonella Vitiello and Linda A. Sherman, Scripps Clinic & Research Foundation, La Jolla, California 92037. In an attempt to identify the epitopes on class I molecules recognized by alloreactive cytotoxic T lymphocytes (CTL) we have examined K^b-specific CTL for their recognition of synthetic peptides with sequences derived from the native K^b molecule. Consecutive overlapping peptides spanning the immunogenic α1 and α2 domains of the K^b molecule were tested for their capacity to inhibit K^b-specific CTL clones in their recognition of cells expressing the native K^b molecule. In these studies inhibition by peptide was found to be an extremely rare event, although one peptide (K^b111-122) did inhibit recognition by a particular CTL clone (clone 13). In a separate set of experiments it was observed that clone 13 could recognize K^b111-122 when presented by H-2^d class I molecules. As clone 13 was of H-2^d origin, this finding led us to conclude that inhibition may be due to class I-restricted recognition of the K^b peptide on the surface of the CTL clone, rather than to direct competition between K^b peptide and native K^b for the T cell receptor. We present evidence in favor of this conclusion.

C 455 A MODIFIED "PEPSCAN" METHOD FOR THE RECOGNITION OF T CELL EPITOPES: VERIFICATION BY T CELL CLONES OF KNOWN SPECIFICITY, Evert J. Hensen[1], Ruurd van der Zee[2], Rob H. Meloen[3], Alie Noordzij[1], Jan D.A. van Embden[2] and Willem van Eden[1], [1]Dept. of Infectious Diseases and Immunology Fac. of Vet. Medicine, Univ. of Utrecht, [2]Lab. of Bacteriol., Natl.Inst.Public Health & Envir.Hygiene, Bilthoven, [3] Central Vet. Institute, The Netherlands

The PEPSCAN method is used for the systematic identification of sequential B cell epitopes in protein molecules (Geysen, Meloen, Barteling. PNAS 81: 3998; 1984). It was designed for the synthesis and subsequent testing for antibody binding of large numbers of overlapping peptides directly on their solid supports. The MHC dependent presentation required for T cell recognition seemed prohibitive for the use of PEPSCAN to identify T cell epitopes. However we now have shown that by a novel modification the peptides can be recovered from their solid supports and used in T cell assays. (Van der Zee et al., to be published). To evaluate the method we used rat T cell clones developed in Adjuvant Arthritis, as well as T cell hybrids derived from these clones, with a specificity for the 180-188 nonapeptide epitope of the mycobacterial 65kD heat-shock protein (Van Eden et al., Nature 331: 171; 1988). All overlapping nonapeptides in and around the critical area of the molecule, and in addition all possible variant peptides with substitutions, insertions and deletions have been generated. The modified PEPSCAN method identified exactly the same sequence to stimulate that was recognized before by much more elaborative methods. This novel application enables the rapid and simultaneous generation of several hundreds of peptides for the mapping and characterisation of T cell epitopes. Furthermore, this method offers us the possibility to study mimicking T cell epitopes by the modifications of the known sequence.

C 456 A SIMPLE METHOD TO DETERMINE THE CELLULAR REQUIREMENTS FOR INDUCING LYMPHOKINE RELEASE FROM ACTIVATED T CELLS BY MITOGENS, Hodgkin, P.D. and Lafferty, K.J. DNAX Research Institute, Palo Alto, California 94304. and John Curtin School of Medical research, A.N.U. Canberra, Australia, 2601.

Non-specific activation by mitogens is a method commonly used to trigger lymphokine release from activated T cells. If lymphokine production was induced by simple ligand (i.e., Con A) binding, then lymphokine titre would be directly related to cell number. However, for many mitogens (Con A, leucoagglutin, wheat germ agglutinin, $NaIO_4$), lymphokine titre is directly proportional to the square of cell number. This relationship suggested that cell contact between T cells was essential for mitogen stimulation. To test this mitogen pretreated killed tumor cells were added in constant amount to T cells at different cell numbers. The relationship became linear. Therefore transmission of the activating signal following mitogen stimulation requires cell contact. This conclusion has implications for studies attempting to identify the intracellular signals induced by mitogen stimulation of single cells. Assessing the relationship between cell Nunber and lymphokine titre is a simple method for determining the cellular interactions involved in mitogenic (and antigen) stimulation.

C 457 ORIGIN OF THE AUTOREACTIVE ANTI TYPE II COLLAGEN RESPONSE, Rikard Holmdahl, Department of Medical and Physiological Chemistry, Box 575 Uppsala University, S-75123 Uppsala, Sweden. Both autoreactive T cells and autoantibodies play an important role in the pathogenesis of type II collagen (CII) induced arthritis in mice. We have earlier reported that only strains with H-2q , H-2w3, H-2w17 and H2-r were responders to autologous mouse CII and only these strains developed arthritis after immunization with autologous or heterologous CII. However, heterologous CII induced a more acute and severe disease and a more pronounced autoantibody response. This findings indicate that 1) the ability to mount an immune response against autologous CII is a prerequisite for the susceptibility to collagen arthritis and 2) that a crossreactive autoantibody response after immunization with heterologous CII may further enhance development of arthritis.

We have now studied activation of autoreactive B cells after primary immunization of DBA/1 mice with rat CII. In hybridoma collections, obtained 9-11 days after immunization, 30-80% of the hybridomas produced IgG reactive with autologous CII, 10-15% produced multispecific IgM and a significant number produced IgG rheumatoid factors. The anti-CII antibodies recognized at least 5 different epitopes on the CII molecule and originated from many different Vh and V kappa gene families. Furthermore, none out of 6 investigated anti-CII hybridoma expressed CD5 RNA message. We therefore suggest that the primary anti-CII autoantibody response involves activation of memory B-cells. These memory B cells have most likely been earlier activated by CII autoreactive T cells. In these aspects the origin of the anti-CII autoantibody response is principally different from the origin of "natural" autoantibodies.

C 458 REPERTOIRE OF T CELL RECEPTORS SPECIFIC FOR BEEF INSULIN IN (HIGH RESPONDER X LOW RESPONDER) F_1 MOUSE, Nobumichi Hozumi, Joan Wither, Terry Delovitch, Laurie Phillips,* Mount Sinai Hospital Research Institute and Department of Immunology, University of Toronto, Toronto, Canada M5G 1X5, *Banting and Best Institute of Medical Research, Toronto.

T cell receptors (TCR) recognize antigen in association with self MHC molecules, usually following processing to smaller peptides. The T cell repertoire to an antigen, therefore, reflects not only the ability of a given MHC molecule to interact with antigen, but also the affects of initial repertoire selection by self MHC. We have been analyzing the TCR repertoire specific for beef insulin (BI) in Balb/c mice ($H\text{-}2^d$), which are high responders to the antigen. These studies revealed that Vβ8.3 is dominantly used in the TCR's specific for BI/A^d and our preliminary data suggests that the Vβ8.3 chain may be involved in MHC restriction. We have now obtained several T cell hybridomas specific for BI from (Balb/cXA/J) F_1, animals. A/J mice ($H\text{-}2^a$) are low responders to BI while the F_1 mice are high responders. Most of the Balb/cXA/J hybridomas were restricted to the $H\text{-}2^d(I\text{-}A^d)$ haplotype and exhibited patterns of antigenic reactivity similar to those found in the Balb/c hybridomas. Interestingly, the analysis of V gene usage demonstrated that Vβ8.3 was not used in the Balb/cXA/J hydridomas. The relevance of these results to the development of the TCR repertoire in different mouse strains will be discussed. This work was supported by the MRC of Canada.

C 459 TOLERANCE AND IMMUNE RECOGNITION IN HLA-A2/K^b TRANSGENIC MICE. Michael J. Irwin, Donata Marchesini, Antonella Vitiello, John Shutter, Jacques Chiller, Norman R. Klinman and Linda A. Sherman. Scripps Clinic and Research Foundation, La Jolla, CA 92037. To investigate the parameters of self recognition with respect to tolerance and repertoire selection, transgenic mice have been constructed which express a human/murine chimeric class I MHC molecule. This molecule is comprised of the first two domains of HLA-A2.1 and the third domain of $H\text{-}2K^b$ ($A2K^b$). Three different transgenic lines have been obtained, each differing in the level of expression of the $A2K^b$ molecule.

Tolerance to $A2K^b$ was demonstrated in animals expressing the $A2K^b$ molecule. CTL specific for the $A2K^b$ molecule were generated from normal splenocytes by *in vitro* culture with $A2K^b$ bearing stimulators. These CTL have been shown to lyse transfected targets expressing HLA-A2 regardless of their murine haplotype, and they specifically kill A2 bearing human target cells. Furthermore, the effector function of these CTL can be inhibited with an HLA-A2 specific monoclonal antibody. Thus, the transgene product functions correctly as a tolerogen and is recognized directly as a class I antigen.

Although transgenic mice have been shown to be tolerant to $A2K^b$ expressed by murine cells, transgenic CTL specific for HLA-A2 on the surface of human cells have been generated. These CTL do not recognize transgenic target cells or HLA-A2 transfected murine target cells.

C 460 SURFACE EPITOPES OF THE ADHESIN OF *MYCOPLASMA PNEUMONIAE* WITH PROLIFERATIVE ACTIVITY, Enno Jacobs*, B. Gerstenecker, B. Mader, R. Röck, and W. Bredt, Institute for Medical Microbiology and Hygiene, University of Freiburg, D-7800 Freiburg.

The major virulence factor of *M.pneumoniae* was shown to be a 168 kDa protein which is located in the tip structure membranes of these cells. Beside the adhesin function this protein is also involved in first massive humoral and cellular responses of the human host during the acute phase of upper respiratory tract infections and interstitiel pneumonia.

Intranasal inoculation of guinea pigs with the isolated 168 kDa protein led to lympho-histiocyte infiltrations around bronchi and small vessels of the lungs which are characteristic infiltrations after an infection with live *M.pneumoniae* cells. Furthermore one peptide (17 amino acids long) which was synthesized according to the amino acid sequence of the adhesin, showed a proliferative activity to *in vitro* cultivated T-cells of bronchial washings, whereas synthetic peptides with the sequences of the direct neighbourhood showed no *in vitro* activity.

Most interestingly this T-cell proliferative activity is located on a surface loop of this protein which is also responsible for the adhesin fuction.

C 461 INDUCTION OF CONTROLLED CELL DEATH (APOPTOSIS) AS A MECHANISM FOR CLONAL DELETION IN THE SELECTION OF THE T-CELL RECEPTOR REPERTOIRE. Eric J. Jenkinson, Christopher A. Smith, Gwyn T. Williams, Rosetta Kingston and John J.T. Owen. Department of Anatomy, University of Birmingham, Medical School, Vincent Drive, Birmingham B15 2TJ, UK. Rearrangement of T-cell receptor a and b chain gene segments during T-cell development results in a diverse array of receptor specificities. To avoid auto-immune responses, cells that have generated self reactive receptors are thought to be eliminated or inactivated, to produce self tolerance. Recent studies have provided compelling evidence that clonal deletion of immature receptor bearing cells within the thymus makes an important contribution to this process, although the mechanisms involved are not uderstood. We have now obtained evidence that engaging the CD3/T-cell receptor complex of immature mouse thymocytes with anti-CD3 antibodies added to thymus organ cultures, induces DNA degradation and cell death through the endogenous pathway of apoptosis. This is in marked contrast to the activation of mature T-cells by the same anti CD3 preparation and is specific to the extent that apoptosis is not induced by either anti-CD-4 or anti-Thy-1. In addition, calcium ionophore (ionomycin) also causes apoptosis when added to organ cultures suggesting a role for changes in intra-cellular Ca++ levels in the signalling pathway leading to the induction of apoptosis in immature cells binding. Thus activation of the process of apoptosis in immature cells binding self antigens may be the mechanism responsible for the selective deletion of cells that could generate an auto-reactive response if allowed to mature.

C 462 IMMUNOGENICITY OF HUMAN COLORECTAL CARCINOMA (CRC) ANTIGENS. J. Milburn Jessup, Kefung Chi, Richard Hostetter, and Susan Kerckhoff. University of Texas M. D. Anderson Cancer Center, Houston, TX 77030
Immunization of patients with BCG and irradiated tumor cells induces specific delayed-type hypersensitivity (DTH) to tumor cells and not to normal colon cells. Since IgG antibodies may require T-cell help, we wished to characterize the IgG-defined tumor-associated autoantigens (TAAA) of human CRC so as to define a subset of the T-cell repertoire for CRC. Western blots of detergent extracts of 73 primary and metastatic human colorectal carcinomas and paired normal tissues were probed with autologous IgG. Nine TAAA were recognized by 20% or more of the sera: 74, 72, 58, 52, 45, 41, 38, 29, and 26 kDa. These TAAA may be normal colon differentiation antigens, since they were present in extracts of normal colon. Autoantibodies are more frequently present to the 41 kDa antigen in patients with metastases (79%) than in primary tumors (47%, $p<0.05$) while there is more reactivity to the 38 kDa antigen in primary tumors (38%) than in metastases (7%, $p<0.05$). Only 1 of 8 patients sensitized with BCG and irradiated autologous tumor cells, developed antibody to new TAAA; whereas 2 of 12 control patients developed antibody to new TAAA without sensitization. The TAAA in the Western blots that stimulate autologous lymphocyte proliferation are greater than 200 kDa. Finally, immunization with a human CRC failed to induce DTH in mice to a syngeneic murine colon carcinoma that expresses at least 1 of the IgG-defined human TAAA. Thus, TAAA that presumably require T-cell help in patients do not appear to be part of the T-cell repertoire for DTH to human CRC.

Section B
Antigen Structure (B and T Cell Determinants, Immunogenicity); Adjuvants and Manipulation of Cellular Immunogenicity; T Cell Activation and Inactivation Generation of the T Cell Repertoire; Autoimmunity and the Immunogenicity of Self Tissues

C 500 UNIQUE EPITOPE SPECIFICITY OF AN ENCEPHALITOGENIC T LYMPHOCYTE LINE IN ACI-STRAIN RATS, R.E. Jones, H. Offner, G. Hashim, Z. Guo and A. Vandenbark, Neuroimmunology Research 151D, Veterans Administration Medical Center, Portland, OR 97201. In several rodent species and strains, the emergence of specificity to a discrete immunodominant epitope of Guinea pig basic protein (GPBP) results from selection of cell lines with whole GPBP. These epitope specificities have been found to be strain-specific and define the specificity of the T helper cells which transfer Experimental autoimmune encephalomyelitis (EAE) in adoptive transfer experiments. We have produced a GPBP-specific T lymphocyte line from ACI-strain rats which was encephalitogenic and specific for a new T cell epitope of GPBP. The cell line responded in vitro to the peptide corresponding to the region 39-54 of GPBP but not to peptides corresponding to other regions of GPBP. This cell line is restricted by I-A but not I-E and transfers a DTH response to GPBP as well as EAE into naive recipients. These results provide support for the hypothesis that the in vitro immunodominant epitope specificity defines the specificity of GPBP-specific encephalitogenic T cells. The definition of a new encephalitogenic epitope in the ACI strain also suggests that the mechanism of EAE in rats depends on strain-specific extrisic properties of antigen recognition such as the MHC class II genotype or the composition of the T cell antigen-receptor repertoire.

C 501 **A STRATEGY TO ISOLATE T CELL SPECIFIC PROTEINS AND THE DESCRIPTION OF A NOVEL ACTIVATION ANTIGEN,** R. Kumar Kadiyala, Bradley McIntyre, Carol Clayberger and Alan Krensky, Dept of Pediatrics, Stanford Medical Center, Stanford, CA 94305. A wide variety of T lymphocyte surface antigens have been isolated and shown to play important roles in the T cell response. We outline here a strategy to isolate and characterize surface proteins unique to activated T cells. A rabbit anti-serum was generated by immunizing with allo-activated human T cells and subsequently absorbing with the T cell tumor HPB-ALL, a tumor that expresses all functionally relevant T cell surface antigens previously described. This absorbed anti-serum was able to recognize an uncharacterized group of surface proteins on T cells that was absent from HPB-ALL. Furthermore, the absorbed anti-serum was able to inhibit *in vitro* cytotoxic and MLR responses; an effect that was abolished by carrying out an additional absorption with the T cells used for the initial immunization. Monoclonal antibodies to activation antigens were generated by immunizing with complexes made by adding the absorbed anti-serum to solubilized CTL. The absorbed anti-serum was also used to screen a λgt11 cDNA library. One cDNA clone so isolated is expressed on CTL as evidenced by Northern analysis. DNA sequence data indicates no similarity to other sequences in available databanks.

C 502 RECOMBINANT HUMAN CYTOKINES STIMULATE PROLIFERATION OF NEW-ONSET TYPE I DIABETIC (IDDM) PERIPHERAL BLOOD MONONUCLEAR CELLS (PBM). Douglas J. Kawahara, Bruce Buckingham, Christy Sandborg and Monique Berman. Childrens Hospital of Orange County, Orange, CA 92668, and University of California, Irvine, CA 92717.

We examined the ability of cytokines to stimulate the proliferation of PBM from 41 new-onset IDDM, 11 long-term IDDM, 10 non-diabetic pediatric control, 5 type II diabetic, and 3 non-diabetic adult control subjects. Initial results demonstrated the ability of human PBM culture supernatants containing peak natural IL-1 activity to stimulate a significantly higher proliferative response from PBM of new-onset IDDM than from PBM of long-term IDDM, type II diabetics, or non-diabetic controls. More recent results have shown that human recombinant IL-1a, -1b, -3, -4 and -6, interferon gamma, lymphotoxin, and tumor necrosis factor, as well as Con A-stimulated rat spleen supernatants, also stimulated a significantly higher proliferative response from PBM of new-onset IDDM than from PBM of controls. However, human recombinant IL-2 stimulated equivalent levels of proliferation from PBM of both new-onset IDDM and controls.

These data indicate that, in new-onset IDDM, there are present PBM which are in an early stage of activation not revealed by increased responsiveness to recombinant IL-2. In the case of T cells, this may represent a stage prior to an IL-2-dependent stage of activation. This would be consistent with the active induction of newly responsive cells during a stage of IDDM when islet cells are still present. This response is not the result of the metabolic aspects of IDDM but a reflection of the immunologically activated state of T cells, B cells and/or monocyte/macrophages in IDDM.

C 503 PREDICTION OF HELPER T CELL EPITOPES, Judy E. Kim, Masaharu Kojima, Richard Houghten, C. David Pendleton, James L. Cornette, Charles DeLisi and Jay A. Berzofsky, Metabolism Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892. Analysis of known helper T cell (Th) epitopes suggests that Th epitopes tend to be amphipathic alpha helices, that is, helices with hydrophobic residues on one side and hydrophilic residues on the other side. Based on this hypothesis, a computer program "AMPHI" was generated which may aid in prediction of Th epitopes. Within residues 1 to 55 of spermwhale myoglobin (SwMb), a Th epitope is known to exist but has not yet been found. As an approach to further test the hypothesis, this undetermined Th epitope was sought both by an unbiased test of ten evenly overlapping 15-residue peptides that span the region, and by computer analysis. Of the ten peptides, only one was able to stimulate two clones that are specific for the 1-55 region. This peptide gave remarkably high stimulation index. Analysis of this region by the computer program also predicted the site covered by this peptide (residues 26-40) to be the most likely site for Th epitope. Therefore, unbiased *in vitro* testing and the computer prediction correlated well. The peptide was able to prime T cells of B10.BR mice *in vivo* for *in vitro* response to the native SwMb as well as to the peptide fragment of residues 1-55. Immunization of mice with SwMb showed that, of the ten overlapping peptides, the major site of response is to the identified peptide. Finally, a peptide of residues 24-42 was made to increase the amphipathic score. This extended peptide induced greater proliferation of the clones. Thus, this study has identified a new dominant epitope of SwMb and confirmed the utility of the amphipathicity hypothesis in a prospective test.

C 504 PROGRAMS FOR PREDICTING T-CELL EPITOPES AND THEIR APPLICATION TO HEPATITIS A VIRUS, A.T.Kozhich, A.E.Gabrielian, V.S.Ivanov, L.D.Tchikin, L.N.Kulik, V.T.Ivanov, Shemyakin Institute of Bioorganic Chemistry, USSR Academy of Sciences, Moscow 117871, USSR. It is known that many T-cell antigenic sites tend to be amphipathic structures (1), and, conversely, amphipathic structures have a high probability to form T-cell epitopes. Keeping this in mind we developed a set of 4 programs to search for T-cell epitopes in proteins: 1) search for amphipathic structures, based on calculation of cross-correlation coefficient between the periodic function and distribution of hydrophobic and hydrophilic amino acids at a selected site; 2) T-cell antigenicity, that is analysis of primary structures with 9- and 11-amino acid templates obtained from database of known T-epitopes; 3) search for characteristic fragments CHHP and CHHHP, where C - charged amino acid or Gly, H - hydrophobic, P - polar or charged amino acid (2); 4) prediction of the secondary structure according to Garnier (3) to exclude sites having high turn or coil forming potential. Comparison of this set with published programs (1,4) shows that it has high predictive power. So we used it to search for probable T-epitopes in hepatitis A virus capsid proteins. Selected peptides were synthesized and their spectral and immunochemical properties were studied.

C 505 T CELL RESPONSE AGAINST HIV ENVELOPE PROTEINS IN IMMUNIZED ANIMALS AND IN INFECTED PRIMATES AND HUMAN BEINGS, K. Krohn, A. Ranki, P. Lusso, L. Arthur, B. Moss, P. Ashorn and R.C. Gallo, University of Tampere, Finland, Helsinki University Central Hospital, Finland, LTCB, NCI, NIH and LVD, NIAID, NIH, Bethesda, and FCRF, Frederick, Md, USA.
The aim of the study was to characterize of the nature of helper T-cell response in infected and immunized humans and animals. Animals were immunized with gp120, recombinant envelope fragment PB-1 or with vaccinia recombinant carrying the envelope gene and tested for proliferative T-cell response using purified HIV envelope proteins or synthetic peptides. A strong group-specific response involving T-cell proliferation and lymphokine secretion was observed in immunized goats, chimpanzees and rhesus monkeys as well as HIV-infected chimpanzees and gibbons. With the use of overlapping set of 15-25 amino acid long synthetic peptided two T-cell epitopes (aa. 386-400, 426-450) were identified. The latter overlaps with the sequence known to be involved in the binding of gp120 and CD-4. In HIV-infected humans, a very low or lacking T-cell response towards whole HIV virions or to HIV native or recombinant proteins were seen. A low level response was seen in some AZT-treated patients as well as in some seronegative sexual partners to HIV positive individuals. No or very low response was seen towards one of the T-cell specific synthetic peptides. That primates but not humans can mount a strong T-cell response towards HIV encelope can partly be based on genetic factors and may be a further reason not to use HIV envelope in the vaccine development.

C 506 POSITIVE SELECTION OF $CD4^-$ $CD8^+$ T CELLS IN THE THYMUS, Ada M. Kruisbeek, Suzana Marusic-Galesic, and Juan Zuniga-Pflucker, BRMP-NCI, National Institutes of Health, BLDG 10, Rm. 12N226, Bethesda, MD 20892. Blocking of intrathymic expression of MHC through *in vivo* treatment with anti-MHC mAb affects the repertoire of developing T cells: $CD4^+$ $CD8^-$ cells do not develop in anti-class II treated mice, and $CD4^-$ $CD8^+$ cells fail to develop in anti-class I treated mice. We next examined T cell repertoire specificities of $CD4^-$ $CD8^+$ T cells under conditions where only one of the class I-MHC encoded molecules is blocked while other class I-MHC glycoproteins are still expressed. Antigen-specific T cells restricted to the blocked class I fail to develop, while generation of other class I-restricted T cells proceeds undisturbed. Additionally, generation of $CD4^-$ $CD8^+$ T cells expressing a particular TCR-Vβ segment was selectively abrogated by blocking some, but not other, alleles of class I-MHC glycoproteins. Thus, development of $CD4^-$ $CD8^+$ T cells with a certain TCR requires expression of particular alleles of class I-MHC glycoproteins. These findings demonstrate that TCR-MHC ligand interactions provide signals crucial to the differentiation of precursor T cells, and support the positive selection theory. The contribution of accessory molecules to the selection process is currently investigated.

C 507 DTH-DEPENDENT ACTIVATION OF MINOR H-SPECIFIC CYTOTOXIC T CELLS WITHIN TUMOR GRAFT REJECTION SITES. B. Ksander and J.W. Streilein, Department of Microbiology and Immunology, University of Miami Medical School, Miami, Florida 33136.

Minor H incompatible P815 tumor cells injected subcutaneously into the conjunctiva of BALB/c mouse eyes formed transient tumors that were rejected within 16 days. It is believed that immune effector cells responsible for graft rejection are activated in the draining lymph nodes and migrate back to the graft site to effect rejection. To trace the steps in specific activation, lymphocytes from draining lymph nodes and from the graft rejection site were analyzed for cytotoxic T cells (Tc- detected in 4 hr chromium release assay), their precursors (pTc - detected by limiting dilution analysis), and for mediators of delayed hypersensitivity (T_{DH} - assayed by ear swelling induced by co-injection of responder cells with irradiated tumor cells). Lymph node cells recovered 14 days post-inoculation contained T_{DH} cells and pTc (freq 1/1,200), but no Tc effector cells. By contrast, graft-infiltrating cells not only contained T_{DH} and pTc (1/1,000), but direct Tc effector cells (21 lytic units). All three sets of T cells were antigen-specific. The finding of significant frequencies of pTc within the lymph nodes and the graft site, but Tc only at the latter, suggests that differentiation of Tc from unprimed pTc may be a multistep process, the first of which occurs in the draining lymph nodes. The data further suggest that the terminal step(s) in this process take place after the clonally expanded pTc have disseminated to the graft site. We suspect that the final step requires help provided uniquely by T_{DH} cells within the graft. The final step may not occur (as might have been expected) in the lymph node because priming of pTc and T_{DH} take place at non-contiguous anatomic sites.

C 508 INTERFERENCE WITH DISTINCT INTRATHYMIC CELL CELL-INTERACTIONS AND T CELL DEVELOPMENT IN VIVO BY T CELL RECEPTOR-SPECIFIC ANTIBODIES AND HYBRID-ANTIBODIES. Bruno A. Kyewski, Institute for Immunology and Genetics, German Cancer Research Center, D-69 Heidelberg, F.R.G.

Intrathymic T cell development probably comprises at least two T cell receptor (TCR)-dependent selection steps; positive selection of self MHC-restricted thymocyte via recognition of cortical epithelial cells and negative selection of self-reactive T cells via recognition of medullary dendritic cells. To further define the sites and mechanisms of these selection events mice were treated postnatally with antibodies against the TCR/CD3 complex. Sustained treatment lead to the arrest of T cell differentiation at the cortex/medulla transition and the lack of mature T cells in the thymus and in peripheral organs. This arrest of T cell development correlated with a blockade in vivo of interactions of immature thymocytes with $I\text{-}A^+$ cortical epithelial cells and $I\text{-}A^+$ medullary dendritic cells while leaving interactions with $I\text{-}A^-$ cortical macrophages intact. In contrast, sustained treatment in vivo with hybrid-antibodies bispecific for a TCR allotype (KJ16) and a non-MHC surface antigen of cortical thymic epithelial cells lead to "overselection" of $KJ16^+$ T cells. These results suggest that binding of TCR^+ thymocytes to cortical epithelial cells is obligatory for thymocytes to proceed from the immature cortical to the mature medullary compartment.

C 509 CD28 ANTIGEN: AN ACCESSORY ACTIVATION SITE OF THE T CELL SURFACE. Werner Lesslauer, Gisela Gehr, Stefan Ryser and *Peter Böhlen, Central Research Units, F. Hoffmann-La Roche & Co., Ltd., CH-4002 Basel, *Lederle Laboratories, Pearl River, NY 10965.

The CD28 antigen has been identified as one of the cell surface sites by which human T lymphocyte growth can be controlled using anti-CD28 mAb as ligand, although its physiologic function remains unknown (eg. Lesslauer et al., Eur.J.Immunol. 16, 1289, 1986). Anti-CD28 mAb directly activate T cells in mononuclear cell culture; in other assays T cell activation mediated by other receptors is either potentiated or inhibited by anti-CD28 mAb. The strong induction of CD28 in cell activation is intriguing. The CD28-protein is a 90 kDa -S-S- bonded dimer of identical subunits. It was purified from HPB-ALL cell membranes and the N-terminal amino acid sequence was determined as (G?)KILVKQSPMLVAYDNAVXLS. This sequence fits exactly that proposed from the complete HPB-ALL cDNA clone identified in expression cloning by other investigators (Aruffo & Seed, PNAS 84, 8573, 1987). The N-terminal amino acid sequence of normal T cell CD28 was found to match the HPB-ALL sequence except in residue 10 and 11 (...TS...). In principle this might reflect somatic mutation in HPB-ALL or CD28-gene polymorphism; erroneous residue assignment in the gas phase sequencing cannot be excluded entirely. To investigate the significance of this sequence variation a genomic CD28 DNA clone of a normal human T cell clone was analysed around residues 10, 11 and found to be identical with the HPB-ALL cDNA sequence. Somatic mutation in HPB-ALL CD28 therefore can be ruled out.

C 510 Comparison between T cell receptor sequence and antigen fine specificity in sperm whale myoglobin specific T cell clones
Alexandra Livingstone, Jayne S. Danska and C. Garrison Fathman

The molecular characterization of T cell receptor (TCR) structure in a panel of mouse T cell clones specific for sperm whale myoglobin (SpW Mb) has shown that 6 1-E^d-restricted clones specific for the SpW Mb region 110-120 use highly homologous V β chains. Four of these clones are indistinguishable in their response pattern to a panel of overlapping synthetic peptides spanning the SpW Mb sequence 110-120, yet use 3 different V α gene segments. We are currently trying to find fine specificity differences among these 4 clones, in order to correlate them with TCR expression. T cell hybridomas have been made from these clones, and are being screened on panels of substituted peptides. We hope that conservative substitutions at positions important for interaction with the TCR will reveal fine specificity differences among these clones.

C 511 NEONATAL TOLERANCE TO Mls ANTIGENIC DETERMINANTS IS SPECIFICALLY AND THYMICALLY INDUCED AND SHOWS HAPLOTYPE SPECIFICITY. Stuart Macphail and Osias Stutman, Sloan-Kettering Institute, New York, NY 10021
We previously showed that clonal deletion, non-suppressor mediated immunological tolerance neonatally induced to Mls allo-determinants was specific in that the frequencies of T cells responding to a variety of other stimuli were unaffected while the frequency of T cells responding to Mls were drastically reduced. Here we show that Mls tolerance is detectable in the thymus prior to a detectable positive response in the periphery (spleen and lymph node). Tolerance induced by spleen cells from Mls^a congenic mice in BALB/c mice is as "potent" as that induced by whole background incompatible DBA/2 spleen cells, indicating that incompatibility at the Mls locus (and possibly closely linked loci) is sufficient and required for induction of the tolerant state. Tolerance to Mls^a is partially cross reactive on Mls^d but not at all on Mls^c. Tolerance induced to Mls^c is minimally cross reactive on Mls^d but not at all on Mls^a. Finally tolerance to Mls^d is cross reactive on both Mls^a and Mls^c. Thus tolerance to Mls determinants shows a pattern of haplotype specificity that is consistent with the model proposed by others; Mls^a and Mls^c are separate non-cross reactive determinants and the Mls^d haplotype contains both Mls^a and Mls^c determinants. These data strongly suggest that Mls loci encode or regulate the expression of alloantigeic determinants which in addition to possessing the property of stimulating a positive immune response, are also capable, without other incompatibility, of inducing haplotype specific tolerance during T cell repertoire selection. S.M. is a Scholar of the Leukemia Society of America.

C 512 $\gamma\delta$ T CELLS IN SHEEP EXPRESS A UNIQUE CELL SURFACE MOLECULE WHICH APPEARS LATE DURING THYMIC DEVELOPMENT, Charles Mackay and Polly Matzinger, Basel Institute for Immunology, Grenzacherstr. 487, Postfach, CH-4005 Basel, Switzerland.

We have defined a cell surface molecule, termed T19, which is expressed exclusively by $CD4^-$, $CD8^-$ $\gamma\delta^+$ T cells in sheep. T19 is recognised by more than four different mAbs, all of which also recognise the equivalent bovine T cell subset. T19 is 215 kd in size, is unrelated to the LCA family and is expressed at different densities, separating the $\gamma\delta$ T cells from blood into two distinct subpopulations.

$T19^+$, $\gamma\delta$ T cells in sheep have the following characteristics:
1. They comprise up to 50 % of blood T cells in lambs and 20-30 % in adults.
2. They are found in high numbers in lymph draining from the skin, and in some regions of gut epithelium, but are absent from conventional T cell areas in lymph nodes. Their distinctive tissue distribution and recirculation pattern suggests that they do not play an immunological role within lymph nodes.
3. They express CD5, CD3 and the $\gamma\delta$ TCR (36, 41-44 and 55 kd) but are negative for CD2, CD4 and CD8.
4. They can be activated in vitro to become specific allo-reactive CTL and their lytic activity can be blocked by mAb to sheep MHC or LFA-1 but not by mAbs to T19, CD2, CD8 or LFA-3.
5. In the adult thymus, T19 cells are found predominately in the medulla, clustered around Hassall's corpuscles. During fetal thymic development, T19 appears at a late stage, at the same time as the single $CD4^+$ and $CD8^+$ T cells, apparently well after the establishment of the $\gamma\delta$ thymic lineage.

We propose that the T19 molecule most likely plays a fundamentally important role relating to the significance of $\gamma\delta$ T cells in the immune response.

C 513 RELATIONSHIP OF THE LY-6 AND T CELL RECEPTOR PATHWAYS FOR ACTIVATION OF NORMAL T LYMPHOCYTES. **Thomas R. Malek, Ethan M. Shevach*, and Kenneth M. Danis, Department of Microbiology and Immunology, University of Miami School of Medicine, P.O. Box 016960, Miami, FL 33101 and *Laboratory of Immunology, NIAID, NIH, Bethesda, MD 20892**

The Ly-6 alloantigens have been shown to participate in the process of T cell activation based on the ability of anti-Ly-6 monoclonal antibodies to induce IL-2 production and proliferation of T lymphocytes. In the present investigation, we have demonstrated that peripheral T lymphocytes from A strain mice exhibited abnormally low proliferative responses after stimulation through Ly-6A/E and Ly-6C molecules when compared to responses of T cells from numerous other mouse strains. The abnormal activation of the Ly-6 pathway of A strain T cells was not due to ineffective Fc receptor cross-linking of the anti-Ly-6 monoclonal antibodies, to inappropriate cellular expression of the Ly-6A/E alloantigen in A strain T cells, or to an active suppressive phenomenon. Identification of this defect provided an opportunity to compare T cell activation by the Ly-6 and TCR pathways. Interestingly, T lymphocytes from A strain mice proliferated normally when the cells were activated by monoclonal antibodies to Thy-1 or the CD3/TCR complex suggesting that this defective activation was selective for the Ly-6 T cell activation pathway. Cell separation studies of T cells and accessory cells demonstrated that this defect was quantitative rather than qualitative and that it was complex, residing at both the T cell and accessory cell levels. These results suggest that activation of T lymphocytes via the Ly-6 molecule may involve unique signalling pathways and that activation via CD3/TCR can proceed normally in the absence of a fully functional Ly-6 pathway.

C 514 IDENTIFICATION OF INFLUENZA A VIRUS PB2 EPITOPES RECOGNIZED BY VIRUS SPECIFIC CYTOTOXIC T LYMPHOCYTES, W. Lee Maloy, Robert W. Anderson, Jack R. Bennick, Jonathen W. Yewdell and J. E. Coligan, Biological Resources Branch, and Laboratory of Viral Diseases, NIAID, NIH, Bethesda, MD 20892.

Previously, influenza A virus specific murine cytotoxic T lymphocytes (CTL) have been shown to recognize the viral hemagglutinin (HA), nucleoprotein (NP), the three polymerases (PB1, PB2, PA) and a nonstructural protein (NS1). Peptidic epitopes recognized by murine CTL have been identified for the HA and NP molecules. The purpose of these studies was to identify viral peptides recognized in the context of H-2D^d with the ultimate objective fo doing binding studies with soluble H-2D^d molecules. To determine if any of the viral proteins were recognized in the context of the H-2D end class I molecules B10.A(5R) mice (H-2K^b,H-2D^d, H-2L^d) were primed *in vivo* and stimulated *in vitro* with influenza A virus A/PR/8/34 and then assayed in a ^{51}Cr-release assay against influenza or mock infected P815 (H-2^d) or L cells (H-2^k) transfected with either the H-2D^d or H-2K^b gene. As previously demonstrated the H-2K^b class I molecule was a poor presenter of influenza antigens, while the influenza infected H-2D^d transfected L cells were lysed by the B10.A(5R) effector cells. The specific viral protein recognized in the context of the H-2D^d molecule was identified by assaying B10.A(5R) effector cells against L cells transfected with the H-2D^d or H-2K^b gene infected with either influenza A or one of several influenza-vaccinia recombinant viruses. The H-2D^d transfected L cells were recognized by the CTL when they were infected with either influenza A virus or a vaccinia recombinant virus encoding the PB2. Thirty-five synthetic peptides derived from PB2, which had either an amphipathic structure or contained a 'Rothbard' epitope, were tested for their ability to render uninfected targets susceptible to lysis by influenza specific effectors. Three epitopes were identified, each of which contained a Rothbard epitope.

C 515 IDENTIFICATION OF HELPER T CELL EPITOPES FROM GP 160 OF HIV THROUGH THE USE OF PEPTIDE-PHOSPHOLIPID CONJUGATES. G. Goodman-Snitkoff and Raphael J. Mannino, Department of Microbiology and Immunology, Albany Medical College, Albany, NY 12208

Our laboratory has developed a new, powerful technique for investigating the immune response to peptide epitopes, involving the covalent coupling of peptide to phosphatidyl-ethanolamine, followed by complexing with additional lipids and phospholipids to form a peptide-phospholipid complex. These complexes can be used to immunize mice in the absence of protein carriers or adjuvants, thus facilitating the study of the immune response to a small chemically defined antigen. Use of this technology has allowed us to identify two T helper cell epitopes in conserved regions of HIV gp 160 not previously identified by computer algorithims, defined by amino acids 485-518 and 585-615. Immunization with these peptides in peptide-phospholipid complexes results in the production IgG_1 and IgG_2 antibodies, which cross react with cloned fragments of the whole protein. Using this technology we have begun to characterize the immune response to individual peptide antigens. The response of H2-k mice to amino acids 494-518 of gp 160 of HIV, has been analyzed. The optimal dose of a peptide containing both B and T_h cell epitopes was found to be 15-30 ug, depending on the route of administration. IM immunization required less antigen for optimum antibody response than did IP. Anchorage in the phospholipid complex is a strict requirement for an antibody response. Additional variables, such as phospholipid composition and method of cross-linking have been studied and will be discussed. We believe that the use of this peptide-phospholipid complex technology will be significant both for studying the immune response to single epitopes and for vaccine development.

C 516 **THE ABNORMAL T LYMPHOCYTES IN *lpr* MICE TRANSCRIBE LYMPHOKINES SPONTANEOUSLY *in vivo*, YET FAIL TO PROLIFERATE IN RESPONSE TO MITOGENIC SIGNALS, Christine Martens, Lesley Murray, and Rosanne Lee, Department of Immunology, DNAX Research Institute, Palo Alto, CA 94304.** We have studied the ability of isolated subpopulations of lymphocytes from the autoimmune mouse model, MRL/MPJ/*lpr/lpr (lpr)*, to proliferate in response to mitogenic signals and to express lymphokine genes *in vivo*. This mouse strain develops a lupus-like disease along with massive accumulation of an unusual subpopulation of $CD4^-$, $CD8^-$, $Thy1^+$, $B220^+$ T lymphocytes. We show that FACS-purified $Thy1^+$, $B220^+$ cells proliferate very little in response to IL-2 or IL-4 plus PMA and ionomycin (I), or to IL-2 or IL-4 in anti-CD3 coated microwells. $Thy1^+$, $B220^-$ ($CD4^+$ or $CD8^+$) cells from the same mice respond normally to lymphokines. Culture with both lymphokines results in additive proliferative effects, suggesting that the two lymphokines affect separate populations. Culture of $CD4^-$, $CD8^-$ T cells for 3 days with IL-2 plus PMA + I resulted in increased numbers of $CD8^+$ and decreased numbers of $B220^+$ cells, and expression of IL-2 receptors. Cultures with IL-4, PMA + I induced similar phenotypic changes with a small increase in IL-4 receptors but little induction of IL-2 receptors. Thus, the $CD4^-$, $CD8^-$ cells do not express IL-2 or IL-4 receptors *in vivo* but can be induced to do so *in vitro*. Finally, freshly isolated $CD4^-$, $CD8^-$ or $Thy1^+$, $B220^+$ cells spontaneously transcribe high levels of IFNγ and TNFα mRNA. These two lymphokines have many synergistic effects on immune function *in vivo*. The fact that the $CD4^-$, $CD8^-$ T cells fail to proliferate in response to lymphokines or other mitogenic signals in their *in vivo* state supports the idea that these accumulating cells may derive from single-positive precursors which do respond to growth factors.

C 517 MAPPING OF IgG SUBCLASS- AND T-CELL EPITOPES ON HIV-PROTEINS BY SYNTHETIC PEPTIDES. Tiit Mathiesen, Per Anders Broliden, Jonathan Rosen and Britta Wahren. Department of Virology, National Bacteriological Laboratory, 105 21 Stockholm, Departments of Neurosurgery, Virology and Immunology, Karolinska Institute, 104 01 Stockholm, Johnson & Johnson Biomedical Research, La Jolla, California,

Pentadecapeptides, sequentially overlapping by 10 a.a., were syntesized based on the HTLV-III sequences of gag and env proteins and used as antigens in IgG-subclass ELISAs and T-cell stimulation assays. Sera and cells were obtained from 30 asymptomatic, HIV-infected homosexuals. Reactivity in all subclasses could be found to parts of the gag-protein. Extensive areas of IgG1 and 3 reactivity were indentified mainly in the p17 and N-terminal half of p24 with an additional region in the N-terminal end of p15. The highest IgG1 and 3 reactivities were detected in a.a. 8-33, rich in glycine, arginine and lysine and thereby hydrophilic with a high segmental flexibility; IgG2 and 4 epitopes were found in the hydrophilic COOH-terminal of p17; the second highest IgG1 and 3 reactivities were found in the hydrophilic N-terminal of p15. For gp120, the IgG1 epitopes identified were in the putative loop-region (a.a. 296-331) and in the hydrophilic COOH-terminal end of gp120 (a.a.489-503). The immunodominant region of gp41 (a.a. 582-617) showed some IgG2, 3 and 4 responses in addition to IgG1. T-cell proliferative responses were detected against many peptides usually in areas not binding IgG. Still, simultaneously T- and B-cell reactive peptides could be detected, and the showed a distinctly preference for IgG1. The variation between different patients was considerable regarding both T-cell reactivity to peptides and the subclasses of reactive IgG. A mapping of subclass restricted epitopes allows an elucidation of anti-viral immunological mechanisms.

C 518 $CD4^+$ CELLS ARE SELECTIVELY GENERATED AFTER INTRATHYMIC (i.t.) TRANSFER OF $CD4^+$,$CD8^+$ THYMOCYTES, Bonnie J. Mathieson, Theresa Gregorio, Linette Edison, John Wine and Kristin Komschlies, Laboratory of Experimental Immunology, BRMP, DCT, and BCDP, Program Resources, Inc., National Cancer Institute-Frederick Cancer Research Facility, Frederick, MD 21701 and Jackson Laboratories, Bar Harbor, ME 04609. Recent suggestions that $CD4^+$,$CD8^+$ "double positive" (DP) thymocytes are precursors for $CD4^+$ and $CD8^+$ mature subsets have been based on indirect evidence with in vivo anti-CD8 or anti-CD4 treatments. We have approached this question directly by isolating subsets of DP thymocytes from fetal or adult thymus and have shown that only a subset of DP cells (<10% of adult thymocytes) can generate donor-derived thymocytes after i.t. injection into irradiated hosts. When day 16 fetal or adult low-density DP cells are injected i.t., up to 20-40% donor-derived cells can be seen at 6 to 18 days after transfer. $CD4^+$ cells can constitute up to 30% of these donor-derived cells at day 10. In contrast, an equivalent i.t. transfer of dull Ly-1/CD5 (dLy-1), early precursor thymocytes would not generate $CD4^+$ cells until days 12-13 but would produce some $CD8^+$ cells by 18 days. Thus we have detected differentiation of $CD4^+$ cells and not $CD8^+$ cells from adult DP cells. To examine DP thymocytes without antibody binding to the cell surface, we depleted host and recovered donor-derived thymocytes 8 to 10 days after i.t. transfer of dLy-1 cells, when most of the donor-derived cells are DP. When retransferred, these DP cells displayed a more rapid progression of thymic localization patterns than dLy-1 cells, but were unable to produce enough donor thymocytes to analyze subsets. Thus $CD4^+$ cells appear to differentiate via a very minor (low density) subset of DP thymocytes. Further, $CD8^+$ cells may not be generated from these intermediate DP precursors but directly from dLy-1 cells.

C 519 ENGAGEMENT OF THE CD4 MOLECULE INFLUENCES CELL SURFACE EXPRESSION OF TcR BY DEVELOPING THYMOCYTES. S. A. McCarthy, A. M. Kruisbeek, I. K. Uppenkamp, S. O. Sharrow and A. Singer. NCI, NIH. Bethesda, MD 20892. The intrathymic differentiation process by which bone marrow-derived progenitor cells develop into mature, immunocompetent T lymphocytes remains poorly understood. The majority of all intrathymic lymphocytes are $CD4^+8^+$ "double positive" cells that have no well-documented function in thymic differentiation, but have been proposed to be either a critical intermediate between $CD4^-8^-$ precursor cells and mature T cells, or, alternatively, a "dead-end" cell type. Furthermore, although signals mediated through the TcR complex are critical for T cell tolerance induction and repertoire selection, the functions of the CD4 and CD8 differentiation antigens expressed by the vast majority of thymocytes are unknown. In the present study we addressed the possibility that CD4 functions as a signalling molecule during T cell development. We engaged cell surface CD4 on murine thymocytes by in vivo injection of anti-CD4 mAb, and monitored the effects of that engagement on distinct thymocyte subsets. $CD4^+8^+$ cells respond to CD4 cross-linking by dramatically increasing their cell surface expression of CD3 and TcR, and decreasing their cell surface expression of CD4. In contrast, mature $CD4^+8^-$ cells respond to CD4 engagement by decreasing their cell surface expression of both CD3 and CD4. These results provide clear evidence that CD4 engagement regulates CD3/TcR expression on developing thymocytes, and, most interestingly, results in increased TcR expression by $CD4^+8^+$ double positive thymocytes. Thus, CD4 engagement is a membrane event to which $CD4^+8^+$ thymocytes respond significantly, indicating that CD4 may be a signalling molecule in T cell development and may promote T cell differentiation by inducing increased CD3/TcR cell surface expression on $CD4^+8^+$ thymocytes in vivo.

C 520 DEFECTIVE ACTIVATION THROUGH CD3/TCR AND CD2 BUT NORMAL ACTIVATION VIA CD28 OF T CELLS FROM HIV-INFECTED ASYMPTOMATIC MEN, F. Miedema, C.J.M. van Noesel, F.G. F.G. Terpstra, P.Th.A. Schellekens and R.A.W. van Lier, Central Lab.Blood Transf.Service, Lab.Exp.Clin. Immunol. of the Univ.of Amsterdam, The Netherlands
T cells can be activated through several membrane molecules that may use distinct intracellular signalling pathways. T-cell activation through both the CD3/TCR complex and CD2 is accompanied by rises in free intracellular Ca^{2+} $(Ca^{2+})_i$ and PKC activation. In contrast, evidence from our laboratory suggests that triggering via CD28 occurs through a PKC-independent route. In this study we used Mab to various T-cell membrane antigens to localize the activation defect in T cells from asymptomatic HIV-infected men that we previously showed to have an intrinsic defect in their proliferative response to soluble anti-CD3 Mab. Compared to HIV- homosexual controls, the monocyte-dependent response of T cells from HIV+ men to soluble anti-CD3 and a mitogenic combination of anti-CD2 Mab was decreased as was the monocyte-independent response to immobilized low-dose anti-CD3. In T cells from both groups a normal rise in $(Ca^{2+})_i$ was induced by anti-CD3 and anti-CD2 Mab. However, T cells from HIV-infected men responded poorly to direct PKC activation by PMA in the presence of healthy donor monocytes, whereas control T cells responded vigorously. In contrast, induction of proliferation by anti-CD28 Mab was comparable in T cells from HIV+ and HIV- men. Our results indicate in HIV+ men a selective qualitative T-cell defect in activation routes that are dependent on PKC activation, whereas PKC-dependent alternative activation appears to be unaffected. These findings will be discussed with repect to HIV immunopathology and normal T-cell physiology.

C 521 ROLE OF PROTEIN KINASE C IN THE REGULATION OF LYMPHOKINE EXPRESSION AND CELLULAR PROLIFERATION IN A TH-2 CLONE (D10.G4.1), Eduardo Muñoz, Juan Muñoz, Ana Zubiaga and Brigitte Huber, Tufts University School of Medicine, Boston, MA 02111.

We have studied the role of the protein kinase C (PKC) in the activation of a prototype TH-2 cell, D10.G4.1. Blocking of this enzyme with the drug H-7 [1-(5-isoquinolinyl-sulfonyl)-2-methylpiperazine] leads to an increase in cellular proliferation of D10 cells activated with specific antigen or mitogen. In addition, an increase in the amount of IL-4 and IL-5 mRNA is seen under these conditions compared to the level observed in mitogen activated cells in the absence of H-7. This superinduction of IL-4 and IL-5 lymphokine mRNA is also detected in mitogen activated D10 cells that are depleted of PKC protein by pretreatment of the cells with phorbol esters. In contrast, mitogen activation of splenic T cells measured by proliferation is blocked in the presence of H-7. These data indicate that PKC has a regulatory role in TH-2 cells by exerting a negative feedback on the events that occur after cell activation avoiding an uncontrolled response. This effect is opposite to that seen in a bulk T cell population that represents mainly TH-1 type cells. The mechanism of signal transmission is, therefore, distinct in the two types of CD4 + T cells.

C 522 IMMUNE TOLERANCE, MHC RESTRICTION AND TISSUE IMMUNOGENICITY IN CLASS II MHC TRANSGENIC MICE. Kenneth M. Murphy*, Melinda L. Elish, Paul Allen*, Casey Weaver*, Emil R. Unanue*, and Dennis Y. Loh, Howard Hughes Medical Institute, Departments of Medicine, Pathology, Microbiology and Immunology, Washington University School of Medicine, St. Louis, MO 63110.

Aberrant expression of Class II MHC proteins on non-immune somatic tissue has been implicated in several autoimmune diseases, but it was unknown whether this expression was involved in the initiation of disease or resulted from induction by the immune reaction. We have engineered several transgenic mouse lines expressing the murine Class II MHC I-A^d protein on exocrine pancreas alone, or on all somatic tissues using either the pancreatic elastase promoter or the Class I K^b gene promoter. The I-A^d on the pancreas is fully capable of presenting peptide antigens to I-A^d restricted hybridomas. H-2^b mice expressing I-A^d on pancreatic acinar cells but not in thymus are not tolerant to I-A^d in mixed lymphocyte reactions in vitro, but do not spontaneously produce an immune response to the pancreas in vivo. Expression of I-A^d under control of the Class I K^b promoter results in functional peripheral antigen presentation, I-A^d restricted immune response, I-A^d expression by T-cells, and widespread somatic I-A^d expression. Thymic expression patterns in these lines varies. One line has strong cortical epithelial and medullary transgene I-A^d expression, whereas another has only medullary expression. The effect that these different patterns have on MHC restriction is being examined. These mice provide an in vivo system for evaluating the possible role of Class II MHC expression in autoimmunity.

C 523 Ca^{++} FLUX ANALYSIS: A RAPID AND DIRECT ASSAY FOR ANTIGEN SPECIFIC T CELL ACTIVATION, AND A MEANS OF ENRICHMENT FOR ANTIGEN SPECIFIC T CELL SUBSETS, Paul T. Nghiem, David R. Parks, and C. Garrison Fathman, Depts of Medicine and Genetics, Stanford U. Med. Ctr., Stanford, CA 94305. A transient increase in the intracellular Ca^{++} ion concentration has been shown to occur rapidly following activation of T lymphocytes. This response is a more immediate and direct indication of T cell activation than either production of interleukins or proliferation. We examined Ca^{++} flux analysis using Indo-1 and the FACS to measure ag-specific T cell activation and also to sort for an ag-specific T lymphocyte subpopulation. Human T cell clones were exposed to either allostimulatory B lymphoblastoid cells (which cause proliferation of the T cells) or nonstimulatory B lymphoblastoid cells. In both CD4+ and CD8+ clones, we observed an ag-specific Ca^{++} flux response similar to an ionomycin positive control. We then attempted to enrich for antigen specific T cells from a mixed population. Two phenotypically different human T cell clones were mixed in equal amounts. After coculture of the cell mixture with B lymphoblastoid cells shown to stimulate only one of the T cell clones, the cell mixture was sorted on the FACS based on specific Ca^{++} flux. A ten fold enrichment was observed for the ag-specific clone. This approach allows rapid observation of T cell activation and provides a means of sorting antigen reactive T cells from a heterogeneous cell population.

C 524 MODULATION OF LECTIN AND ALLOANTIGEN INDUCED T-CELL PROLIFERATION IN THE PRESENCE OF BETA-2-MICROGLOBULIN, Mogens H. Nissen, Thomas Tscherning and Mogens H. Claësson, Laboratory of Experimental Hematology and Immunology, Institute of Medical Anatomy, Department A, University of Copenhagen, The Panum Institute, Blegdamsvej 3C, DK-2200 Copenhagen N, DENMARK.

Previously attemps to characterize the function of beta-2-microglobulin (beta-2-m) have used anti-beta-2-m antibodies, to block the functional activity of the protein. We now demonstrate that human beta-2-m and the proteolytic derivates beta-2-m Lys58 and beta-2-m desLys58 added in nanomolar concentrations to allogeneic, PHA and Con-A stimulated mouse lymphocyte cultures modulate the response induced. Thus beta-2-m and especially beta-2-m desLys58 when added day 0 and 1 augment the generation of specific cytotoxic T lymphocytes in MLC, problably due to stimulation of endogeneous lymphokine production in the culture. Both the Con-A and PHA induced response were modulated. In the presence of beta-2-m and its proteolytic derivates the proliferation was optimal at 48 hours, as opposed to the 24 hours optimum in the abscence of beta-2-m. These results strongly suggest an active role of beta-2-m or proteolytic derivates hereof in the T cell activation and generation of specific cytotoxic T lymphocytes.

C 525 **SPECIFICITIES AND FUNCTIONS OF IN VITRO CULTURED DOUBLE NEGATIVE THYMOCYTES,** Atsuo Ochi and Yojiro Kawabe, Department of Molecular Immunology and Medical Genetics, Mount Sinai Hospital Research Institute, University of Toronto, 600 University Avenue, Toronto, Canada M5G 1X5.

In order to evaluate the maturational stage of double negative cells and to obtain some information about their specificity and functional role in thymocyte development, we have developed a culture system for double negative thymocytes. These unique thymus-derived double negative cell lines manifest both auto-reactivity and allo-reactivity in the same line. Furthermore, we have found that these cells produce IL-2, IL-3 and glamurocyte macrophage colony stimulating factor (GM-SCF) in response to mitogenic stimuli. These observations, together with the presence of the CD3 surface antigen, indicate that these double negative cells are not as yet self-tolerant but are highly functional in responding to environmental antigens and secreting series of lymphokines. Our observations support the concept that self-tolerance occurs during maturation from double negative cells to single positive cells (possibly at the double positive cell stage) by eliminating highly autoreactive cells; double negative cells, therefore, belong to the preselected population and are reactive to self-antigen. The results on the study of phenotypes, antigen-specific activation, function, and finally differentiation of in vitro established double negative T cell lines will be discussed.

C 526 ENCEPHALITOGENIC T CELL CLONES WITH VARIANT RECEPTOR SPECIFICITY, Halina Offner, G. Hashim, Y. Chou, B. Celnik, R. Jones and A. Vandenbark, Neuroimmunology Research 151D, Veterans Administration Medical Center, Portland, OR 97201. We explored antigenic differences between GP-BP, Rt-BP and respective peptides from the encephalitogenic region for Lewis rats by comparing the fine specificity of T lymphocyte lines and clones selected from animals primed with these antigens. Encephalitogenic T cell lines specific for GP-BP or Rt-BP predictably recognized the corresponding 72-89 and to a lesser degree the 72-84 (S55S) amino acid sequence. T cell lines selected from rats primed with GP-S55S responded preferentially to GP-S55S compared to other peptides. A T cell line raised to Rt-S55S, however, was unresponsive to the intact GP-BP or Rt-BP. Clones selected from the Rt-S55S line had two distinct patterns of response: Clones that recognized both of the BPs and the S55S peptides transferred delayed type hypersensitivity (DTH) and EAE. These clones also recognized residues 69-81 (S67) but not peptide S75-89. In contrast, T cell clones that responded only to peptides GP-S55S and Rt-S55S but not to the parent BPs transferred DTH but not disease. The same clones failed to respond to either the S67 or the S75-89 sequences. These results demonstrate that the encephalitogenic Rt-S55S sequence houses a minimum of two T cell epitopes, one which is immunodominant and resembles the encephalitogenic region of the intact BP molecule, and the second which is non-encephalitogenic is not shared by the parent BPs, the S67 or the S75-89 sequences. Both types of Rt-S55S-specific clones differ in fine specificity from encephalitogenic GP-BP-specific clones, thus indicating that uniformity of T cell recognition of the encephalitogenic epitope is not an absolute condition for T cells to be encephalitogenic.

C 527 ANTI-CD3 AND CON A CAUSE A RAPID INCREASE IN LYMPHOCYTE F-ACTIN, Charles H. Packman, Pradyumna D. Phatak, and Marshall A. Lichtman, Hematology Unit, University of Rochester, Rochester, NY 14642. Attachment of anti-CD3 or Con A to the human T cell receptor complex (TCR) results in activation of protein kinase C (PKC) and an increase in intracellular Ca^{++}. Activation of PKC results in a rapid increase in F-actin in T cells (J. Immunol. Nov. 88, in press). We thus examined whether monoclonal anti-CD3 or Con A could cause an increase in T cell F-actin. Human blood lymphocytes were stimulated, fixed, made permeable, stained for F-actin with NBD-phallacidin, and analyzed by flow cytometry. Cellular F-actin increased after incubation with anti CD-3 (1.4 fold) and Con A (1.2 fold). The increase peaked at 2 min and returned almost to baseline by 10 min. PMA 0.2 nM caused a similar but sustained increase in F-actin. The Ca^{++} ionophore A23187 caused an increase in F-actin (1.2 fold). Irrelevant ligands (FMLP, monoclonal anti-vimentin) and an antibody that inhibits sheep E rosetting (anti CD2) did not cause an increase in lymphocyte F-actin. Thus, attachment of lectin or antibody to the TCR results in an early transmembrane signal evidenced by a change in the G- and F-actin equilibrium. F-actin could play a role in T cell recognition and attachment, or may be a link in signal transduction for proliferation and mitogenesis.

C 528 Growth-Associated Vimentin Gene Expression in Cloned T Helper Cells
Patricia L. Podolin, Daniel E. Sabath, Charles V. Clevenger and Mike B. Prystowsky, Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA 19104
To determine the identity of genes in T lymphocytes that are regulated in a growth-associated manner, and thus the gene products that are necessary for the process of T cell proliferation, a cDNA library was constructed using poly(A)+ RNA from 20 hr IL2-stimulated murine T helper cells. Differential screening of this library allowed for the selection of clones whose expression is upregulated in late G_1/S phase cells as compared to resting cells. Sequencing of a high frequency, 1800 bp clone identified it as the gene encoding vimentin, an intermediate filament protein. Like the hamster vimentin gene, this murine vimentin cDNA contains an extended 3' untranslated region when compared to a human vimentin cDNA (296 bp vs. 57 bp, respectively). Vimentin mRNA levels in T cells increase 20-fold following 20 hr of IL2 stimulation (late G_1/S phase), and reach 40 times the vimentin mRNA level of resting cells by 70 hr (at which time the cells are cycling asynchronously). Vimentin protein levels increase approximately 2-fold as the cells enter the S, G_2 and M phases of the cell cycle. This apparent discrepancy in the degree of accumulation of vimentin mRNA vs. protein as T cells progress through the cell cycle is not currently understood, and will be addressed in future studies. Supported by NIH grants.

C 529 THE GENE DEFECTS CAUSING HUMAN X-LINKED IMMUNODEFICIENCIES INTERRUPT THE DEVELOPMENT OF DISTINCT LYMPHOCYTE LINEAGES.
Jennifer M. Puck and Mary Ellen Conley, Joseph Stokes, Jr. Research Institute, Children's Hospital of Philadelphia, Phila, PA 19104.
The molecular bases of human X-linked agammaglobulinemia (XLA) and severe combined immunodeficiency (XSCID) are unknown, but may be approached by defining affected cell lineages and by reverse genetics. We hypothsized that obligate carrier women have normal immune function because their cell lineages targeted by these gene defects are composed of cells in which the mutant gene is on the inactive X chromosome. We used nonrandom X inactivation in carriers as an indicator of cell lineages primarily affected by each gene defect. In XLA carriers only B, and not T cells had exclusively the normal X active; however, in XSCID carriers both B and T cells, but not monocytes had nonrandom X inactivation. Moreover, XSCID carriers' immature IgM B cells showed random X inactivation, while more mature IgG and IgA B cells had only the normal X active. We conclude that the XLA gene product is required for B cell development, while the XSCID gene product is required for both T and B cell maturation. Gene mapping has placed XSCID in Xq13-21.3, linked to phosphoglycerate kinase (PGK), lod 3.1, θ=0. Mouse xid, which affects only B cells, is also linked to mouse PGK, suggesting the possibility that XSCID and xid belong to related gene families.

C 530 MAPPING OF THE GENES WHICH CONTROL Vβ3 EXPRESSION, Ann M. Pullen, Philippa C. Marrack and John W. Kappler, Howard Hughes Medical Institute at Denver, National Jewish Center, Denver, CO 80206.

The generation of a hamster monoclonal antibody specific for V$\beta3^+$ T cell receptors has facilitated the demonstration that V$\beta3^+$ T cells are absent in the periphery of mice expressing Mls-2^a (formerly Mls^c). As is the case for all the different Vβs studied so far, these cells are eliminated in the thymus upon contact with bone marrow-derived cells which express the particular self antigen-MHC combination.

Mapping data for the BXD and AKXD recombinant inbred strains shows that the MHC and the products of at least two other genes control the expression of Vβ3. One of these is linked to Ly-7, and the second for the AKXDs is linked to MTV-13 on chromosome 4. A backcross of B10.BR x (C3H x B10.BR)F1 (Mls-2^b x (Mls-2^a x Mls-2^b)) has been used to confirm the linkage of one of these controlling genes to Ly 7.

Using the Vβ3-specific monoclonal antibody, exceptions have been found to the general rule, that strains which have eliminated T cells expressing a particular Vβ specific for a defined self antigen in the thymus, also express this self antigen on their spleen cells, and so these cells are capable of stimulating T cell hybridomas bearing the particular Vβ in question.

C 531 A MOUSE MODEL TO STUDY ANTI-SELF RECOGNITION IN THE THYMUS AND IN THE SPLEEN, Paola Ricciardi-Castagnoli, Laura Pirami, Suzy Shammah, Marica Sassano, Marco Righi, Nicoletta Malgaretti°, Roberto Mantovani°, Paola Comi* and Sergio Ottolenghi°, CNR Center of Cytopharmacology, Dept. of Pharmacol., Univ. of Milan, °Dept. Genetics, Univ. of Milan, *CNR Center Study Cell Pathology, Milan.

The T cell receptor (TCR) of the alloreactive T cell clone 2C recognizes the H-2L^d specificity. The cloned α (Vα 3.1) and β (Vβ 8.2) TCR cDNAs have been inserted into pLJ retroviral vectors containing LTR and ψ regions of the MoMuLV and neomycin resistance genes. Three different constructs have been used to transfect ψ_2 cells. G418-resistant clones showing the highest titer of viral particle production have been selected, and injected subcutaneously into newborn Balb/c mice (H-2 L^{d+}) or into the congenic strain dm2 lacking the H-2L^d gene. Mice injected with the ψ_2 cells producing retroviruses carrying the α gene have been examined for the expression of the new specificity on the infected T cells. Northern blot analysis using the Vα and the neo-probes showed, in the lymphoid tissues, a transcript with an expected size of 3.6 Kbs hybridizing with both probes. In the spleen of treated Balb/c mice, but not in dm2 mice, the expression of the exogenous Vα 3 resulted in proliferation of infected T cells, as assessed by an auto-MLR proliferative assays.

C 532 ROLE OF CD3 MOLECULE IN THE Ti/CD3 COMPLEX OF CTL, A. Ratner*, D. Rosen, G. Berke, Weizmann Institute, Rehovot, Israel.*U.C.L.A., Los Angeles, CA

It has been previously demonstrated that PEL (*in vivo* primed CTL) treated with trypsin loose most of their lytic activity as well as the ability to form conjugates with specific TC. Preliminary experiments show that SPDP-anti-CD3-derivatization of specific TC overcomes the trypsin effect, so that both conjugation and lysis are observed between trypsin-treated PEL and SDPD-anti-CD3-derivatized specific TC. As has been asserted by many investigators, the Ti/CD3 complex on PEL surface is central to specific lysis. The observed inhibition of trypsin may be due to its action upon it. Since trypsin-treated PEL could be triggered toward conjugation with and lysis of TC by anti-CD3, the CD3 at least must have remained intact and is the important molecule for both the critical conjugation step and lysis. Trypsin treatment possibly disturbs the interaction of TC with Ti/CD3 of PEL so as to prevent proper engagement of the CD3. Concanavalin A (Con A) treatment of TC appears not able to bring back the lytic activity or the ability to form conjugates of trypsin-treated cells, indicating a dichotomy in the mechanism of killing by derivatization with anti-CD3 versus Con A mediation. The importance of the CD3 is indicated further by comparing PEL and their blasts (PEB). Unlike PEL, PEB do not show LDCC or lytic activity against SPDP-anti-CD3-derivatized nonspecific TC. LDCC by Con A has been proposed to function via Ti/CD3 interaction. Immunolabeling both cells with anti-CD3 revealed that unlike PEL, PEB also do not show fluorescence. It may be that the Ti/CD3 complex on PEB differs from the one on PEL. At the same time, total lack of CD3 is contraindicated for PEB kill specifically. The region important in Ti interaction for LDCC and that recognized by anti-CD3 may be within the aberrant area of CD3, so neither the Con A nor the anti-CD3 trigger could be transmitted on PEB.

C 533 COMBINATION OF CD4$^+$ AND CD8$^+$ ISLET SPECIFIC T CELL CLONES ARE PATHOGENIC IN THE NOD MOUSE, Eva-Pia Reich and Charles A. Janeway, Jr., Department of Pathology, Section of Immunobiology, Yale University School of Medicine, 310 Cedar St., New Haven, CT 06510.

Although diabetes in the NOD mouse is thought to be initiated by T lymphocytes, thus far no T cell clone has been derived from NOD islets. We therefore isolated islets from acutely diabetic NOD mice and islet derived lymphocytes were propagated in IL-2 and stimulated every 2 weeks with fresh mitomycin-C treated NOD islets to maintain islet specificity (i.e. a proliferative response to NOD islets). T cell clones were generated by limiting dilution followed by recloning by single-cell manipulation. FACS analysis showed that all clones were positive for Thy-1$^+$ and CD3$^+$, confirming that receptor bearing T cells had been cloned. CD4$^+$CD8$^-$ and CD4$^-$CD8$^+$ clones were isolated that proliferated in the presence of NOD islets. The two CD4$^+$ clones respond to NOD but not BALB/c or B10.BR islets, while the two CD8$^+$ clones respond to NOD and BALB/c islets but not B10.BR. This suggests that the CD4$^+$ clones are islet-specific, I-A^{NOD} restricted, while the CD8$^+$ clones are islet-specific and D^d restricted.

Injection of individual cloned lines into young, irradiated NOD mice leads to no overt or microscopic disease. However, mixture of CD4$^+$ and CD8$^+$ cloned lines causes a syndrome of dehydration, rapid deterioration and death about 10-14 days after transfer. Mice sacrificed at this time show lymphocytic infiltration around the islets. These cloned lines may be useful in the search for islet cell autoantigens.

C 534 MERCURIC CHLORIDE INDUCES AUTOANTIBODIES AGAINST U3 SMALL NUCLEAR RIBONUCLEOPROTEIN IN SUSCEPTIBLE MICE, Rolf Reuter*, Gabriela Tessars*, Hans-Werner Vohr[1], Ernst Gleichmann[1] and Reinhard Lührmann*, * Max-Planck-Institut für molekulare Genetik, Otto-Warburg-Laboratorium, Ihnestrasse 73, D-1000 Berlin 33 (FRG), [1] Division of Immunology, Medical Institute of Environmental Hygiene at the University of Düsseldorf, D-4000 Düsseldorf 1 (FRG). Autoantibodies to nucleolar components are a common serological feature of patients suffering from scleroderma, a collagen vascular autoimmune disease. While animal models which spontaneously develop abundant anti-nucleolar antibodies have not yet been described, high titers of such antibodies may be induced by treating susceptible strains of mice with mercuric chloride. We have identified the nucleolar autoantigen against which the $HgCl_2$-induced IgG autoantibodies from mice of strain B10.S are directed. It is a protein of apparent molecular weight of 36 kDa and pI value of about 8.6 which is associated with the nucleolar snRNA U3, and by these criteria, is identical with a polypeptide called fibrillarin. It is striking that scleroderma patients spontaneously form autoantibodies against the same U3 RNP protein. The $HgCl_2$-induced murine and the scleroderma-specific human anti-U3 RNP autoantibodies were indistinguishable in their reactivities towards fibrillarin. They further resemble each other in so far as both recognize epitopes on the 36-kDa protein which have been highly conserved throughout evolution. Our results provide a basis to investigate at the molecular level whether similar immunoregulatory dysfunctions may lead to the preferential anti-U3 RNP autoantibody formation in the animal model and in scleroderma patients.

C 535 HUMAN T-CELL RESPONSES TO MYELIN BASIC PROTEIN (BP), J.R. Richert, E.D. Robinson, G.E. Deibler, R.E. Martenson, L.J. Dragovic, M.W. Kies. Georgetown Univ. Med. Ctr., Washington, DC 20007; NIMH, Bethesda, MD; Medical Examiner's Office, Baltimore, MD. Forty BP-specific $CD4^+$ T-cell clones were isolated from the peripheral blood of a patient with multiple sclerosis. Studies with a panel of xenogeneic BPs of known amino acid sequence and with large fragments of the BP molecule demonstrated the existence of at least 10 epitopes recognized by human T-cells. At least 7 of these epitopes were potentially clustered in the middle or at the C terminus of the molecule. In studies with synthetic peptides corresponding to residues 86-105 and 152-170 of human BP (HBP), 9 clones proliferated in response to 86-105 and 19 recognized 152-170. CTL assays were carried out with targets consisting of an autologous B-cell line coated with HBP. Nineteen of the 40 clones demonstrated BP-specific CTL activity; these were the same 19 that had recognized peptide 152-170 in proliferation assays. These clones also killed targets coated with peptide 152-170 but not targets coated with 86-105. Clones that proliferated in response to peptide 86-105 or to epitopes located outside of these two regions failed to kill HBP-coated targets. The lack of killing of targets coated with 86-105 could not be ascribed to a lack of binding of 86-105 to the target cell, as the same B-cell line successfully presented peptide 86-105, in proliferation assays, to clones that had previously recognized this peptide when presented by E^- cells. Thus, the epitope specificity of CTL activity against BP is more restricted than is the specificity demonstrated for proliferative activity.

C 536 **ISOLATION AND CHARACTERIZATION OF IL2-INDUCED GENES IN A CLONED T LYMPHOCYTE** Daniel E. Sabath, Patricia L. Podolin, and Michael B. Prystowsky, Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA
As an approach to studying regulatory factors important during T cell proliferation, the expression of genes induced during IL2-stimulated T cell growth was examined. To identify genes induced by IL2, a cDNA library was made from IL2-stimulated cloned T cells in late G_1 of the cell cycle and was screened by differential hybridization, selecting those clones which had higher steady-state mRNA levels after IL2 stimulation. Of 40,000 clones originally screened, 90 positive clones were identified, which represented 21 different genes. When partially sequenced and compared with sequences in the NIH and EMBL databases, 6 clones were identified as cDNAs encoding glycolytic enzymes, 4 were cDNAs of cytoskeletal genes, 3 were cDNAs coding for proteins important in protein synthesis, and 8 clones were not identified. The maximal steady state mRNA levels generally occurred during late G_1/early S phase, and the induction of most RNAs was not inhibited by cycloheximide at a dose that inhibited cell proliferation. Nuclear run-on transcription demonstrated an increase in the rate of transcription for GAPDH. In addition, there is post-transcriptional regulation of expression, as some mRNAs were stabilized upon IL2 stimulation. Having cloned these mRNAs, we can now begin to study the regulation of expression of selected genes. Supported by NIH grants.

C 537 INDUCTION OF ORGAN-SPECIFIC AUTOIMMUNE DISEASE IN MICE BY NEONATAL ADMINISTRATION OF CYCLOSPORIN A, Shimon Sakaguchi and Noriko Sakaguchi, Department of Medicine Division of Immunology and Rheumatology, Stanford University School of Medicine, Stanford, CA 94305.
Cyclosporin A (CsA), a potent immunosuppressive drug, caused organ-specific autoimmune disease, such as gastritis with anti-parietal cell autoantibodies or oophoritis with anti-oocyte autoantibodies, in BALB/c mice when the drug was administered daily for one week to newborns. Administration to adult mice did not. CsA abrogated the production of $L3T4^+$ T cells and $Lyt\text{-}2^+$ T cells in the thymus. Consequently, these T cells were substantially depleted from the peripheral lymphoid organs, especially when the drug was administered from the day of birth. Autoimmune disease was prevented when CsA-treated newborn mice were inoculated with splenic T cells from normal syngeneic mice. On the other hand, removal of the thymus immediately after neonatal CsA treatment produced autoimmune disease with a higher incidence and in a wider spectrum of organs, i.e., thyroiditis, sialoadenitis of the salivary gland, gastritis, insulitis of the endocrine pancreas, adrenalitis, oophoritis, or orchitis. Each autoimmune disease was accompanied by the development of autoantibodies specific for the corresponding organ antigens. It is likely that CsA caused organ-specific autoimmune disease, when administered to newborns, by depleting certain regulatory T cells (suppressor T cells) controlling self-reactive clones. (This work was supported by a grant from the Lucille P. Markey Charitable Trust.)

C 538 **B- AND T-CELL RESPONSE TO A HIGHLY REPETITIVE ANTIGEN OF PLASMODIUM FALCIPARUM.** Artur Scherf, Philippe Dubois, Marika Pla* and Luis Pereira da Silva, Institut Pasteur, 25-28, rue du Dr. Roux, 75015 Paris, *U93 INSERM, Hopital St. Louis, 2, place du Dr. Alfred Fournier, 75010 Paris, France.
We isolated from a genomic expression library of the malaria parasite *P. falciparum* a clone coding for an antigen associated with the membrane of infected erythrocytes. Intensive sequence analysis of this gene, called 11-1, showed that the major part of this molecule consists of at least 200 repeats of nine amino acids. The consensus sequence of these degenerated repeats is E-E-V-V-E-E-V-V-P and secondary structure analysis predicts that they have a high tendency to form amphipathic alpha helices.
The B- and T-cell response to the two most frequent types of repeat were analysed in five different H-2 congenic mice strains using two synthetic peptides (P9A: Y-P-(E-E-V-V-E-E-V-V-P)$_2$-K; P9B: Y-P-(E-E-L-V-E-E-V-I-P)$_2$-K). The B-cell response to both peptides is restricted to the $H\text{-}2^d$ and $H\text{-}2^k$ haplotype. P9A and P9B specific antibodies do not discriminate between the two peptides (measured by ELISA). The MHC restriction was confirmed by analysing the T-cell response to both peptides. T-cell lines specific for each of the peptides P9A/P9B were derived from $H\text{-}2^d$ responding mice. Although the peptides differed in four positions of the hydrophobic part of the helices (valine replaced by isoleucine and leucine) no significant differences were observed in the antigen presentation between peptide P9A and P9B. In contrast, both peptides were unable to induce a proliferation of the heterologous T-cell line, suggesting that the variable hydrophobic amino acids of the repeats are involved in T-cell recognition. We are currently investigating if other variant repeats of the 11-1 antigen are restricted to a different MHC haplotype and that the totality of repeats may be subjected to no restriction. Given the type of amino acid replacements within the two repeats tested, the T-cell receptor appears to discriminate between very similar amino acids.

C 539 MOLECULES INVOLVED IN T CELL RECEPTOR DEPENDENT AND INDEPENDENT ACTIVATION OF CYTOLYTIC T CELLS, Anne-Marie Schmitt-Verhulst, Nathalie Auphan, Claude Boyer, Annick Guimezanes, Claire Langlet, Centre d'Immunologie INSERM-CNRS de Marseille-Luminy, Case 906, 13288 Marseille, Cedex 9, France.
The γ-interferon gene is activated upon cell surface triggering of the transmembrane T cell receptor (TcR)/CD3 complex or via phosphatidylinositol anchored Thy-1 molecules of cytolytic T cell clones. Both these activation events are inhibited by antibodies directed at the Lyt-2/Lyt-3 molecules and are lost on TcR α-chain deletion variants (1, 2). Biochemical analyses failed to reveal any association between the TcR/CD3 complex and either Lyt-2/Lyt-3 or Thy-1 molecules. However, such studies consistently revealed a non-covalent, cis-type association between Lyt-2/Lyt-3 and H-2 class I products as well as between Thy-1 and H-2 class I products. The structural features of the molecules required for such interactions are being determined using hybrid engineered molecules and possible implications of such interactions for T cell activation are being evaluated.
(1) Schmitt-Verhulst, A.-M. et al. Nature **325**, 628-631 (1987).
(2) Guimezanes, A. et al. Cellular Immunol. **113**, 435-446 (1988).

C 540 DIRECT ANALYSIS OF THE SIZE OF THE T CELL REPERTOIRE: A MOLECULAR AND CELLULAR APPROACH, R.-P. Sekaly, S. Liu, N. Labrecque, V. Foster, and T.W. Mak*, Laboratory of Molecular Immunology, Clinical Research Institute of Montreal, Canada and *Department of Biophysics, Ontario Cancer Institute, Toronto, Canada. The T cell antigen receptor (TcR) which recognizes antigens and the MHC gene products is a cell surface protein heterodimer consisting of an α and ß chain. Direct structure-function relationships between T cell specificities and TcR α/ß chain primary structures have been difficult to establish. It is increasingly apparent that the association of both chains is critical to reconstitute the Ag-MHC binding site. Combinational mechanisms for generating diversity play a major role in increasing the total size of the repertoire. However, evidence for preferential associations have been described between other heterodimeric proteins of the immunoglobulin gene superfamily. It is therefore important to determine if association between different sets of Vαs and Vßs occur at random or if, alternatively, preferential associations exist. We have devised a system which should enable us to determine, by DNA mediated gene transfer of cloned cDNAs encoding different Vαs or Vßs, if such preferential associations do exist. We have also used a panel of monoclonal antibodies specific for a particular Vα or Vß to derive T cell clones expressing these Vαs or Vßs. In a polyclonally activated T cell population expressing a specific Vα or Vß chain, we will present data indicating that the association between these chains is preferential or random.

C 541 POSITIVE AND NEGATIVE SELECTION OF AN ANTIGEN RECEPTOR ON T CELLS IN TRANSGENIC MICE. William C. Sha, Christopher A. Nelson, Rodney D. Newberry, David M. Kranz**, John H. Russell*, and Dennis Y. Loh, Howard Hughes Medical Institute, Departments of Medicine, Genetics, Microbiology, Immunology, and Pharmacology*, Washington University School of Medicine, St. Louis, MO 63110 and Department of Biochemistry**, University of Illinois, Urbana, IL 61801.

Interactions between T cells and MHC molecules are thought to select a T-cell repertoire skewed towards recognition of antigens in the context of self MHC molecules. In addition, T cells which react strongly to self MHC molecules are eliminated by a process called self-tolerance. We have recently described generation of transgenic mice expressing the αß T-cell receptor (TCR) from the cytotoxic T lymphocyte 2C (Sha et al., 1988 NATURE 335:271). The clone 2C was derived from a BALB.B ($H\text{-}2^b$) anti-BALB/c ($H\text{-}2^d$) MLC and is specific for the L^d class I MHC antigen. In transgenic $H\text{-}2^b$ mice, a large fraction of T cells in the periphery expressed the 2C TCR. These T cells were predominantly $CD4^-CD8^+$ and were able to specifically lyse target cells bearing L^d. In the periphery of transgenic mice expressing L^d, functional T cells bearing the 2C TCR were deleted. This elimination of autoreactive T cells appears to take place at or before the $CD4^+CD8^+$ stage in thymocyte development. In addition, we report that in $H\text{-}2^s$ mice, a non-autoreactive target haplotype, large numbers of $CD8^+$ T cells bearing the 2C TCR were not found, providing strong evidence for the positive selection of the 2C TCR specificity by $H\text{-}2^b$ molecules.

C 542 CD4 EXPRESSION IS REQUIRED FOR THYMUS SELECTION OF CLASS-II SPECIFIC T CELLS DURING T CELL DIFFERENTIATION, Susan O. Sharrow, Ingeborg Uppenkamp and Dominick DeLuca. Experimental Immunology Branch, NCI, NIH, Bethesda, MD 20892 and Dept. of Biochem. and Molecular Biol., Medical University of South Carolina, Charleston, SC 29425

The requirements for CD4 expression during T cell differentiation in fetal thymus organ culture (FTOC) were investigated by analysis of the effects of addition of anti-CD4 Mab. Control FTOC cultured for 5 days contained predominantly $CD4^+CD8^+$ cells as well as CD4 or CD8 single positive cells expressing high levels of CD3. T cells from FTOC cultured for 5 days in the presence of anti-CD4 Mab expressed markedly diminished CD4 expression. When anti-CD4 Mab was removed 20 hours prior to phenotypic analysis, it was found that CD4 was re-expressed on $CD4^+$ $CD8^+$ T cells. After 12 days of culture, control FTOC contained high frequencies (20-30%) of $CD4^+CD8^-$ and $CD4^-$ $CD8^+$ thymocytes expressing high levels of CD3. In contrast, anti-CD4 treated cultures, while containing $CD4^-$ $CD8^+$, $CD3^+$ T cells, were well depleted of cells which reacted with anti-CD4 antibody. When anti-CD4 Mab was removed 20 hrs prior to analysis, CD4 was re-expressed on $CD4^+CD8^+$ cells, and low frequencies (<5%) of $CD4^+$ $CD8^-$ T cells were detected, but these CD4 single-positive cells did not express high levels of CD3 or detectable αß heterodimers. While normal frequencies of $CD4^-CD8^+$,$CD3^+$ cells expressed Vß8 determinants, the anti-CD4 treated cultures were relatively enriched in $CD4^-CD8^+CD3^-$ and $CD4^-CD8^-CD3^-$ T cells compared to control 12 day FTOC. These data demonstrate that while $CD8^+CD3^+$ T cells can differentiate in the presence of anti-CD4 Mab, differentiation of $CD4^+CD3^+$ cells is inhibited and cell surface CD4 is down-modulated. $CD4^+CD8^+$ cells can develop in the presence of anti-CD4 Mab and do express T cell receptors. These results suggest that CD4 expression, while not required for development of $CD3^+CD4^+CD8^+$ T cells, is necessary for differentiation of mature $CD4^+CD8^-$ $CD3^+$ T cells, and that the interaction of CD4 with its ligand is critical for thymus selection of Class-II specific T cells.

C 543 CRITICAL ROLE OF THE TcR IN THYMOCYTE DIFFERENTIATION: EVIDENCE FROM THE IMMUNODEFICIENT SCID MOUSE, Elizabeth W. Shores, Susan O. Sharrow, Ingeborg Uppenkamp, and Alfred Singer, Experimantal Immunology Branch, NIH, Bethesda, MD 20892.
In order to investigate the role of the T cell receptor (TcR) in thymocyte maturation, we have studied thymocytes from the immunodeficient C.B-17/scid mouse, which due to a genetic defect, is unable to express TcR genes. Despite this mutation, scid thymocytes were functional as they produced lymphokines and proliferated in response to stimuli (PMA, ionomycin, IL-2, IL-4). Phenotypic analysis revealed that, like normal immature thymocytes, scid thymocytes were large, Thy 1.2^+, $CD4^-$, $CD8^-$, TcR^- and were enriched in $CD5^{+dull}$, IL-$2R^+$, Pgp-1^+, and HSA^+ cells. However, other TcR^- populations present in normal mice, (i.e. $CD4^-8^+TcR^-$ and $CD4^+8^+TcR^-$ cells) were absent from scid mice. In order to determine if TcR expression was required for CD4 and CD8 expression, we analyzed thymi from the occasional scid mouse which possessed small numbers of TcR-bearing thymocytes. Interestingly, expression of CD4 or CD8 was detected only in those scid thymi that possessed TcR^+ thymocytes, although CD4 and CD8 expression was not confined to those TcR-bearing cells. Further, the introduction of TcR^+ cells from Thy 1 congenic normal mice into TcR^- scid mice consistently promoted the expression of CD4 and CD8 (but not TcR) by scid thymocytes. Moreover, scid thymocytes from these chimeras possessed $CD4^+8^+$ cells, however no mature, single positive thymocytes of scid origin were detected. Together, these results suggest that TcR-bearing cells within the thymic milieu are required for the expression of CD4 and CD8 and for the differentiation into $CD4^+8^+$ cells. However, final maturation into mature single positive thymocytes requires that the thymocyte itself express TcR.

C 544 ANALYSIS OF THE CD4-p56^{lck} COMPLEX EXPRESSED IN NIH3T3 FIBROBLASTS. Scott C. Simpson, Joseph B. Bolen and Andrē Veillette, Laboratory of Tumor Virus Biology, NCI, Bethesda, Maryland 20892.
The CD4 T-cell surface receptor has been implicated in the stabilization of intercellular interactions. In addition, it is felt to mediate the transduction of an independent intracellular signal. We have recently shown (Veillette et al., in press) that the CD4 molecule expressed in T-lymphocytes is complexed to the internal membrane tyrosine kinase p56^{lck}, suggesting that alterations in the properties of this enzyme mediate the CD4 related signal. To gain further insight into the structure and function of the CD4-lck complex, we have generated NIH3T3 fibroblasts that co-express the CD4 and lck proteins. NIH3T3 fibroblasts expressing a murine lck cDNA were transfected by calcium-phosphate precipitation with a construct containing a murine CD4 cDNA and the neomycin resistance gene. Stably transfected clones were selected for resistance to the aminoglycoside G418. We have now demonstrated that the CD4 and p56^{lck} expressed in these non-lymphoid cells can form a stable non-covalent complex. The structure and function of the CD4-lck complex expressed in murine fibroblasts is currently being evaluated.

C 545 EVIDENCE FOR A HOLE IN THE T CELL RECOGNITION REPERTOIRE, Bhagirath Singh, Zuzana Novak, Arun Fotedar, Michel Boyer, Michel W.J. Sadelain and Esther Fraga, Department of Immunology, University of Alberta, Edmonton, Alberta, Canada T6G 2H7
Bone marrow radiation-induced chimeras were used to demonstrate that H-2^b T cells, non responders to an immune response (Ir) gene controlled antigen Poly-18 Poly EYK(EYA)$_5$, educated in a responder H-2^{bxd} environment do not make the full spectrum of Poly-18 clonotypes found in responder H-2^d animals. Two major T cell clonotypes (Type A and B) are induced by Poly-18 in H-2^d, H-2^{bxd} and H-2^{bxd}→H-2^{bxd} responder animals. Type A clones require lysine-containing peptide EYK(EYA)$_5$ and Type B clones use non lysine-containing peptide (EYA)$_5$ of Poly-18 for activation. H-2^b T cells from H-2^b→H-2^{bxd} responder animals fail to generate one (Type A) of these two major clonotypes. The H-2^{bxd} environment is permissive for the T cell specificities of both Poly-18 clonotypes (Type A and B) and also provides the determinants for their selection. The absence of Type A clones in the H-2^b→H-2^{bxd} chimeras can therefore only be attributed to the failure of H-2^b T cells to generate the appropriate T cell receptors. These results suggest a genetic hole in the T cell recognition repertoire of H-2^b mice for the lysine-containing epitope of the Poly-18 antigen.

C 546 MOLECULAR CHARACTERIZATION OF HLA-DR AND HLA-DQ ALLELES ASSOCIATED WITH SUSCEPTIBILITY TO HUMAN AUTOIMMUNE DISEASE, A.A. Sinha*, L. Steinman+, and H.O. McDevitt#, Departments of +Neurology, *Microbiology and Immunology, and #Medicine, Stanford University, Stanford, CA 94305

We have used oligonucleotide-primed gene amplification to study the association between HLA class II alleles and autoimmune disease. Pemphigus vulgaris (PV) is a severe autoimmune skin disease highly associated with DR4 and DRw6. We have shown that the nucleotide sequence of the first (and most polymorphic) domain of a new DQ_β allele is found in 13/13 Israeli DRw6 PV patients but only 1/13 DRw6 healthy Israeli controls. This allele differs at only position 57 from two other DQ_β alleles not associated with PV. Further, we show that a shared sequence in the third hypervariable region of the first domain of two common $DR_{\beta 1}$ subtypes of DR4 and DRw6 cannot be the sole determinant of susceptibility to PV. None of 7 DR4+/DRw6+ PV patients had this epitope. We also report a new $DR_{\beta 1}$ first domain sequences from a DRw6 PV patient. The frequencly of this allele in patients and controls is being assessed. Similar studies to identify disease associated class II sequences are underway in patients with multiple sclerosis and rheumatoid arthritis.

C 547 A MALARIA T CELL EPITOPE RECOGNIZED IN ASSOCIATION WITH MOST MOUSE AND HUMAN MHC CLASS II MOLECULES, Francesco Sinigaglia, Maria Guttinger, Jochen Kilgus, Hugues Matile, Howard Etlinger, Dieter Gillessen and J. Richard L. Pink. Central Research Units, F. Hoffmann-La Roche & Co. Ltd., 4002 Basel, Switzerland.

To investigate the interaction of synthetic peptides derived from the P.falciparum Circumsporozoite (CS) protein with HLA class II molecules, we establish a competition assay. One of five CS-peptides comprising together about 50% of the CS-protein sequence was found to compete with the binding of different stimulator peptides to DR5 and DRw6. The competing CS-peptide, CS(378-398), was then shown to be able to induce primary in vitro responses of T cells from donors with different HLA-DR haplotypes.
The analysis of the MHC restriction of CS(378-398)-responsive T cell clones revealed that CS(378-398) associates with at least 7 DR proteins. Furthermore, 6 different mouse strains, genetically unresponsive to the repetitive sequence (NANP) of the CS protein can produce anti-(NANP) antibodies if the $(NANP)_3$ sequence is coupled to the CS(378-398) sequence, implying that peptide CS(378-398) is recognized in association with many different mouse as well as human MHC class II molecules.

C 548 FACTORS MODULATING IMMUNOLOGICAL NON-RESPONSIVENESS AND AUTOIMMUNITY IN INSULIN/T ANTIGEN TRANSGENIC MICE, Jacek Skowronski, Susan Alpert and Doug Hanahan, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. 11724

Transgenic mice harboring the rat insulin promoted SV40 T antigen gene vary in their immune responses to the transgenic protein. Mice of different lineages are heritably either tolerant to the SV40 large T antigen, or not. These two distinct phenotypes are characteristically associated with the developmental timing of the transgene expression. The prenatal presence of T antigen results in the tolerant phenotype. Adult mice of the nontolerant lineages, where T antigen expression is not detectable until 2-3 months of age, spontaneously develop an immune response against T antigen. The frequency of these autoimmune responses is determined genetically, and is essentially 100% in the projeny derived in crosses of transgenic mice to some inbred strains of mice (e.g. those of $H\text{-}2^d$ haplotype), and yet only 10% in others (e.g. $H\text{-}2^b$).
Recently, we initiated an analysis of the immunological nonresponsiveness in two lineages of insulin/T antigen transgenic mice with early onset of expression of the transgene (RIP-1 Tag #2 and RIR Tag #2). While the presence of the transgenic protein during the prenatal/neonatal period is the primary requirement for the nonresponsive phenotype, it further appears that the levels of T antigen expression during embryonic development quantitatively influence the subsequent nonresponsiveness in adult life. An additional modification is exerted by a genetic component which seems to map to the mouse major histocompatibility complex (H-2). Interestingly, it appears that the $H\text{-}2^d$ haplotype, which is associated with the most complete impairment of immune responses when T antigen is expressed prenatally, is also associated with the highest frequency of autoimmune responses when the developmental expression of the transgene is delayed. This correlation is considerably strengthened by the observation that outcrosses to C57B1/6J ($H\text{-}2^b$) produce mice with either a weaker tolerance in the case of the early expressors, or a less frequent and weaker autoimmune response in lines with a delayed onset of expression of the transgene. Thus, the two distinct biological consequences of the presentation of this antigen (tolerance or autoimmunity) are likely to result from differences of the developmental maturity of the immune system, and both effects appear to be similarly MHC restricted.

C 549 Mapping the NZB genetic contribution to lupus-like autoimmune disease. Pam Smarnwarawong[1], Eva Eicher[2], Brian Kotzin[1] and Ed Palmer[1]
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NZB mice carry a gene(s) that contributes to the lupus-like autoimmune disease seen in (NZW X NZB) F1 hybrids. However, the precise locus of this gene has yet to be determined. To map its location, we are breeding a panel of NZBxSM/J recombinant inbred (RI) with NZW mice. By determining which of the RI strains produce F1 animals that develop autoimmunity, the location of this NZB gene can be deduced based on the strain distribution pattern of the known genetic markers among the NZBxSM/J RI strains.

C 550 THYMUS SELECTION PROCESS INDUCED BY HYBRID ANTIBODIES, Fred Zepp and Uwe D. Staerz, Basel Institute for Immunology, Grenzacherstr. 487, Basel, Switzerland.
The peripheral T lymphocyte repertoire is skewed towards recognition of antigen in context of self-MHC and towards tolerance to self-antigens. During T lymphocyte development in the thymus, this repertoire is formed by the interaction of T cell antigen receptor (TCR) with MHC molecules resulting in positive and negative selection phenomena. It should be possible to mimic selection processes in normal animals with hybrid antibodies (HAb) that specifically link members of a TCR family to MHC molecules on the thymic stroma. Here, we present our observations probing T lymphocyte development with HAb linking $V_{\beta 8}$ positive TCR to either class I or class II MHC products in thymic organ culture. Thymocytes exposed to either HAb in an early stage of maturation respond with significant increase in the frequency of $V_{\beta 8}$ positive thymocytes are depleted. These results illustrate the succession of positive and negative selection in the developing thymus of normal mice.

C 551 MACROPHAGE SECRETED PGE_2 POTENTIATES IMMUNE COMPLEX-INDUCED UNRESPONSIVENESS OF SMALL DENSE B CELLS. Sidney H. Stein and Richard P. Phipps. Cancer Center and Depts. of Microbiology and Immunology and Dental Research, University of Rochester, School of Medicine and Dentistry, Rochester, New York 14642. We previously demonstrated that immune complex (IC) pulsed macrophages delivered a potent negative signal to B cells, which resulted in B cell unresponsiveness to an immunogenic challenge. The negative signal consisted of two components: an antigen specific one supplied by the IC (composed of a monoclonal anti-fluorescein (FL) antibody (AF1-9) and FL-keyhole limpet hemocyanin) and products of macrophage arachidonic acid metabolism. The purpose of this study was to examine if specific populations of B cells differed in their sensitivity to IC-induced unresponsiveness and if prostaglandins could potentiate the effect. Purified B cells were prepared by depleting spleen cells of adherent cells and T ymphocytes. The B cells were then fractionated on a percoll gradient to obtain small dense "resting" B cells and large "activated" B cells. The small dense B cells demonstrated increased inhibition at every dose of IC examined, when compared to large B cells. For instance, pulsing with 10 ug of IC resulted in a 96% reduction in anti-FL, but not anti-trinitrophenyl, plaque forming cell (PFC) response for small dense B cells. In contrast, the PFC response of large "activated" B cells was reduced only 66%. In addition, when subtolerogenic doses of IC were used (10 ng) prostaglandin E_2 ($5X10^{-8}M$) could function as a potent secondary negative signal rendering these B cells susceptible to negative signalling. We concluded that small dense B cells are more sensitive to IC induced unresponsiveness and that prostaglandin E_2 may further sensitize B cells along this pathway. This work was supported by CA-42739 and K16 DE 00159-03.

C 552 TRIGGER MECHANISM FOR LYTIC FACTOR RELEASE FROM NK AND RESTING T LYMPHOCYTES, Joan Stein-Streilein and Judy Guffee, Department of Medicine, University of Miami School of Medicine, Miami, FL 33101.

Moab G1.4 and D7.5 were developed in the mouse against antigens present on humans, hamster and rat NK and T lymphocytes. Pre-incubation of lymphocytes with Moab did not block the ability of the NK cells to bind to targets but did block their ability to lyse NK sensitive targets. The pre-incubation media contained lytic factors that lysed NK sensitive targets in a 20 h chromium release assay. Interestingly pre-incubation of thymus cells or resting peripheral T lymphocytes with the Moab induced the T lymphocytes to release lytic factors and acquire NK activity against NK-sensitive targets. Lytic factors and acquired lytic activity were unable to lyse LAK targets. Signalling events did not involve cross linking or Fc receptor participation since factors were released during incubation of the lymphocytes with Fab fragments of either Moab. Immunoprecipitation and western blot analysis using the Moabs defined a molecule of 27 kD on the surface of thymus or lung mononuclear cells. Isolation and amino acid sequence analysis is being done to assist in the characterization of the G1.4 and D7.5 antigens. Moab made to the idiotypic determinants on Moab G1.4 and D7.5 bound to NK sensitive targets and not to NK-insensitive targets. Moabs G1.4 and D7.5 and their respective anti-idiotype Moabs may define the actual molecules involved in signals for lysis by the NK effector cells and their targets. The reason for the expression of a functional trigger molecule for NK lytic factors on T lymphocytes is unknown but may suggest that the TrgS expression and function precedes thymic differentiation. Supported in part by NIH HL 33372 and HL 33709

C 553 THE POTENTIATION OF LEVELS AND AFFINITY OF ANTIBODY RESPONSES TO SYNTHETIC ANTIGENS, Michael W Steward, Colin R Howard and Graeme P Holland, Immunology Unit, Department of Clinical Sciences, London School of Hygiene and Tropical Medicine, London WC1E 7HT, United Kingdom.

There is an urgent need for the development of new approaches to immunization which result in the enhancement of the immunogenicity of synthetic antigens with potential as new vaccines.In addition to potentiating antibody levels, these new approaches should also result in an enhancement in the affinity of the antibody response since the biological effectiveness of high affinity antibody is superior to that of antibody of lower affinity.

We have investigated a number of substances with adjuvant potential in terms of their ability to enhance both levels and affinity of antibody to synthetic antigens in mice both individually and in combination in order to look for possible synergistic effects.Furthermore, we have also studied the effect on these two parameters of the humoral immune response of different routes of immunization.Particular emphasis has been placed on exploring the efficacy of the oral route for the induction of systemic antibody responses to synthetic antigens.

C 554 Bacterial Polysaccharides Bound to Proteins by Heterobifunctional Thiolating Agents. Shousun C. Szu, Chiayung Chu and John B. Robbins. National Institute of Child Health and Development, NIH

Capsular polysaccharides (CPS) and the O-specific side chains (O-SSC) of LPS are virulence factors and protective antigens of invasive bacteria. CPS have limitations as vaccines because of their age-related and T-dependent immunogenicity. O-SSC are too small to be immunogenic. In order to increase their immunogenicity in infants and to confer T-dependent properties, synthetic schemes for forming conjugates of polysaccharides (PS) with proteins by controlled formation of disulfide bonds between tetanus and diphtheria toxoids were devised.

Toxoids were thiolated with N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), a heterobifunctional reagent. PS with carboxyls were thiolated by carbodiimide-mediated (EDAC) amide formation with cystamine. PS without carboxylic acids but with vicinal hydroxyls, were activated with CnBr and thiolated with cystamine or reacted with adipic acid dihydrazide (ADH). ADH was reacted with reducing end groups of O-SSC by reductive amination. The activated PS wase either thiolated by reacting with cystamine or with ADH. The hydrazide derivatized PS were thiolated through EDAC activation of 3-3'-dithiopropionic acid. Conjugates were formed by combining the thiolated PS with SPDP-toxoids. Several CPS and O-SSC protein conjugates enhanced polysaccharide antibody responses in mice and primates. Re-injection showed a booster polysaccharie antibody response. The conjugates elicited antibodies with neutralizing activities to the toxoid carriers.

C 555 INDUCTION OF CELL SURFACE T CELL RECEPTOR FOLLOWING CULTURE OF CD4-, CD8$^-$ THYMOCYTES WITH IL 1 + IL 2. Tohru Takashi, Alfred D. Steinberg, and William C. Gause. NIAMS, National Institutes of Health, Bethesda, MD 20892

We have been studying in vitro activation of CD4$^-$, CD8$^-$ thymocytes. In the present studies, highly purified double negative thymocytes were cultured with IL 1 with or without IL 2. Following a 72 hour culture with IL 1 + IL 2 (in the absence of mitogens) significant proliferation was detected, although all cells remained CD4$^-$, CD8$^-$. The frequency of cells staining for CD3 cell surface protein increased from 10% to 60% and TCR $V_{beta}8$ from 4% to 40%. FACS sorted CD3$^-$ cells from the CD4$^-$, CD8$^-$ population showed similar increases in cell surface TCR suggesting differentiation. Northern blot analyses showed an increase in c-myc RNA at 24 hours, no change in CD3 delta or epsilon expression at 24, 48, and 72 hours, and a marked increase in the ratio of 1.3 to 1.0 TCR beta transcript between 24 and 48 hours. These data suggest that IL 1 + IL 2 can induce CD3 cell surface protein expression on progenitor thymocytes. Furthermore, this induction is not associated with increased CD3 transcription, but rather is associated with an increased proportion of functional TCR beta transcripts.

C 556 CORRELATION BETWEEN PHYSICO-CHEMICAL PREDICTIONS AND B OR T-CELL EPITOPES LOCALIZATION IN A VACCINATING PROTEIN

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The P28 antigen of *Schistosoma mansoni* has been shown to be able to induce protective immunity against schistosomiasis in rodents and primates. Synthetic peptides were used to localize the major B and T-cell epitopes of this protein. The primary structure of the P28 molecule was analyzed using various predictive algorithms :
- surface localization, hydrophilicity, flexibility, secondary structure, amphipathicity of predicted α-helical regions, Rothbard pattern.
According to these criteria, several peptides were synthesized and tested in immunological assays *in vitro* and *in vivo*.
Correlations between these results and the physico-chemical predictions will be shown.

C 557 MAPPING OF H-2D^b RESTRICTED SV40 T ANTIGEN EPITOPES BY SYNTHETIC PEPTIDES Satvir Tevethia[1], Yuetsu Tanaka[1], Robert Anderson[2], and W. Lee Maloy[2]; [1]Department of Microbiology, Penn State College of Medicine, Hershey, PA 17033; and [2] Biological Resources Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892.

Simian virus 40 (SV40) T antigen is recognized by cytotoxic T lymphocytes (CTL) in association with class I major histocompatibility antigens.We have identified four distinct antigenic sites on SV40 T antigen defined by H-2D^b restricted CTL clones. We have therefore attempted to map these antigenic sites precisely by using overlapping synthetic peptides corresponding to SV40 T antigen amino acids. The synthetic peptides of 15-20 amino acid length were incubated for five hours with ^{51}Cr labelled-non-SV40 transformed H-2^b cells in the presence of CTL clones specific for each of the four antigenic sites; and the released radioactivity was used as a measure of specific recognition of synthetic peptides by the CTL clones. The results demonstrated that the antigenic site I resides between amino acids 205-219; sites II and III between amino acids 220-233. Site III can be separated from site II as it was found to reside between amino acids 225-239. The antigenic site V was localized between amino acids 489-503. These results show that SV40 T antigen is the target protein for CTL and the regions of T antigen recognized by CTL are tightly clustered in the amino terminal half. Attempts to identify amino acids that selectively interact with the class I antigen and the T cell receptor are in progress.

C 558 SPECIFIC RESPONSE OF T-LYMPHOCYTES SUBSET TO TUMOR-TISSUE EXTRACTS. M. Deutsch, R. Tirosh, A. Weinreb and S. Chaitchik * Department of Physics, Bar Ilan University, Ramat Gan, Israel and *Department of Onclology, Tel Aviv Medical Center, Israel. Peripheral blood lymphocytes of cancer patients demonstrate an early humoral and specific response to application of tumoral extracts. The response is measured as a change in the spectrum of fluorescence polarization of fluorescein molecules. This is probably a measure of sol-gel transformations induced in the cytoplasm upon cellular activation of resting lymphocytes. This measurement is carried by epifluorescent microscopy on single lymphocytes, sitted on a regular holes matrix that is scanned under computer control. It is possible to follow changes within each cell in a population of upto ten thousand cells. Since the cells are sitted safely in their holes, it is possible to rinse them with various reagents and follow the kinetics of individual responses by repeated scanning. Further studying of the stimulative process might be useful in identifying the responding lymphocytes and the tumoral reagents. This test of the immune system is intended for early and specific detection of cancer and for follow-up of immuno-therapeutic manipulations as well.

C 559 IMPLICATION OF A UBIQUITOUS NUCLEAR PROTEIN IN TRANSMITTING SIGNALS FROM THE T CELL RECEPTOR TO THE INTERLEUKIN-2 GENE. Katharine S. Ullman, J.P. Shaw, Elizabeth Emmel, and Gerald Crabtree. Stanford University, Stanford, CA 94305.

Following antigenic stimulation, T cells undergo an orderly series of changes that result in cell division and immunologic function. In hopes of working backward in the cascade which links the membrane events to the genes essential for these differentiated functions, we have begun an analysis of the nuclear proteins responsible for gene activation in stimulated T cells. In previous studies, using a panel of internal deletion mutations of the IL-2 enhancer, we found that when the region from -73 to -82 was mutated, the level of induction dropped 20-fold. This region is protected in a DNAase footprinting assay by a protein, NF-IL2-A. Although the protection is seen with nuclear extracts from both stimulated and resting Jurkat cells, in vivo this is a site of DNAase hypersensitivity found only in activated Jurkats and peripheral blood T cells. A tetramer of the protected site directs transcription from an unrelated promoter in Jurkat cells only when the cells are stimulated by lectin or antibodies to the antigen receptor. Furthermore this activation can be blocked by 1 ng/ml of cyclosporin A. As a means of detecting more subtle changes in protein binding following stimulation, the methylation-interference patterns of stimulated and non-stimulated Jurkat cell nuclear extracts were compared and found to be identical. We propose that though the DNA-binding protein, NF-IL2-A, is present constitutively, it becomes functional only following specific signalling through the antigen receptor. Further characterization of this protein will help to clarify its particular role in the activation of IL-2 expression following T-cell stimulation.

C 560 SEQUENCES FLANKING THE MINIMAL IMMUNODOMINANT PEPTIDE IN STAPHYLOCOCCAL NUCLEASE EXERT NEGATIVE EFFECTS ON T CELL RECOGNITION. Melanie S. Vacchio, Alison Finnegan*, Jay Berzofsky†, John A. Smith@, and Richard J. Hodes, Experimental Immunology Branch, NIH, Bethesda, MD., *Dept. of Rheumatology, Rush Medical School, Chicago, IL., †Metabolism Branch, NIH, Bethesda, MD., @Harvard Medical School, Boston, MA. Although peptides have been utilized extensively to characterize the minimal essential immunodominant sites on model protein antigens, little work has focused on the effect that sequences flanking these minimal recognition sites may exert on T cell recognition. Previous work with Staph. nuclease demonstrated that I-E^k restricted clones recognize the peptide 81-100 while I-A^b restricted clones recognize the overlapping peptide 91-110, and that there is no crossreactivity in recognition of these two peptides. Further analysis with truncated peptides reveals that the minimal peptide recognized by I-E^k restricted clones is 91-100. Addition of residues 86-90 (i.e. to give the peptide 86-100) enhances recognition substantially, whereas addition of residues 101-105 produced a 91-105 peptide with no detectable stimulatory ability. Single amino acid substitutions within 91-105 produced peptides which induce responses comparable to to those seen with 91-100. These results suggest that interactions between the antigenic 91-100 peptide and residues in the flanking 101-105 sequence have negative consequences for presentation of the immunodominant epitope to T cells. Amino acid substitutions which remove these negative interactions lead to restoration of stimulatory ability. The negative effect of flanking sequences on T cell recognition of immunodominant sites presents new considerations for development of synthetic vaccines as well as for understanding the biology of antigen processing and presentation.

C 561 STUDIES ON THE ROLE OF PROTEIN KINASE C ACTIVATION IN HUMAN T-CELL TRIGGERING, Rene van Lier, Miranda Brouwer, IJsbrand Kramer, Lucien Aarden and Arthur Verhoeven
Central Lab. Blood Transf.Service, Lab. Exp. and Clin. Immunology of the Univ. of Amsterdam, The Netherlands
The hydrolysis of phosphatidylinositol-diphosphate and the formation of inositol-triphosphate (IP3) and diacylglycerol (DG) are among the first events that can be monitored after T-cell triggering. IP3 mobilizes Ca^{2+} from intracellular stores, whereas DG is the physiological activator of Protein Kinase C (PKC). Although it is suggested that activation of PKC is essential in the induction of mitogenic T-cell responses, no direct evidence for this hypothesis has been presented yet. In this study we used two novel PKC inhibitors, i.e. 1-alkyl 2 methyl-glycerol (AMG) and staurosporine to assess the role of PKC activation in human T-cell stimulation. We observed that the inhibitors did not interfere with the anti-CD3 or anti-CD2 monoclonal antibody (mAb) induced Ca^{2+} rises in peripheral blood T cells, whereas cytoplasmatic alkalinization initiated by the same stimuli or the phorbol ester PMA was strongly reduced. AMG as well as staurosporine gave a dose dependent inhibition of both Interleukin 2 (IL-2) production in Jurkat cells and T-cell proliferation. In contrast, the induction of IL-2 receptor expression was highly resistent to the action of both compounds. It was shown that anti-CD28 Mab were able to partly overcome the action of both inhibitors. These studies support the important role of PKC-activation in human T-cell triggering. Moreover, since anti-CD28 mAb were able to counteract the action of the PKC-inhibitors, it is suggested that mitogenic stimuli generated via different T-cell membrane molecules may use distinct intracellular signal transduction pathways.

C 562 RESPONSE OF HUMAN T LYMPHOCYTE LINES TO MYELIN BASIC PROTEIN: ASSOCIATION OF DOMINANT EPITOPES WITH HLA CLASS II RESTRICTION MOLECULES, Arthur A. Vandenbark, Yuan K. Chou, Margarita Vainiene, Ruth Whitham, Dennis Bourdette, Selene C.H.-J. Chou, George Hashim and Halina Offner, Neuroimmunology Research 151D, Veterans Administration Medical Center, Portland, OR 97201. In animals, the selection in vitro of T cell lines to myelin basic protein (MBP) can define immunodominant and encephalitogenic epitopes which are preferentially associated with Class II major histocompatibility (MHC) molecules of each strain. These principles were used to evaluate the specificity and MHC restriction of fourteen human MBP-reactive T cell lines selected from normal individuals and patients with multiple sclerosis (MS) or other neurological diseases (OND). The four normal T cell lines recognized single, separate immunodominant MBP epitopes which were restricted by HLA molecules from the DR or in one case the DP Class II locus. In contrast, the MS and OND T cell lines recognized multiple MBP epitopes, each in association with a discrete Class II MHC molecule from the DR or DQ locus. Overall, HLA-DR molecules were used preferentially to associate with epitopes on human MBP, restricting 26/33 responses. Thus, as predicted from animal studies, T cells from each individual responded to different immunodominant epitopes on human MBP in association with distinct MHC Class II molecules. These data provide the first evidence of genetically restricted human T cell recognition of potentially encephalitogenic epitopes of MBP.

C 563 UNRESPONSIVENESS TO A FOREIGN ANTIGEN CAUSED BY TOLERANCE TO SELF ANTIGEN

Damir Vidović and Polly Matzinger, Basel Institute for Immunology, CH-4005 Basel. In mice, two sets of genes govern the immune response to the synthetic antigen GT. One maps to the MHC and behaves like a typical immune response (Ir) gene. The second is a background gene encoding a cell surface structure found on B cells. Mice which express (and are therefore tolerant of) one form of this structure do not respond to GT. Thus tolerance of self generates holes in the T cell repertoire, partially crippling the immune system. The significant difference between the frequencies of specific anti-GT-TCR-bearing T cells in responder and nonresponder mice suggests clonal deletion as a mechanism of immune tolerance.

C 564 MOLECULAR DEFINITION OF A MINOR HISTOCOMPATIBILITY ANTIGEN OF MITOCHONDRIAL ORIGIN, Chyung-Ru Wang, Bruce Loveland, Hiromichi Yonekawa, Evan Hermel and Kirsten Fischer Lindahl, Howard Hughes Medical Institute, Departments of Microbiology and Biochemistry and Immunology Graduate Program, Univ. of TX Southwestern Med. Ctr., Dallas, TX 75235-9050.

The maternally transmitted antigen (Mta) of mice consists of two components: a MHC class I-like restriction molecule (Hmt), encoded distal to H-2 on Chr. 17, and the maternally transmitted factor (Mtf) whose inheritance implies a mitochondrial origin. Four alleles of Mtf (α, β, γ, & δ) have been identified with antigen-specific cytotoxic T cells. Three of the mtDNA genomes were sequenced (the β, γ, & δ types) and compared with the published L cell (α type) mtDNA sequence. A candidate antigen sequence was found at the N-terminus of the protein, a 30 kd subunit of NADH dehydrogenase. Each of these highly conserved hydrophobic sequences had a different residue at position 6. Three peptides of α type (residues 4-26, 1-26, & 1-17) and one of β-type (residues 1-17) were synthesised and tested for antigenicity by incubating them overnight with target cells before adding Mtf antigen specific cytotoxic T cells. Three of the peptides behaved as Mtf gene products. After incubation with ND1α1-26 or α1-17, targets of β phenotype were lysed by anti-α killer cells. The antithesis was also observed: α phenotype targets were lysed by anti-β T cells after incubation with ND1β1-17. ND1α4-26 was not recognised. We conclude that the Mtf antigen is derived from a mitochondrial gene product, a hydrophobic peptide from N-terminus of ND1. The alleles are created by amino acid substitutions at position 6. This is the first molecular definition of a minor histocompatibility antigen, and of a unique antigen of mitochondrial origin.

C 565 LTac: A NOVEL HUMAN T CELL-SPECIFIC LATE ACTIVATION ANTIGEN. Peter Wang, Carol Clayberger, and Alan Krensky. Department of Pediatrics, Stanford University Medical Center, Stanford, CA 94305.

We have developed a monoclonal antibody that recognizes an apparently T cell-specific antigen, LTac, expressed only in the late stages of T cell activation. When peripheral blood lymphocytes are activated with allogeneic stimulators or PHA and examined by FACS, LTac is expressed only after day 6 or 7. LTac is expressed by all T cell clones tested, both $CD4^+$ and $CD8^+$. A wide range of cell types were tested and found negative for LTac expression, including B cells, fibroblasts, endothelium, and neural cells; thus LTac expression seems to be restricted to the T cell lineage. Immunoprecipitation of cell-surface labelled proteins and SDS-PAGE revealed a 160 kD band under reducing conditions and three bands of 240, 180, and 160 kD under non-reducing conditions. LTac is distinct from all other reported T cell activation antigens on the basis of its pattern of expression and molecular weight. We have purified LTac protein using wheat germ agglutinin-agarose chromatography followed by monoclonal antibody-agarose chromatography, and have used it to produce an antiserum suitable for screening bacterial cDNA expression libraries. The regulation and function of molecules expressed in the late stages of T cell activation is an important frontier in understanding the immune system, and reagents such as monoclonal antibodies may have important diagnostic and therapeutic uses.

C 566 T CELL RECEPTOR REPERTOIRE SPECIFIC FOR BEEF INSULIN IN BALB/C MICE, Joan Wither, Laurie Phillips,* Terry Delovitch,* Nobumichi Hozumi, Mount Sinai Hospital Research Institute and Department of Immunology, University of Toronto, Toronto, Canada M5G 1X5,* Banting and Best Institute of Medical Research, Toronto.

Balb/c Mice ($H\text{-}2^d$) are high responders to beef insulin (BI). To examine the TCR repertoire to this small soluble antigen we have generated a panel of 28 T cell hybridomas reactive to BI obtained from 4 independent fusions. The hybrids can be divided into 3 groups based upon their patterns of reactivity to beef, sheep and pork insulins, BI peptides, and allogeneic cells. Group I hybrids are 2 to 5 fold more reactive to beef than sheep insulin and exhibit little or no reactivity to pork insulin. Group II hybrids respond equally well to beef and sheep insulins, while Group III hybrids are reactive to beef, sheep and pork insulins. Our preliminary molecular analysis of TCR gene usage has revealed that Vβ8.3 is the dominant gene utilized in all 3 groups. In contrast, α gene usage appears to be more restricted. Vα3.2 (previously isolated from a BI/A^b restricted hybridoma AF.3.G7) was found only in Group I hybrids, where approximately 40% of the hybrids used this gene. Sequence data will be presented and the significance of the results will be discussed with reference to the structure of the TCR - antigen - MHC complex. This work was supported by the MRC of Canada.

C 567 ALTERED IMMUNE RESPONSES IN MICE WITH A LARGE DELETION IN THE T CELL RECEPTOR β LOCUS.

David Woodland, Brian Kotzin and Ed Palmer, Basic Sciences Division, Department of Pediatrics, National Jewish Center for Immunology and Respiratory Medicine, Denver, Co 80206.

The T cell receptor (TCR) β locus of New Zealand White (NZW) mice contains an 8.8kb deletion which encompasses the Cβ1, Dβ2 and Jβ2 genetic elements. Theoretically, NZW mice are capable of generating only 30% of the TCR diversity of other mouse strains. To experimentally assess the affect of this deletion we have bred the NZW TCR β allele onto a BALB/c background and tested the ability of this new congenic strain (BALB.H.β7) to respond to a panel of 24 random antigens. BALB.H.β7 mice were capable of responding to all 24 antigens tested but the magnitude of the response to some of these antigens (11 of 24 antigens) was reduced 2-5 fold when compared with BALB/c mice. Responses to the remaining antigens were either comparable (11 of 24 antigens) or occasionally even enhanced (2 of 24 antigens) compared to BALB/c mice. These results suggest that Jβ2 and Dβ2 elements are required to maintain the strength of the T cell repertoire and that, in the wild, mice carrying this deletion could be at a significant selective disadvantage.

C 568 THYMIC STROMAL CELLS ARE EFFICIENT ANTIGEN PRESENTING CELLS BUT DO NOT STIMULATE AUTOREACTIVE T CELL LINES IN VITRO, Maurice Zauderer, Kannan Natarajan and Dvora Burstyn, Cancer Center, University of Rochester Medical School, Rochester, NY 14642

We have compared the ability of thymic stromal cells to stimulate proliferation of self Ia-specific autoreactive T cells and to present antigen to Ia-restricted, antigen-specific T cell lines. We determined that thymic stromal cells present antigen more efficiently than similarly treated spleen cells to cloned antigen-specific T cell lines. In sharp contrast, spleen cells but not thymic stromal cells efficiently stimulate proliferation of autoreactive T cell lines.

Several factors could account for the failure of thymic stroma to stimulate autoreactive T cells. 1) Thymic stroma may induce tolerance in Ia-specific T cells. 2) Autoreactive T cells may be specific for Ia in association with other self molecules that are not expressed on thymic epithelial cells. 3) Activation requirements for autoreactive T cells may be different than for antigen-specific T cells.

It does not appear that tolerance induction can account for the failure of thymic stroma to stimulate autoreactive T cells since stimulation of autoreactive T cell lines with thymic stroma does not suppress the response to syngeneic spleen cells. Preliminary experiments indicate that the determinants recognized by autoreactive T cells are expressed on the thymic stroma and suggest that the failure to stimulate autoreactive T cells is due to a requirement for additional activation signals.

Vaccine Design; Accessory Molecules for T Cell Activation; T Cell Activation at Different Stages of Development/Maturation; Regulatory Effects on Immunogenicity; The Immune Response in Bacterial, Viral and Parasitic Infection

C 600 CYTOTOXICITY LINKED TO A POLYSPECIFIC MONOCLONAL ANTIBODY TO GROUP A STREPTOCOCCI: DO AUTOANTIBODIES AGAINST INFECTIOUS AGENTS PLAY A ROLE IN AUTOIMMUNE DISEASE?

Susan M. Antone, Daron G. Street and Madeleine W. Cunningham, Department of Microbiology and Immunology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190.

Autoantibodies to heart may be produced in response to streptococcal infection and may result in the development of rheumatic carditis. We have studied the specificity of human and murine monoclonal antibodies (mAbs) that cross reacted with group A streptococcal and human heart proteins in an attempt to identify the antigens responsible for the cross reactivity. Polyspecific mAb 36.2.2 was found to react with the alpha-helical coiled-coil tail region of myosin and with actin and keratin. In this study we investigated the cytotoxicity of our mAbs (all IgM_κ) for cultured rat heart cells and human fibroblasts in ^{51}Cr release assays. mAb 36.2.2 was highly cytotoxic for rat heart cells (0.63 μg/ml) but not for fibroblasts in the presence of complement. Two other mAbs were cytotoxic for the heart cells at immunoglobulin concentrations of ≥20-40 μg/ml. Concentrations up to 150 μg/ml were tested on fibroblasts with little to no effect. These data suggest that mAb 36.2.2 which reacts with intracellular alpha-helical proteins was effective at interacting with the surface of the heart cell. To further substantiate this hypothesis, mAb 36.2.2 was reacted with unfixed heart cells and found to bind to their surface as detected by indirect immunofluorescence. Other mAbs equally as reactive with streptococcal proteins, myosin or actin or DNA did not react as well with the surface of the heart cells and were not highly cytotoxic. These data support the hypothesis that infectious agents such as group A streptococci activate B cell clones which may then produce potentially harmful autoantibodies.

C 601 **ACCESSORY CELL FUNCTION: COMPARATIVE STUDIES ON THE SYNTHESIS AND RELEASE OF BIOACTIVE IL-1α AND IL-1ß.** H. Ulrich Beuscher, Christian Günther, Ivan G. Otterness* and Martin Röllinghoff, Institute for Clinical Microbiology, University of Erlangen-Nürnberg, 8520 Erlangen, F.R.G. and the *Department of Immunology and Infectious Diseases, Pfizer Inc., Groton Conn. 06340, U.S.A.
The identification of Il-1 was carried out in radiolabeled murine macrophages by immunoprecipitation and SDS-PAGE, using antibodies to Il-1α and Il-1ß protein. Cell associated Il-1α and Il-1ß were predominately of M_r 31.000 and 37.000, respectively. As assessed by estimating radioactivity in each band, Il-1α was about 4 fold more abundant than Il-1ß. Most of the cell associated Il-1 activity, as measured by the D10.G4.1 proliferation assay, could be inhibited by anti Il-1α whereas no inhibitory effect was observed with anti Il-1ß. After removal of Il-1α from cell lysates by immunoadsorbtion the residual activity accounted for only about 5 % of the total Il-1 activity, and was completely neutralized by anti Il-1ß. However, in culture supernatants anti Il-1ß reduced the Il-1 activity by approx. 50 % whereas a combination of both, anti Il-1α and anti Il-1ß completely neutralized the activity to background levels. Interestingly, only one radiolabeld polypeptide of M_r 37.000 was precipated from the extracellular medium by the anti Il-1ß antibody. These data therefore suggest, that externalization is required in order to generate bioactive Il-1ß molecules, but apparently a proteolytic processing step of the precursor is not involved. Moreover, in view of previous reports, demonstrating that cellular interactions during antigen presentation can be mediated via a membrane form of Il-1, our experiments substantiate the hypothesis that membrane-associated, biologically active Il-1 is predominantely Il-1α.

C 602 AN ALTERNATIVE TO CARBODIIMIDES FOR PREPARING IMMUNOGENIC CONJUGATES David L. Brandon and Joseph W. Corse, USDA Agricultural Research Service, Western Regional Research Center, Albany, CA 94710
In order to prepare antibodies which would recognize O-glucosides of cytokinins (purine phytohormones), we investigated an alternative to carbodiimide reagents. Carbodiimides induce the formation of amide bonds, and thus can be used to couple haptens containing carboxyl or amino groups to proteins. The use of water-soluble carbodiimides is limited by the sparing solubility of some haptens in water and by the formation of the intermediate O-acylisourea, which can be less soluble than the hapten and which can itself act as an unwanted hapten. To overcome these problems, we have used 2-morpholinoethyl isocyanide and a suitable catalyst to effect amide bond formation. These reagents are the basis of recently developed chemical procedures for peptide synthesis (Aigner et al., 1980). The reaction conditions permit coupling compounds which are soluble or insoluble in water to proteins in a simple, homogeneous system. We have demonstrated that this procedure is widely applicable for preparing enzyme-labeled and immunogenic conjugates of cytokinins and other biologically relevant compounds with an available carboxyl group. We hypothesize that conjugates made with these reagents will not induce the allergic responses and unwanted crossreactivities associated with the use of carbodiimides.
Supported, in part, by BARD Grant US-711-83.

C 603 MAPPING OF A SIGNIFICANT B-CELL EPITOPE IN THE MAJOR MEROZOITE SURFACE ANTIGEN OF A MURINE MALARIAL PARASITE, James M. Burns, Jr., Thomas M. Daly, and Carole A. Long,
Malaria Research Group, Dept. of Microbiology, Hahnemann University, Philadelphia, PA 19102
The 195 kDa major merozoite surface antigen of *Plasmodium falciparum* (Pf PMMSA) is a potential candidate for the development of a blood-stage malarial vaccine. We have focused on an analogous 230 kDa antigen of the murine malarial parasite, *Plasmodium yoellii*, as a model vaccine antigen. Previously we reported the cloning and sequencing of a 2.1 kb portion of the gene encoding the C-terminal 77 kDa of the Py PMMSA, containing the B-cell epitope recognized by a passively protective monoclonal antibody, McAb 302. To localize this B-cell determinant, we have produced recombinant peptides of 74, 40, 34, 17, and 10 kDa which bear the epitope recognized by McAb 302. The results indicate that this B-cell epitope is located within the most carboxyl region of the Py PMMSA which contains a series of ten cysteine residues, also found in the PMMSA of *P. falciparum*. Reduction of disulfide bonds as well as the deletion of Cys-607 of the cloned sequence, results in the loss of the expression of this epitope. The localization of this immunologically relevant B-cell epitope to a region of the molecule displaying considerable homology with the Pf PMMSA, may impact on the development of an effective blood-stage vaccine. (Sponsored by NIH grant AI21089 and WHO grant T16-181-M2-36C.)

C 604 HUMAN IL-2, NOT MOUSE IL-2, STIMULATES FUNCTIONALLY-IMMATURE THYMOCYTES TO SECRETE IL-4 AND BECOME CYTOTOXIC. Priscilla A. Campbell, Kim E. Stedman and Louis B. Justement, Department of Medicine, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO 80206. Human rIL-2, not mouse rIL-2, induces a functionally-immature subset of mouse thymocytes to secrete IL-4, whichin turns induces cells within this subpopulation to become cytotoxic. Normal mouse thymocytes become CTL in response to Con A ± IL-2, and that the CTL responses induced by these agents are blocked completely by the addition of the anti-IL-4 mAb 11B11. In addition, normal mouse thymocytes produced IL-4 in response to Con A ± IL-2. The functionally-mature lobster agglutinin (LAg1)-positive thymocyte subset responded to Con A ± IL-2 in a similar manner. When the functionally-immature subset of LAg1-negative thymocyte subsets was incubated with Con A ± mouse rIL-2, they did not become CTL and did not produce IL-4. However, when LAg1-negative thymocytes were cultured with Con A ± human rIL-2, cells within this subpopulation developed good CTL activity and also secreted IL-4. These data demonstrate that CTL generation and IL-4 production occur concomitantly in all thymocyte populations studied. Moroever, stimuli which fail to induce CTL generation also fail tostimulate endogenous IL-4 production. These experiments indicate that IL-4 is a required factor for all mouse thymocytes to differentiate into polyclonal CTL.

C 605 HETEROGENEITY OF HUMAN T CELL CLONES SPECIFIC FOR THE SURFACE PROTEIN ANTIGEN (SPA) OF TYPHUS GROUP RICKETTSIAE. M. Carl and G.A. Dasch, Naval Medical Research Institute, Bethesda, Maryland 20814

Immunity to the typhus group of rickettsiae is largely dependent on the effector function of several classes of T lymphocytes including those which produce gamma interferon. Since the surface protein antigen (SPA) derived from typhus group rickettsiae has been shown to be an effective immunogen in animal models, human T cell clones specific for the SPA of Rickettsia typhi were isolated and tested for their antigenic specificity as well as for their ability to produce gamma interferon. Eighteen CD4-positive clones specific for the SPA of R. typhi exhibited considerable diversity in their response to the SPA's derived from two strains of R. prowazekii and from R. canada. The vast majority of clones also recognized the SPA's from R. prowazekii but not from R. canada. Two heteroclitic clones demonstrated significantly higher proliferative responses to the SPA's derived from one or both of the of R. prowazekii strains than to the SPA of R. typhi, and one clone demonstrated a significantly higher response to the SPA of R. typhi as compared to the other SPA's. All 18 clones produced gamma interferon in response to SPA stimulation. One of the clones demonstrated significant proliferative responses to a fragment of the SPA produced in E. coli infected with a recombinant lamda gt 11 phage containing a 1 kb fragment of the SPA gene from R. typhi. We conclude that the SPA's from typhus group rickettsiae can elicit both a diverse T cell response in humans as well as the efficient stimulation of gamma interferon-mediated immunity.

C 606 IMMUNOGENICITY OF SELF-ERYTHROCYTES FOLLOWING REMOVAL OF IDIOTYPE-SPECIFIC SUPPRESSOR T CELLS IN NORMAL AND AUTOIMMUNE MICE. Michael J. Caulfield*, Deborah Stanko*, Daniel H. Schulze#, Robert D. Miller, and Catherine E. Calkins+, *Research Institute of the Cleveland Clinic Fndn., Cleveland, OH 44195, #The University of Texas Medical Branch, Galveston, TX 77550, and +Thomas Jefferson University, Philadelphia, PA 19107.

A monoclonal anti-mouse RBC antibody (G-8) has been prepared which appears to represent a pathogenic autoantibody related to those that arise spontaneously in aging NZB mice and which cause autoimmune hemolytic disease (AIHD). This autoantibody recognizes native erythrocytes from mice but not from other species, and Northern blot analysis revealed strong hybridization with the J558 probe but not with the 5 other Vh gene family probes tested. Thus, G-8 is clearly distinct from autoantibodies that recognize bromelain-treated MRBCs and which belong to a unique Vh gene family. When G-8-producing hybridoma cells were grown as tumors in BALB/c mice, the mice developed AIHD characterized by a decrease in the number of erythrocytes (hematocrit) and the development of Coombs-positivity. An anti-idiotypic antibody (E8) was prepared against G-8 and was found to recognize an idiotypic determinant present on most autoantibody forming cells derived from old (Coombs-positive) NZB mice. Previously, we demonstrated that treatment of spleen cells from young NZB mice with G-8 + C or with anti-Ly2 antibody + C resulted in the elimination of suppressor cells and the generation of AFC specific for MRBC. Treatment with a control NZB IgM mAb (G-4) + C or with a rabbit anti-MRBC antiserum + C had no effect on the AFC response to MRBC. Similar deletion of suppressor cells was obtained following treatment of spleen cells from BALB/c mice with G-8 + C. Greater than 80% of the resulting AFC expressed the G-8 idiotype. The results indicate that the response to self-erythrocytes is comprised of autoantibodies that express a recurrent idiotype.

C 607 NATURE OF THE CELL SURFACE RECEPTORS PRESENT ON AN ALLOREACTIVE, RADIORESISTANT POPULATION OF REGULATORY T-CELLS. B. H. Devens, J. A. Kapp, C. M. Sorensen, & D. R. Webb. Syntex Research, Palo Alto, Ca.,94303 Depts. of Pathology, and Microbiology & Immunology, Washington University School of Medicine, St. Louis, Mo. 63110.
During the development of a mixed lymphocyte reaction (MLR) in vitro, a population of radioresistant T-cells can be detected that have the capacity to suppress the development of allospecific CTL to the same stimulatory cells. These allospecific regulatory cells are not themselves cytolytic nor do the block the development of CTL by absorbing growth factors such as IL-2. Recent studies have shown that these regulatory cells may be killed by using monoclonal antibodies directed against antigen specific suppressor factors (TsF) in the presence of complement. These data imply that these cells have suppressor factor like molecules on their cell surface. Current studies are directed at determining whether these regulatory cells also possess CD3 antigens and T-cell receptors. These data show that during the development of alloreactive cells in culture regulatory cells arise that can modulate this process.

C 608 SYNTHESIS OF ANTI-PATHOGEN VACCINES: EFFECT OF MOLECULAR WEIGHT AND EPITOPE DENSITY ON IMMUNOGENICITY.
Howard M. Dintzis, Andrew Lees and Renee Z. Dintzis. Departments of Biophysics and Cell Biology and Anatomy, Johns Hopkins School of Medicine, Baltimore, MD 21205
In an attempt to determine the optimal molecular parameters for the synthesis of anti-pathogen vaccines, we have studied the murine antibody responses to two types of model vaccines. The vaccines were: (1) Purified polysaccharide Type 3 derived from pneumococcal bacteria, and (2) Synthetic conjugates made from a peptide region of the circumsporozoite coat protein of the murine malaria pathogen, P. berghei, coupled with dextran of high molecular weight. In both cases the molecular size was systematically varied, and an optimal size was found to be important in determining the level of immune responsiveness. In the case of the malaria peptide-dextran conjugate, epitope multiplicity (epitope density) was also found to be an extremely important parameter, immunogenicity increasing with increasing epitope density.

C 609 AGE-RELATED CHANGES IN ACTIVATION OF ANTIGEN-SPECIFIC SUPPRESSOR T CELLS. Gino Doria, Laboratory of Pathology, ENEA C.R.E. Casaccia (Rome), Italy. The effects of aging on the cellular and molecular components of the 4-hydroxy-3-nitrophenyl acetyl (NP)-specific suppressor T (Ts) cell circuit were analyzed in vitro using inducer (Tsi), transducer (Tst), effector (Tse) cells and activating factors (Tsi F and Tst F) derived from young or old mice. Activation of Tst cells is age-restricted as it is efficiently induced by Tsi cells only if both cell populations are derived from mice of the same age. Moreover, Tsi F originated from young mice activates Tst cells from young, but not from old, mice. Since age restriction is also involved in stimulation of Tse cells by Tst cells or Tst F, these findings altogether suggest a loss of Tsi, Tst, and Tse cell subsets in old mice. However, triggering of Tse cells by small amounts of Tst F is more efficient when both are derived from old rather than from young mice while the same level of maximum suppression is attained. Higher affinity of the interactions involved in Ts cell activation may compensate for loss of Ts cell subsets in old mice. No age restriction was found for antigen presentation to Tsi cells and for the interaction between Tse cells and target B cells. Thus, the effects of aging on NP-specific immunosuppression result from changes within the Ts cell circuit.

C 610 EXPRESSION OF CD2 ON MURINE T CELLS. Pascale Duplay and James P. Allison. Cancer Research Laboratory, University of California, Berkeley, CA. 94720.

We have produced an antiserum to a synthetic peptide corresponding to a portion of the murine CD2 gene product. This antiserum reacted specifically with cells expressing CD2. Flow cytometric analysis of normal lymphoid tissues revealed that thymocytes were heterogenous in the level of CD2 expression while splenic T cells expressed CD2 at uniformly high levels. CD2 expression with that of other T cell differentiation antigens (IL-2R, J11d, CD5 and CD3) in the murine young thymus and during fetal ontogeny was analyzed using three-color flow microfluorometry. Our results indicate that CD2 expression is correlated with the stage of thymocytes maturation and demonstrate that IL-2R expression in the thymus is not induced via stimulation of the CD2 pathway.

C 611 A SYNERGISM BETWEEN INTERLEUKIN 1 AND INTERLEUKIN 6 INDUCES PROLIFERATION IN RESTING HUMAN T CELLS, Frank Emmrich, Christina Zadikian, Max-Planck-Gesellschaft, Klinische Argeitsgruppen fur Rheumatologie/Immunologie,Schwabachanlage 10, 8520 Erlangen. Accessory cell depleted resting (Ia) T cells are not able to produce autocrine growth factors when stimulated via the T cell receptor complex (TCR), although IL-2 receptor expression takes place. The addition of accessory cells restores the proliferative capacity. We have tested a series of recombinant lymphokines and growth factors for their ability to substitute this accessory cell function for human CD8 and CD4 T cells. Triggering via the TCR was achieved by a combinatorial stimulation with anti-CD3 and either anti-CD4 or anti-CD8 that gives rise to IL-2 receptor expression only on the corresponding T cell subpopulation. IL-3, TNFα, IFNγ, IL-1, IL-4, IL-6 and GM-CSF were either negative or induced only a minor proliferative response. However, the combination of IL-1 with IL-6 was nearly as efficient as IL-2 in CD4 T cells. CD8 T cells were much less stimulated by IL-1+ IL-6. Other lymphokine combinations displayed no synergistic effect, which is in contrast to some reports on accessory-cell depleted mouse T cells whose function require costimulation with IL-1. This observation might be important for the understanding of T cell proliferation in chronic inflammatory tissues in which IL-1 as well as IL-6 is produced in large amounts.

C 612 ANALYSIS OF T CELL RECEPTOR GENES IN A T CELL HYBRIDOMA PRODUCING A DNP-SPECIFIC/H-2K^d-RESTRICTED SOLUBLE SUPPRESSOR MOLECULE, Robert L. Fairchild*, Ed Palmer*+ and John W. Moorhead*, *Department of Microbiology and Immunology, University of Colorado Health Sciences Center, Denver, CO 80262, and +Basic Sciences Division, Department of Pediatrics, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO 80206. Lyt 2$^+$ T suppressor cells from dinitrobenzene sulfonate-tolerized mice produce DNP-specific/class I MHC-restricted suppressor molecules which inhibit the transfer of contact sensitivity to 2,4-dinitrofluorobenzene. Structural and serological studies of these secreted effector molecules have suggested that they are disulfide-linked dimers bearing T cell receptor α and β chain determinants. A T cell hybridoma, MTs 79.1, constitutively producing a DNP-specific/H-2K^d-restricted suppressor molecule was generated and studied. Antibody staining indicated MTs 79.1 expresses a T cell receptor containing a Vβ8 element. Northern and Southern analyses indicated MTs 79.1 used Vβ8 and Vα4 genes to encode the T cell receptor. The secreted MTs 79.1 suppressor molecule was bound by monoclonal anti-Vβ8 antibodies and by antibodies specific for constant region determinants of the α chain. To assess the role of α and β chain genes in encoding the secreted suppressor molecule, we have generated Vβ8- and Vα4-negative variants from the MTs 79.1 parent. Experiments performed to date indicate that the loss of the Vβ8 gene results in the inability to produce the suppressor molecule. The results are consistent with the idea that the T cell receptor α and β chain genes are used to encode these hapten-specific/MHC-restricted secreted suppressor molecules. Supported by UPHS Grant AI-12993.

C 613 SIGNAL TRANSFER FROM TCR$\alpha\beta$ TO CD3 MAY BE INEFFICIENT IN IMMATURE T CELLS. Terri H. Finkel, Mary P. Happ, John W. Kappler, Philippa Marrack, Ralph Kubo and John C. Cambier, Dept. of Immunol., Natl. Jewish Ctr. for Immunol. and Resp. Med., Denver, CO 80206. Sensitivity to clonal deletion and selection for MHC restriction appears to be a feature of a particular stage of thymocyte development, specifically late cortical $CD4^+8^+$ cells. In recent studies we have shown that early events in signal transduction in response to antigen receptor crosslinking differ in the immature $CD4^+8^+$ population and the mature $CD4^+8^-$ or $CD4^-8^+$ population. Results indicate that antigen receptors on both immature and mature receptor-positive T cells transduce signals via calcium mobilization, however the magnitude of influx of extracellular Ca^{++} which follows binding of anti-receptor antibody differs between these populations. Specifically, immature cells show a much reduced Ca^{++} influx response compared to mature cells. We show here that although ligation of TCR$\alpha\beta$ has different consequences with regard to Ca^{++} mobilization in mature and immature cells, no such difference is seen following ligation of the receptor's transducer, CD3. The results suggest that the signalling cascade leading to the influx of extracellular Ca^{++} is intact when CD3 is ligated, but is incomplete when TCR$\alpha\beta$, the physiological ligand, is ligated. In addition, ligation of CD4 or CD8 on immature T cells induces influx of extracellular Ca^{++} comparable to that seen in mature T cells. A clonal population has been isolated from immature thymocytes which has the characteristic signal transduction properties of the bulk of immature thymocytes. These findings suggest that "signal" transfer from TCR$\alpha\beta$ to CD3 may be inefficient in $CD4^+8^+$ cells. Structural analysis of the TCR$\alpha\beta$/CD3 complex in immature and mature T cells is in progress.

C 614 NATURAL ADJUVANT PROPERTIES OF A TUMOR-SPECIFIC TRANSPLANTATION ANTIGEN IN THE MANIPULATION OF ANTIGEN-SPECIFIC IMMUNE RESPONSES IN VIVO. Patrick M. Flood and Alan Friedman, Dept. of Pathology, Yale University School of Medicine, New Haven, CT, 06510. Protective immunity to the ultraviolet (UV) light-induced sarcoma 1591-RE is directed toward a single tumor-specific transplantation antigen expressed by the 1591-RE tumor cells, termed the A antigen. A progressive variant line of 1591-RE, termed 1591-PRO4, lacks only the expression of this A antigen. Immunization of mice with 1591-RE tumor cells haptenated with trinitrophenyl (TNP-1591-RE) leads to the subsequent rejection of TNP-haptenated progressive tumors and to increased delayed-type hypersensitivity and CTL responses to TNP in normal, immunocompetent syngeneic mice. However, little or no humoral immunity to TNP is seen in animals injected with TNP-1591-RE tumor cells. In contrast, animals injected with TNP-1591-PRO4 did not exhibit TNP-specific tumor protective or cell-mediated immunity, but rather exhibited tolerance to subsequent immunizations with more immunogenic forms of TNP. Biochemical and molecular genetic studies have revealed the A antigen to exist on the cell surface as a complex of 3 class I MHC-like molecules. Transfection studies with DNA encoding each of these molecules into 1591-PRO4 reveals that the expression of one, and only one, of these three molecules mediates this increased immunity. These experiments suggest that an MHC-like antigen expressed on the 1591-RE sarcoma acts as a natural adjuvant to increase cell-mediated but not humoral immunity to linked antigens. The mechanism of this increased immunity is discussed. This work was supported by NIH grant CA-29606.

C 615 GENETIC CONTROL OF REGULATION OF PARASITE POPULATIONS IN CATTLE. Louis C. Gasbarre, Eldin A. Leighton, K. Darwin Murrell, and Christopher J. Davies, Helminthic Diseases Laboratory, Livestock and Poultry Sciences Institute, USDA, Beltsville, MD 20705; and Wye Research and Education Center, Univ. of Maryland, Queenstown, MD 21658. Efforts to immunize cattle against economically important gastrointestinal nematodes showed that immunity is manifested by: 1) a response that reduces the fecundity of established and subsequently acquired worms, and/or 2) a reduction in the number of worms developing upon challenge infection. However, the ability of individuals to mount such immunity is highly variable. Extending these studies to naturally infected populations indicate that there is a great difference in the number of eggs excreted by individual young calves on pasture. To delineate whether these differences were the result of host genetics and to begin to elucidate the mechanisms of resistance to parasite infection, a genetically defined cattle herd was assessed for parasite levels by determining fecal eggs per gram. Three years of sampling of the calves during their first grazing season indicates that: 1) certain individuals in the herd will consistently excrete high or low numbers of parasite eggs, 2) the high or low phenotype is significantly controlled by the genetic make-up of the calf, and 3) the high or low phenotype is highly heritable (heritability = 40). The role of the bovine MHC in controlling this resistance/susceptibility is currently under investigation. The MHC class I alleles carried by the calves have been determined and MHC class II typing is currently in progress. This information is being used to assess the role of the bovine MHC in controlling immunoresponsiveness to parasite antigens.

C 616 IL 1 + IL 2 INDUCES IL 4 PRODUCTION BY CD 4$^-$, CD 8$^-$ THYMOCYTES. William C. Gause, Tohru Takashi, and Alfred D. Steinberg. NIAMS, National Institutes of Health, Bethesda, MD 20892
We have been studying the differential effects of IL 1 + IL 2 versus IL 4 on the growth and differentiation of CD 4$^-$, CD 8$^-$ thymocytes. Culture of highly purified CD 4$^-$, CD 8$^-$ thymocytes with IL 1 (4 U/ml) + IL 2 (100 U/ml) resulted in marked proliferation and increased cell size without change from the CD4$^-$, CD8$^-$ phenotype. Culture with IL 1 or IL 2 alone did not cause proliferation. A substantial contribution to the proliferation was secondary IL 4 release: addition of anti-IL 4 (11B11) blocking antibody inhibited proliferation induced by culture with IL 1 + IL 2. IL 4 mRNA was demonstrated by Northern blot analyses after 48 and 72 hours of culture with IL 1 + IL 2 whereas none was detected at culture initiation and very little was present at 24 hours. Effects of IL 1 + IL 2 on IL 4 transcription rate will also be reported.

Despite a marked inhibition of proliferation with anti-IL 4, there was no affect on expansion of CD 3$^+$ cells following culture with IL 1 + IL 2 (increase from 10% to 60% in 72 hours). Thus, in this system, IL 4 enhances proliferation of progenitor thymocytes but does not contribute to induction of T cell receptor.

C 617 ANTIGEN PRESENTATION BY NEONATAL MURINE SPLEEN CELLS. Harriet Gershon and Ditza Levin, Dept. of Immunology, Faculty of Medicine, Technion, P.O.B. 9649, Haifa, ISRAEL.
The ability of murine neonatal spleen cells to present antigen to D10-G4.1 (D10) T-helper cells and produce growth factors in response to subsequent cellular interactions were studied. The ability of neonatal spleen cells to present antigen and to stimulate D10 cells is low. During antigen presentation antigen presenting spleen cell populations produce IL-1. However, neonatal spleen cells do not respond to the same levels as do adult spleen cells. The addition of exogenous IL-1 cannot repair the antigen presentation by neonatal cells. Experiments in which the antigen processing and presentation steps were separated from those requiring growth factor induction and secretion, demonstrate that neonatal spleen cells are impaired in their ability to perform adequate antigen processing and presentation. Neonatal spleen cells are as competent as adult cells to cooperate with T-helper cells and secrete growth factors, provided antigen processing and presentation is performed by fully competent adult spleen cells. No suppressor mechanisms responsible for the low antigen presentation of neonatal cells could be detected. The impairment of neonatal spleen cells in the initial stages of antigen processing and presentation leads to low levels of growth factor production and is the major determinant in the ineffectual stimulation of T-helper cells by neonatal spleen cells.

C 618 IDENTIFICATION OF HELPER T CELL EPITOPES FROM GP 160 OF HIV THROUGH THE USE OF PEPTIDE-PHOSPHOLIPID CONJUGATES. G. Goodman-Snitkoff and Raphael J. Mannino, Department of Microbiology and Immunology, Albany Medical College, Albany, NY 12208
Our laboratory has developed a new, powerful technique for investigating the immune response to peptide epitopes, involving the covalent coupling of peptide to phosphatidyl-ethanolamine, followed by complexing with additional lipids and phospholipids to form a peptide-phospholipid complex. These complexes can be used to immunize mice in the absence of protein carriers or adjuvants, thus facilitating the study of the immune response to a small chemically defined antigen. Use of this technology has allowed us to identify two T helper cell epitopes in conserved regions of HIV gp 160 not previously identified by computer algorithims, defined by amino acids 485-518 and 585-615. Immunization with these peptides in peptide-phospholipid complexes results in the production IgG_1 and IgG_2 antibodies, which cross react with cloned fragments of the whole protein. Using this technology we have begun to characterize the immune response to individual peptide antigens. The response of H2-k mice to amino acids 494-518 of gp 160 of HIV, has been analyzed. The optimal dose of a peptide containing both B and T_h cell epitopes was found to be 15-30 ug, depending on the route of administration. IM immunization required less antigen for optimum antibody response than did IP. Anchorage in the phospholipid complex is a strict requirement for an antibody response. Additional variables, such as phospholipid composition and method of cross-linking have been studied and will be discussed. We believe that the use of this peptide-phospholipid complex technology will be significant both for studying the immune response to single epitopes and for vaccine development.

C 619 FRESH AND CULTURED LANGERHANS CELLS PRESENT ANTIGEN EQUALLY WELL TO PRIMED T CELLS, BUT ONLY THE LATTER PRESENT ANTIGEN TO UNPRIMED T CELLS.
S.Grammer and J.W.Streilein, Department of Microbiology and Immunology, University of Miami School of Medicine, Miami, Florida 33136

Based on assays in which T cell proliferation was induced via oxidative mitogenesis and exposure to MHC alloantigens, it has been reported that Langerhans cells (LC) isolated from normal mouse skin acquire maximum capacity to activate T cells only after 72 hours of culture. We have studied LC from BALB/c mouse skins for their capacity to present ovalbumin (OVA) and Ia alloantigens to unprimed T cells and to antigen-specific T cell hybridomas. The data reveal that both fresh and cultured LC presented OVA and alloantigens with equal efficiency to previously primed responders (and with 10 fold greater efficiency than spleen cells or the B cell lymphoma A20.1-11). By contrast only cultured LC displayed the capacity to present antigen to unprimed T cells. We propose that the antigen presenting potential of freshly prepared and cultured Langerhans cells, respectively, reflect the in vivo functional properties of intraepidermal LC and of LC that have picked-up antigen in the epidermis and migrated via dermis to the regional lymph node. If so, these data suggest that resident epidermal LC are fully prepared to present cutaneous antigens to memory/effector T cells (efferent limb), whereas resident LC must leave the influence of the epidermis in order to develop the capacity to meet the more stringent conditions required for antigen presentation to unprimed T cells (afferent limb).

C 621 DEVELOPMENTAL POTENTIAL OF MURINE THYMOCYTE SUBSETS, C.J. Guidos, B. Adkins, and I.L. Weissman, Department of Pathology, Stanford University, CA 94305.
Understanding how and at what developmental stage selection of the T cell repertoire for antigen occurs requires knowledge of the precursor-product relationships among mature ($CD4^+8^-$ and $CD4^-8^+$) and nonmature ($CD4^+8^+$) thymocyte subsets. We first used 4-color flow cytometry to identify candidate precursor populations, and then assessed the developmental fate of highly purified thymocyte subsets following intrathymic (IT) injection into unirradiated Thy-1 congenic host mice. At various times post-injection, CD3, CD4, and CD8 expression on donor-derived cells were analyzed by 3-color flow cytometry. We thus identified an immature ($CD3^-J11d^{hi}$) subset of adult $CD4^-8^+$ thymocytes which arises at day 15 of fetal life, prior to nonmature $CD4^+8^+$ and functional $CD4^+8^-$ and $CD4^-8^+$ thymocytes. Most $CD3^-4^-8^+$ thymocytes are in the G_2 + S phases of the cell cylce and produce $CD3^+4^-8^+$ (14-20%) and $CD4^+8^+$ (76-83%) progeny within 16 hr of IT injection. $CD3^+4^+8^-$ progeny (15-20%) were only detected 2-3 days after the $CD4^+8^+$ progeny, suggesting a precursor function for $CD4^+8^+$ thymocytes. This was confirmed by demonstrating that highly purified $CD4^{lo}8^+$ and $CD4^+8^+$ blast cells give rise to both $CD3^+4^+8^-$ and $CD3^+4^-8^+$ thymocytes within 3-5 days of IT injection. These studies provide the first direct evidence that $CD3^-4^-8^+$ and at least some $CD4^+8^+$ thymocytes are intermediate stages in the development of mature T cells. Implications for mechanisms of repertoire selection will be discussed.

C 622 STRUCTURAL REQUIREMENTS OF A MINIMAL T CELL DETERMINANT,
Ann Haberman and Walter Gerhard, Wistar Institute, Philadelphia, PA 19104
We have investigated the structural restrictions placed on residues contained within a minimal T cell determinant, using the Balb/c class II restricted T cell response to the site 1 determinant of the influenza hemagglutinin molecule as a model system. To delineate which of the residues comprising the site 1 determinant are involved in interaction with the T cell receptor, we have determinaed the response of a large panel of site 1 specific T cell hybridomas to a collection of peptide analogs differing by single conservative or non-conservative substitutions at 9 positions. The fine specificity patterns of the T cell panel is extremely diverse; T cells varied in both the location and number of residues within the antigenic peptide that effected recognition. Our results implicate at least 6 out of 9 residues within the antigenic pepetide as being involved in interaction with the T cell receptor. This result suggest that peptides comprising the site 1 determinant do not form alpha helical structures when in association with MHC molecules.

C 623 THE KINETICS OF RUBELLA SPECIFIC ISOTYPE AND IGG SUBCLASS RESPONSES TO RUBELLA IMMUNIZATION. Elizabeth J. Hancock, Fiona P. Yong, Kees Pot, and Aubrey J. Tingle, Departments of Pediatrics and Pathology, University of British Columbia, Vancouver, B.C. CANADA.

Rubella-specific isotype and IgG subclass responses were evaluated using ELISA techniques in 44 rubella HAI seronegative adult females undergoing rubella immunization (RA 27/3 strain). Responses were evaluated prior to immunization and at 1,2,3,4,5,6,12 and 24 wks post-immunization. Pre-immunization sera showed detectable levels of rubella-specific antibody in the IgG class (17/44); IgA class (32/44) and in one or more of IgG subclasses (22/44). Post-immunization, 11 subjects failed to develop IgM class responses by the HAI (SDG) technique while 43/44 developed IgM antibody by ELISA techniques. IgA responses were detected at low levels in all vaccinees beginning at 3-4 wks and declining by 24 wks post-immunization. All individuals produced detectable antibody in IgG1 and IgG3 subclasses by 2-3 wks post-vaccine with sustained IgG1 levels but significant decline in IgG3 levels noted between 6 and 24 wks post-immunization. No seroconversion was noted in the IgG2 subclass although 9 individuals had detectable pre-immunization IgG2 rubella antibody present. IgG4 levels were detected in all vaccinees post-vaccine with a delayed and progressive rise over the study period. Subsequent correlation was then performed between rubella-specific antibody responses and the presence or absence of adverse joint reactions occurring in association with rubella vaccine administration.

C 624 T LYMPHOCYTE RESPONSES TO VARICELLA ZOSTER VIRUS. Anthony Hayward, Abbas Vafai, Roger Giller & Eileen Villanueba. Departments of Pediatrics and Microbiology, University of Colorado School of Medicine, Denver CO 80262 & University of Iowa School of Medicine, Iowa City IO

The proliferative response of blood lymphocytes from varicella zoster virus (VZV)-immune donors to live VZV, extracted VZV antigens or purified glycoproteins is predominantly by $CD4^+$, HLA-D restricted T cells but little is known of the specificies of the responder cells. We restimulated T cells cloned by limiting dilution from VZV-stimulated cultures with purified VZV glycoproteins gpI, gpII and gpIII and found that T cell clones with specificity for each of these mediated both help for antibody responses and HLA-DR restricted VZV-specific cytotoxicity. 5 polypeptides of 10 to 14 amino acids length corresponding to predicted amphipathic sequences in the primary structures of gp I, gp II and gp IV were synthesised. Proliferative responses were observed to 3 of these peptides (one from each glycoprotein) with responder cell frequencies in the $1:10^5$ blood T cells range. The gp I peptide additionally defined an epitope recognised by serum antibody.

C 625 AN IMMUNOMODULATORY APPROACH TO TREATING HSV-1 CORNEAL DISEASE, **Hendricks R.L., Departments of Ophthalmology, and Microbiology/Immunology, University of Illinois School of Medicine, Chicago, IL 60612**

Herpes simplex virus type I (HSV-1) corneal infections are a leading cause of blindness worldwide. We and others have demonstrated that the cellular immune response to HSV-1 contributes to the elimination of virus from the cornea, but in doing so causes the tissue destruction that is responsible for the blinding complications of the disease. We have demonstrated that specifically suppressing the cytotoxic T lymphocyte (CTL) response to HSV-1 renders mice resistant to corneal disease following topical corneal HSV-1 infection. In agreement with this observation was our recent finding that in vivo depletion of $L3T4^+$ (T helper/inducer, and most DTH effector cells) neither reduced susceptibility to corneal disease, nor increased susceptibility to disseminated disease. The corneal lesions in L3T4 depleted mice contained numerous Lyt-2 (T suppressor/cytotoxic) cells, and no L3T4 cells. The L3T4 depleted mice exhibited normal HSV-specific CTL precursor frequencies. Experiments designed to determine the effect of in vivo Lyt-2 depletion on susceptibility to corneal disease are in progress. Our goal is to identify cellular immune responses to HSV-1 that maximize protection, while minimizing immunopathology in the cornea, and identify HSV-1 epitopes that preferentially activate those responses.

Supported by NIH Grant EY05945

C 626 IMMUNOGENICITY OF IDIOTYPES: ANTI-ANTI-BSA IN ORDINARY IMMUNE SERA, AND * ANTI-ANTI-SELF MHC ANTIBODIES IN AUTOIMMUNITY. Tracy A. Kion, Robert B. Forsyth and Geoffrey W. Hoffmann, Departments of Microbiology and Physics, University of British Columbia, Vancouver, B.C., V6T 1W5.
We found that affinity purified antibodies to BSA, KLH and diptheria toxoid all contain a substantial amount of specific anti-idiotypic activity. Chicken antibodies affinity purified against BSA react with mouse anti-BSA antibodies, which suggests that we are dealing with internal image antibodies. * MRL-*lpr/lpr* mice develop spontaneous autoimmunity. We found that these mice make anti-anti-(self H-2) antibodies prior to making appreciable amounts of pathological autoantibodies such as anti-DNA, anti-RNP.Sm, and rheumatoid factor. The anti-anti-self antibodies are detected using an inhibition of antibody mediated cytotoxicity assay, that also detects anti-anti-(self H-2) in ordinary allogeneic anti-sera. The antibodies are not rheumatoid factors, although the animals do make rheumatoid factors later in the development of the disease. The anti-anti-self activity is fully developed at 2 months, when the other autoantibodies are typically barely detectable. We conclude that anti-anti-self antibodies could play an important role in the etiology of the disease.

C 627 FEEDBACK REGULATION OF IL-1 SYNTHESIS IN MONOCYTES BY T CELL PRODUCTS: DUAL EFFECT OF IL-4. Mikko Hurme, Tessa Palkama and Marja Sihvola, Department of Bacteriology and Immunology, University of Helsinki, SF-00290, Helsinki, Finland.
IL-1 production of human monocyte/macrophages is regulated by several cytokines some of which are themselves able to activate the IL-1 production (e.g. TNF and IL-2) while others (e.g. IFN-γ) modulate the production activated by other signals. We have now examined the effect of IL-4 on the IL-1 synthesis. IL-4 alone did not induce any IL-1 bioactivity or IL-1α or IL-1ß mRNA expression in freshly isolated peripheral blood adherent cells. In contrast, IL-4 effectively suppressed the LPS induced IL-1 production. This suppression took place without any decrease in the steady-state levels of IL-1α and IL-1ß mRNA, suggesting that this downregulative effects is posttranscriptional. Monocyte/macrophages are known to rapidly loose their ability to produce IL-1 when cultivated in vitro. If IFN-γ is present in the culture fluid, the cells remain capable of producing IL-1. As IFN-γ and IL-4 have been reported to have similar "priming" effects on macrophages (e.g. increasing the tumoricidal capacity and MHC class II antigen expression) we cultivated monocytes for 24 h in the presence of either IFN-γ or IL-4, and after washing the cells they were stimulated with LPS. IL-1 activity could be detected both in the IFN-γ and IL-4 preincubated cultures (but not in the cultures preincubated with medium alone). These data suggest that IL-4 can also display a similar upregulatory function in IL-1 production as IFN-γ.

C 628 DIFFERENCES IN FRIEND VIRUS-INDUCED SPLENOMEGALY AND FRIEND VIRUS-SPECIFIC CTL GENERATION IN BALB STRAINS CONGENIC AT H-2. Sally T. Ishizaka, David Polsky and Frank Lilly. Department of Genetics, Albert Einstein College of Medicine, Bronx, NY 10464.
BALB mice congenic at the H-2 complex differ in their susceptibility to erythroleukemia induced by Friend virus (FV). BALB.B, G and 2R are all relatively resistant to FV as measured by splenomegaly, while BALB/c, 5R, A and K develop splenomegaly rapidly. This localizes resistance to the D end of the H-2 complex. (BALB.B x BALB/c)F_1 are susceptible, indicating that susceptibility is dominant. A similar pattern is seen in (C57BL/6J x BALB)F_1. This H-2 effect is seen by 12 days after infection, suggesting that early immune reactivity may be important in this disease. Resistance is not a reflection of differing infectivity of the virus in congenic strains, as all strains tested show similar numbers of virus-induced spleen foci 9 days after infection. The congenic strains also possess identical levels of natural killer activity.
We have tested the ability of CTL from (BALB.B x BALB congenic)F_1 mice immunized with FV-infected BALB.B cells to lyse cells expressing FV-encoded antigens. Levels of CTL activity in these mice correlate with their resistance to splenomegaly; BALB.B and its crosses to 2R and G show high CTL generation while crosses to BALB/c, K and 5R have low to moderate CTL activity. Preliminary data from limiting dilution assays suggest that these variations in CTL response reflect differences in the frequency of CTL precursors responsive to Friend antigens. Thus strains congenic at H-2 appear to differ in their repertoire of FV-reactive CTL.

C 629 ANTIBODY RESPONSE TO MURINE IMMUNOGLOBULIN IN MARROW TRANSPLANT RECIPIENTS TREATED FOR GRAFT-VERSUS-HOST DISEASE, A. Jackson, C. Anasetti, P. J. Martin, J. Hansen, S. Harkonen, M. Quist, T. A. Reichert, and N. L. Warner, Becton Dickinson Monoclonal Center, Mountain View, CA 94043 and Fred Hutchinson Cancer Research Center, Seattle, WA 98104

Allogeneic Marrow transplant recipients with Graft-versus-Host disease refractory to treatment with corticosteroids were injected daily for 7 days with 0.1 to 1.0 mg/kg of a monoclonal antibody (MAb) to the IL-2 Receptor (clone 2A3). Serum samples were drawn before and one hour after each antibody injection and approximately three weeks after treatment. ELISA assays for circulating murine IgG1 were performed on all samples throughout the treatment period and showed that a trough serum level greater than 10 ug/ml was achieved consistently in patients treated with 0.5 or 1 mg/kg/day. ELISA values for human antibodies to murine IgG on post treatment samples were compared to background values of pretreatment serum and to a positive control value of serum from a cynomolgous monkey immunized with a murine IgG1 MAb specific for CD4 (Leu 3a). Sera assayed during the treatment period showed no signs of immune elimination of the mouse IgG. Two of two patients receiving 1.0 mg/kg and two of four treated with 0.2 mg/kg developed IgM antibodies to murine Ig and one of each group made also IgG. There was a difference between the magnitude of the IgG response to an irrelevant murine IgG1 (anti-KLH) and the immunizing MAb in one individual, indicating an anti-idiotype response. Patient data will be presented in correlation with time intervals between injections and antibody response.

C 630 QUALITATIVE DIFFERENCES IN THE PROFILE OF ANTIBODIES TO MYCOBACTERIAL ANTIGENS IN DIFFERENT TYPES OF LEPROSY PATIENTS AND THEIR CONTACTS., M.M.James and VR. Muthukkaruppan, Department of Immunology, School of Biological Sciences, Madurai Kamaraj University, Madurai - 625 021, India.

In order to identify the mycobacterial antigens reacting with serum antibodies and to see whether there is any qualitative differences among lepromatous (LL) and tuberculoid (TT/BT) leprosy patients and their contacts with reference to their anti-mycobacterial antibodies, the techniques of SDS-PAGE and western blot analysis were employed. It was found that of the 33 major bands in BCG sonicate after SDS-PAGE, the lepromatous patients had antibodies to 32 bands and in tuberculoid patients to 28 bands. More importantly, four of these bands (56 Kd, 48 Kd, 32 Kd and 20 Kd) were unique to lepromatous sera and one (44 Kd) to tuberculoid sera. Antibodies to these bands were not present in the other types of patients or healthy individuals tested. Further, it was found that only IgG antibodies demonstrated such differences among the patients and normal groups. And, by 2-D gel electrophoresis followed by immunoblot, an additional BCG protein of pI 5.5 and molecular weight of 42 Kd was identified to have antibodies only in LL patients. Similar studies were also conducted with M. leprae sonicate. Of the 20 bands by SDS-PAGE, antibodies of IgG class were detected for 16 bands in LL sera and 13 bands in TT/BT sera. Three bands of 56 Kd, 21 Kd and 20 Kd were unique to the LL sera. Thus, a total of seven qualitative differences could be identified between the serum antibodies of LL and TT/BT patients.

C 631 CHARACTERIZATION OF IMMUNODOMINANT T-CELL ANTIGENS THAT INDUCE PROTECTIVE IMMUNITY TO SPOTTED FEVER GROUP RICKETTSIAE, Thomas R. Jerrells, Ken Gage, Amy Roehrig, and David H. Walker, Department of Pathology, University of Texas Medical Branch, Galveston, TX 77550 Development of immunity to members of the spotted fever group of rickettsiae is a T-cell dependent response. We have used T-cell hybridomas and cloned T-cell lines from immune animals and convalescent humans to identify the rickettsial antigens that induce antigen-responsive T-cells. In these studies we found that the 155 kDa antigen of Rickettsia rickettsii, the causative agent of Rocky Mountain spotted fever, is one of the immunodominant T-cell antigens. T-cells from immune animals and humans were responded in culture to a recombinant 155 kDa antigen. Both sources of T-cells were of the T-helper type ($L3T4^+$ and $CD4^+$ respectively) and produced IL-2 and interferon. It was found that soluble antigenic material of R. rickettsii obtained by extraction with hypotonic buffer maximally stimulated the T-cell lines. This material was enriched for the high molecular weight polypeptides of 155 kDa and 120 kDa. Also, R. rhipicephali will induce a long-lived immunity against infection with R. rickettsii. Infected guinea pigs develop a minimally cross-reactive antibody response to R. rickettsii. In contrast, a strong cross-reactive T-cell proliferative response is produced. Studies are in progress to determine the nature of the common protective antigen of R. rhipicephali. Supported in part by NIH grant AI21242

C 632 INDUCTION OF ANTIGEN SPECIFIC CLONED CYTOTOXIC HUMAN T-CELLS BY T. GONDII. Lloyd H. Kasper, Kendall A. Smith and Imtiaz A. Khan. Department of Medicine and Microbiology, Dartmouth Medical School, Hanover, NH 03756

Infection with T. gondii has become a major cause of morbidity in patients with AIDS implying the importance of T-cell immunity against this pathogen. We have recently demonstrated the induction of parasite specific cytotoxic T-cell splenocytes in mice using a highly purified membrane protein (P30) of T.gondii. We used a whole parasite extract to stimulate an in vitro proliferative response of PBM cells from a seropositive individual. Whole extract responder cells were expanded in culture in the presence of irradiated PBM and rIL-2. Responder cells were cloned by limit dilution and three were selected for analysis. All three clones express the CD3+ phenotype. One clone was able to reduce extracellular T. gondii plaque forming units by almost 50% when incubated at an effector:target ratio of 20:1 or greater. A radioactive release assay indicated that the inhibitory effect of the immune cells resulted from direct cytotoxicity. Analysis of antigen specificity demonstrated this clone to respond to a low molecular weight antigen. Thus, we have identified an antigen specific subset of cloned CD3+, responder T-cells that are directly parasiticidal and exhibit cytotoxicity possibly independent of MHC restriction.

C 633 Mhc RESTRICTION OF THE ANTIBODY REPERTOIRE TO A MAJOR ALLERGEN OF THE PARASITIC NEMATODE ASCARIS. Malcolm W. Kennedy, Jacqueline F. Christie, Lesley A. Tomlinson and Eleanor M. Fraser, Wellcome Laboratories for Experimental Parasitology, University of Glasgow, Bearsden, Glasgow G61 1QH, Scotland, U.K.

Humans infected with the parasitic nematode Ascaris lumbricoides vary considerably in antibody responsiveness to a 14 kDa component of the parasite. This molecule is secreted by the parasite, and is also abundant internally. This heterogeneous reactivity has been modelled in laboratory rodents, and the antibody response to it is H-2- and RT1-restricted in mice and rats, respectively. Using inbred and congenic animals, only mice of $H\text{-}2^{s}$ and rats of $RT1^{u}$ were, so far, found to be responders, and this restriction only operated in the context of infection. The specificity of the IgE response in these animals was assayed by Passive Cutaneous Anaphylaxis, and in an IgE-specific ELISA assay. The data show that the above Mhc restriction also applied to the specificity of the reaginic antibody response, although animals of all Mhc haplotypes responded to other Ascaris allergens. Amino acid analysis of the 14 kDa equates it to a previously identified "Allergen A" of the parasite, and we now have its sequence available. These findings have implications for the genetic control of allergic responses in general, and, in particular, to the hypersensitivity responses which are such a feature of infections with parasitic nematodes. There are also implications for the generation of hypersensitivity responses by recombinant vaccines involving certain parasite antigens.

C 634 IMMUNE RESPONSES TO RETROVIRAL INFECTIONS WITHIN THE CENTRAL NERVOUS SYSTEM, Jonathan M. Korostoff, Kenneth J. Blank*, and Glen N. Gaulton. Division of Immunobiology, Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA 19104 and *Department of Microbiology and Immunology, Temple University School of Medicine, Philadelphia, PA 19140. We have previously shown that exposure of neonatal BALB.B mice to a variant of Gross Murine Leukemia Virus, WB91-GV, within the first 24 hours after birth, resulted in a selective white matter infection within the CNS. Immunohistochemial analysis of both frozen sections prepared from the brains of animals immunized in this manner and of highly enriched glial cell subpopulation cultures for viral gp70 expression indicated that oligodndrocytes and possibly a subset of astrocytes were the targets of this infection. Further, microscopic analysis of frozen sections failed to reveal any overt signs of gross pathologic changes associated with the viral infection. We have been able to demonstrate the presence of virus specific antibody in the serum of these mice as well as virus specific cytolytic T cells in the peripheral lymphoid organs. Experiments are currently underway to determine whether the lack of pathology associated with WB91 infection in light of the previously shown virus specific immune responses in these mice is due to a failure of antigen presentation within the CNS or some other form of immunoregulatory phenomena.

C 635 REGULATION OF THE T CELL PROLIFERATIVE RESPONSE TO PIGEON CYTOCHROME C IN ALLOGENEIC, B10.A$_{(b.m.)}$→B10.A(3R) or (5R)$_{(irr)}$ BONE MARROW CHIMERAS, Zdenko Kovac and Ronald H. Schwartz, LCMI, NIAID, NIH, Bethesda, MD 20892
The T lymphocyte proliferative response to pigeon cytochrome c in B10.A mice is restricted to the $E_\beta k$:$E_\alpha k$ Ia molecule and specific for the C-terminal determinant comprised of residues 93-104. B10.A(3R) and B10.A(5R) mice are nonresponders to pigeon cytochrome c. Nonetheless, the T cell repertoire of B10.A(3R) or (5R) contains some T cell clones capable of recognizing and proliferating to pigeon cytochrome c when presented by B10.A antigen-presenting cells (APC). Therefore, one would expect to stimulate such clones in allogeneic bone marrow chimeras of the type B10.A$_{(b.m.)}$→B10.A(3R) or (5R)$_{(irr)}$, which have B10.A APCs and a B10.A(3R) or (5R) T cell repertoire, respectively. When such chimeras were primed with pigeon cytochrome c fragment 93-104 or a synthetic fragment of moth cytochrome c, they showed a good antigen specific proliferative response in vitro. Surprisingly, however, if pigeon cytochrome c was used for priming, no response was detected, even at priming doses as high as 400 nmol per mouse. Priming with fragment 81-104 could only be achieved by treating the allochimeras with an anti-CD8 monoclonal antibody in vivo during the priming step. Such treatment did not influence the priming of clones specific for purified protein derivative (PPD) in the same chimera. Thus the regulation which involves CD8 positive cells is antigen specific. Transfer of pigeon cytochrome 81-104 primed lymph node cells from the chimera into naive B10.A mice prevented priming of the recipient for a T cell proliferative response to pigeon 81-104, but not priming to the moth synthetic fragment. These results suggest the existence in these chimeras of an antigen-specific suppression mechanism involving CD8 positive cells.

C 636 CROSS-REACTIVE IDIOTYPE IN TUMOR-SPECIFIC CTL RESPONSE: Kagemasa Kuribayashi, Yuji Matsubayashi, Tohru Masuda, Eiichi Nakayama, and Hiroshi Shiku. Institute for Immunology, Faculty of Medicine, Kyoto University, Kyoto 606, and Department of Oncology, Nagasaki University School of Medicine, Nagasaki 852, Japan. Sera from B6 mice immunized with a syngeneic CTL specific for FBL-3 tumor of B6 origin blocked the cytotoxic activity of only the immunizing CTL clone. Therefore, a monoclonal antibody (mAb) N9-127 was produced by fusion of the B6 spleen cells immune to a syngeneic FBL-3-specific CTL clone (No. 8). The specificity of the mAb N9-127 was confirmed by immunoprecipitation, blocking of cytolytic activity, stimulation of proliferation, and induction of TCR-mediated nonspecific cytolysis of the CTL clone No. 8. In some B6 mice, 3-13% of the anti-FBL-3 MLTC cells were positive for this N9-127-defined idiotype, and formed a well demarcated population upon examination by flow cytometry. Even in mice in which no such population was observed some CTL clones established by limiting dilution culture were also positive for this idiotype (10 out of 89 clones from 3 mice). The cytotoxic activities of these CTL clones were blocked by N9-127, which in turn induced the nonspecific cytolysis in redirected assay. However, no positive cells were detected in non-cultured normal or FBL-3-immune spleen and lymph node cells. This indicates the presence of cross-reactive (dominant) idiotype in the B6 anti- FBL-3 cytotoxic T cell responses and may provide a potent tool for analyzing the idiotype-mediated regulation of the anti-tumor immune responses.

C 637 REGULATION OF MONOCYTE INTERLEUKIN-1 (IL-1) mRNA INDUCTION DURING ANTI-CD3 MEDIATED T CELL ACTIVATION R. Clive Landis*, Richard I. Fisher+, Thomas M. Ellis+*, +Section of Hematology/Oncology and *Department of Microbiology, Loyola University School of Medicine, 2160 S. 1st Ave, Maywood IL 60153. The regulation of human monocyte IL-1 mRNA induction was investigated during primary immune activation in an anti-CD3 (α-CD3) mitogenesis model. Unstimulated normal human monocytes displayed a transient elevation of IL-1 α and IL-1 β mRNA levels following their isolation from peripheral blood and adherence to plastic, which returned to undetectable levels by 24-40 hr of culture. IL-1 α & β mRNA expression by monocytes was reinduced after 40 hr of adherence by the addition of purified, autologous T lymphocytes plus α-CD3 mAb, but not by the addition of T lymphocytes or α-CD3 alone. The addition of T lymphocytes plus a non-mitogenic α-CD5 mAb failed to induce IL-1 α or β mRNA, suggesting that IL-1 mRNA induction by α-CD3 was associated with a specific activating signal rather than a non-specific F_CR binding event or the formation of monocyte:T cell conjugates. Kinetics experiments showed that IL-1 β mRNA was detectable in monocytes as early as 4 hr, peaked at 24 hr and decreased by 48 hr following induction by T lymphocytes plus α-CD3 mAb. In contrast to IL-1 β mRNA, IL-1 α mRNA levels were maintained at 48 hr following induction. IL-1 mRNA induction was associated with secreted IL-1 activity in supernates of monocyte/T cell/α-CD3 cultures. These studies demonstrate that monocyte IL-1 mRNA is induced during α-CD3 mitogenesis and requires an appropriate T cell signal for triggering. Experiments are in progress to identify the signal(s) required for IL-1 mRNA induction during α-CD3 mitogenesis.

C 638 THE T CELL RESPONSE TO ANTIGENS OF PLASMODIUM CHABAUDI, Jean Langhorne, Sally Slade and Sylvie Gillard, Max-Planck-Institut für Immunbiologie, D-7800 Freiburg, Federal Republic of Germany

T cells play an essential role in the protective immune response to malaria and are associated with some of the pathological consequences of the disease. However, the nature of their responses and the antigens to which they respond are not well defined. We have developed a limiting dilution assay system in which specific T cell responses to malaria antigens can be monitored at the clonal level. It is possible to determine the nature of the responding T cell by the growth factors they secrete and by their ability to act as helper cells for the antibody response to malaria antigens. Our data suggest that the T cell response changes during the primary infection and in hyperimmune animals. One to two weeks after initiation of a blood stage infection the major $CD4^+$ T cell which proliferates in response to parasite antigens secretes IL-2 and IFN-γ but is not an efficient helper cell for antibody responses. In contrast later in infection and in immune animals there is an effective helper cell response and many of these cells are distinct from those secreting IFN-γ and IL-2. We are currently investigating whether these cells retain these phenotypes when grown in long-term in vitro culture and whether defined antigens of the erythrocytic parasite elicit different T cell responses.

C 639 NEUTRALIZATION EPITOPES OF CORONAVIRUSES AND THE MECHANISM OF IMMUNODOMINANCY. J.A. Lenstra, J.G. Kusters, R.J. de Groot, W. Luytjes, W.J. Spaan & B.A.M. van der Zeijst, Veterinary Faculty, State University of Utrecht, and W.P.A. Posthumus & R.H. Meloen, Central Veterinary Institute, Lelystad, The Netherlands.

We have localized linear neutralization epitopes on the coronaviruses IBV, MHV, FIPV and TGEV. The results can be summarized as follows:

1. Linear epitopes of the spike proteins (1162-1452 residues) could be mapped to a resolution of a single residue by expression of gene fragments in the prokaryotic pEX plasmids and/or PEPSCAN peptide synthesis.
2. The length the epitopes varied from 4 to at least 20 amino acid residues. We present evidence that the larger epitopes, although conformation-independent according to operational criteria, are nevertheless discontinuous.
3. In IBV, we localized several overlapping but different epitopes within an immunodominant region of 30 residues. This region is recognized by all polyclonal antisera tested. We propose that its immunodominancy is a consequence of its structure and function and does not depend on antigen presentation or idiotypic networks.

C 640 EXPRESSION OF HUMAN LDH-C_4 FROM *E.coli*: DEVELOPMENT OF A CONTRACEPTIVE VACCINE

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An immune response against the mouse testis-specific antigen LDH-C_4 reduces fertility by 70 percent in female baboons. An immune reaction to human LDH-C_4 would be expected to be more effective in primates. Since the human testis enzyme is not readily available in large quantities, recombinant DNA technologies were used to create a source of human LDH-C_4. Antibodies to mouse LDH-C_4 were used to screen a λgt11 human testis cDNA expression library. A full length human *Ldh-c* clone was identified, sequenced, and the *Ldh-c* cDNA was engineered for expression in *E.coli*. The 5' and 3' untranslated sequences were removed by restriction enzyme digestion, and synthetic linkers were added adjacent to the start and stop codons of translation. The modified cDNA was subcloned into the prokaryotic expression vector pKK223-3 and introduced into W3110 *lac* I^q cells. Cells were grown to mid-log phase, and induced with IPTG for positive regulation of the strong hybrid *tac* promoter. Induced cells overexpressed the 35 KD subunit which spontaneously formed the enzymatically active 140 KD tetramer. Human LDH-C_4 was purified 196-fold from liter cultures of cells by two step affinity chromatography to a specific activity of 90 I.U./mg. The 20 N-terminal amino acids sequenced were identical to those predicted from the nucleic acid sequence. Antibodies to synthetic peptide epitopes of human LDH-C_4 cross-reacted with the enzyme produced in *E.coli*. Two mg human LDH-C_4 were expressed per liter of bacterial cells. The purified protein is now available for immunogenicity and fertility studies.

Supported by NIH Grant HD 05863.

C 641 INTERLEUKIN SECRETION AND MEMBRANE IL-1 EXPRESSION BY NEONATAL SPLEEN CELLS DURING ANTIGEN PRESENTATION. Ditza Levin and Harriet Gershon, Dept. of Immunology, Faculty of Medicine , Technion , P.O.B. 9649, Haifa, ISRAEL.

The T-helper cell line (D10-G4.1), specific for conalbumin presented on syngeneic antigen presenting cells and dependent on IL-1 for its proliferation, was used as an indicator cell for the ability of neonatal murine spleen cells to present antigen and produce IL-1 and IL-2. The antigen presenting capacity of neonatal spleen cells is low. During antigen presentation there is an augmentation of IL-1 and IL-2 production by the antigen presenting spleen cell population. However, neonatal spleen cells do not respond as well as adult cells. The low levels of IL-1 can not be attributed to a low potential for producing IL-1 since neonatal cells produce high levels of IL-1 after induction by a crude IL-1 Inducer Factor (IL-1-IF). The spontaneous expression of membrane IL-1 by neonatal cells is low. Membrane IL-1 levels on neonatal cells can be brought to adult levels by induction with IL-1-IF. Neonatal spleen cells have an impaired capacity to process and/or present soluble antigen. This impairment leads to a decreased stimulus of the T-helper cell to produce inducer factors which leads to low levels of IL-1 and IL-2 production by the neonatal cells during antigen presentation. No suppressor mechanisms responsible for the low interleukin production were detected.

C 642 ***Demonstration of p-azobenzene-arsonate-L-tyrosine (ABA-Tyr) Specific T cells in Low Responder H-2^b Mice by IL-1 Supported T Cell Proliferation.*** J. Colin R. Hunter and George K. Lewis, Department of Microbiology and Immunology, University of Maryland Medical School, Baltimore, MD 21201

Previous studies have shown that H-2^b mice immunized with ABA-Tyr fail to produce ABA specific delayed-type hypersensitivity and show little or no T cell proliferation in vitro to ABA-Tyr. These observations suggest that H-2^b mice are deficient in TH1 cells that respond to ABA-Tyr. By contrast, immunization of H-2^b mice with TNP conjugates of ABA-Tyr revealed good cognate help, suggesting that these mice do possess ABA-Tyr specific TH2 cells and that such cells are not revealed in conventional lymphoproliferative assays. Because such assays are widely used to evaluate Ir gene control and to map T cell epitopes, the databases generated from such studies may seriously under represent the total number of responder phenotypes and T cell epitopes. Because of this concern, we established culture conditions that will support ABA-Tyr specific T cell proliferation in H-2^b mice. In these studies, C57BL/6J mice were immunized s.c. with ABA-Tyr and 7 to 14 days later the draining lymph nodes were cultured with varying doses of ABA-Tyr or with varying doses of ABA-Tyr and varying doses of recombinant IL-1 alpha (rIL-1a), a known co-stimulator of TH2 cells. Culture with ABA-Tyr alone produced no proliferation. By contrast, culture with ABA-Tyr and rIL-1a revealed T cell proliferation that titrated with the dose of ABA-Tyr and the dose of rIL-1a. These data suggest that the conventional lymph node proliferative assay may underestimate the number of responder phenotypes because it underestimates TH2 proliferation. Furthermore, addition of rIL-1a to such cultures may correct for this deficit. Supported by NIH Grants AI-25682, NS-2665, and ACS Grant FRA-254.

C 643 REGULATION OF IMMUNITY IN LEISHMANIASIS BY LYMPHOKINES, F. Y. Liew, Department of Experimental Immunobiology, Wellcome Biotech, Beckenham, Kent BR3 3BS, England.

It is now generally accepted that the principal effector mechanism in the host's defence against leishmaniasis is gamma-interferon (IFN-γ) which activates infected-macrophage to eliminate intracellular parasites. Resistance can be achieved in highly susceptible BALB/c mice by prior sublethal whole body irradiation or treatment with anti-IgM or anti-CD4 antibody. Protection can also be induced by repeated intravenous or intraperitoneal immunisation with killed parasites or purified antigens. T cells from the recovered or protectively immunised mice produce little or no IL-3 or IL-4 but substantially elevated levels of IFN-γ when stimulated with leishmanial antigens in vitro. The culture supernatant of lymphoid cells from BALB/c mice with progressive disease can inhibit the MAF (macrophage activating factor) and leishmanicidal activities of the culture supernatant of lymphoid cells from mice recovered from L. major infection. Furthermore, the active ingredient of MAF appears to be IFN-γ, whereas the MAF inhibiting factors are IL-3 and IL-4. The whole system can be reproduced with recombinant IFN-γ, IL-3 and IL-4 and the MAF inhibiting activity of the suppressive supernatant can be reversed by specific anti-IL-3 and anti-IL-4 antibodies. Thus it appears that the two subsets of $CD4^+$ T cells modulate the outcome of the disease by influencing the ability of macrophage to kill the intracellular parasite.

C 644 T CELL REPERTOIRE EXPRESSION TO CIRCUMSPOROZOITE PROTEIN OF *PLASMODIUM BERGHEI* IS INFLUENCED BY B CELLS, Heidi T. Link, W. Ripley Ballou, Urszula Krzych. Dept. of Biology, The Catholic University of America and Dept. of Immunology, Walter Reed Army Institute of Research, Washington, D.C.

The development of efficacious vaccines against malaria requires an understanding of the mechanisms involved in protective immunity. Previous studies with *Plasmodium berghei* demonstrated that sporozoite immunity is dependent upon antibody responses specific for the repeat region of the circumsporozoite (CS) protein and cell mediated mechanisms involving CD8+ T cells. In this study we analyzed the splenic T cell repertoire directed against epitopes on the CS protein of *P. berghei* and determined whether sporozoite-immune CD4+ and CD8+ T cells respond to shared or distinct epitopes. Sporozoite-immune spleen cells, CD4+ and CD8+ enriched T cell populations of Balb/c (H-2d), C3H (H-2k), and C57Bl/6 (H-2b) mouse strains were cultured in the presence of irradiated sporozoites or synthetic peptides representing 70% of the complete CS protein. Surprisingly, none of the cultures proliferated to any of the peptides tested, although proliferative responses to sporozoites were observed in unfractionated spleens and CD4+ T cell populations. CD8+ T cells did not respond to any of the antigens tested, even in the presence of exogenously added Il-2. Titration of CD8+ cells into proliferating CD4+ cell cultures did not suppress the anti-sporozoite response. The lack of anti-peptide reactivity contrasts with uniform responses to sporozoites and may be the result of the context in which CS antigens are presented to T cells. Functional analysis of accessory splenic B cells and macrophages revealed that while the anti-sporozoite proliferative responses were not affected by the removal of macrophages, sporozoite-primed B cells were essential for the responses. These data suggest that the CS protein on sporozoites is not processed extensively by macrophages to yield many potential T cell epitopes, but instead is presented by immunodominant B cells that restrict responses to a limited number of T cell clones.

C 645 THE ANTIGENIC SPECIFICITY OF HERPES SIMPLEX VIRUS (HSV-1) SPECIFIC CYTOTOXIC T LYMPHOCYTES INDUCED BY RECOMBINANT VACCINIA VIRUS CONSTRUCTS DOES NOT NECESSARILY REFLECT THE SPECIFICITIES OF THOSE INDUCED BY HSV-1 INFECTION, Stephen Martin, Jerry P. Weir, and Barry T. Rouse, Department of Microbiology, College of Veterinary Medicine, University of Tennessee, Knoxville, Tennessee 37996-0845.

Infection of mice with HSV-1 induces a brisk CTL response which is necessary for the subsequent resolution of the primary infection and is also required for optimum protection against reinfection. Current studies have demonstrated that relatively few of the viral antigens tested to date (7 viral envelope glycoproteins or 2 nonstructural nuclear proteins) are recognized by HSV-1 immune CTL populations generated in several different strains of mice (H2 haplotypes $H2^b$, $H2^d$, or $H2^k$). This failure of HSV specific CTL to recognize the cloned gene products in *in vitro* assays was demonstrable at the clonal level and could not be attributed to a peculiarity of the recombinant vaccinia constructs used because studies with adenovirus vectors or tranfected L cell constucts yielded the same results. Surprisingly, despite their inability to be recognized by HSV specific CTL *in vitro*, when used to immunize mice several of the vaccinia virus constructs would induce memory CTL populations capable of lysing HSV-1 infected autologous cells. For example, HSV-1 glycoprotein C (gC) was recognized by $H2^b$ restricted but not $H2^k$ restricted HSV specific CTL. However, immunization of either haplotype of mice with a vaccinia gC recombinant induced CTL populations which upon *in vitro* restimulation with HSV-1 would lyse histocompatible cells infected with HSV-1. This demonstrates that despite the presence of suitable epitopes (intrinsic factors) the context of the immunogen (extrinsic factors) will also influence it's ability to induce CTL. The results of further studies into the nature of these extrinsic factors will be presented and discussed with relevance to future sub-unit vaccine design. Work supported by Public Health Service Grants, AI 14981 and AI 24471 from the National Institute of Allergy and Infectious Diseases.

C 647 ENHANCEMENT OF THE RESPONSE TO ALLOANTIGEN BY IL1 AND IL6, Douglas McKenzie, Department of Biology, University of California at San Diego, La Jolla, 92037.

We have examined the requirements for non-activated allogeneic B lymphocytes to serve as stimulator cells in primary mixed lymphocyte cultures(MLC). The generation of a response was totally dependent on the use of low density B cells; small B cells did not stimulate a response under any conditions. Moreover, the ability of low density B cells to stimulate the response was dependent on the number of stimulator cells added to the cultures. At high cell input levels low density B cells stimulated a response without any further modification to the cultures. In contrast, at low cell input levels the generation of an optimal response required the addition of both IL1 and IL6 to the culture. Neither lymphokine by itself enhanced the ability of allogeneic B cells to stimulate the MLR. Our results suggest that low density B cells have attained a differentiation state that allows them to stimulate an mixed lymphocyte response. Furthermore, when the dose of alloantigen is limiting, both IL1 and IL6 enhance the primary proliferative response of T lymphocytes.

C 648 STRUCTURAL ANALYSIS OF INTERNAL IMAGE EXPRESSION: EPITOPE MIMICRY BY ANTI-IDIOTYPIC SEQUENCES IN REVERSE ORIENTATION, D.W. Metzger[1], C.W. Naeve[2], and V.H. Van Cleave[1]. Departments of [1]Immunology and [2]Virology and Molecular Biology, St. Jude Children's Research Hospital, Memphis, TN 38101.
We have investigated the structural basis for antigen mimicry by anti-idiotypic internal image antibodies. Two mouse monoclonal antibodies (mAbs) that bear internal images of a well-defined protein epitope, i.e., the rabbit immunoglobulin (Ig) a1 allotype, were produced and the variable region sequences were determined by RNA primer extension sequencing. The results showed that the mAb light chains did not contain any allotype-related residues; however, both heavy chain V regions contained a unique sequence homologous to the nominal antigen but in opposite orientation. This reversed sequence was expressed within CDR2 of both mAbs. Synthetic peptides corresponding to the putative antigenic regions of rabbit Ig and the mAb internal images, respectively, were tested for the ability to mimic the a1-like determinant. Although the homologous residues were presented in opposite orientations, both peptides completely inhibited at similar concentrations the binding of rabbit Ig to anti-a1 antibody. A paired Thr and Glu was necessary for expression of the a1 epitope as revealed by conservative substitutions in the peptide sequence. Computer-generated, energy-minimized models of rabbit Ig and the mAbs revealed that the critical a1 residue side chain placements could be almost superimposable in either context. Thus, it appears that an antigenic epitope can be determined solely by the molecular environments of the amino acid side chains independently from the orientation of the protein carbon backbone. (Supported by AI 18880, CA 21765, and ALSAC)

C 649 ANALYSIS BY IN SITU HYBRIDIZATION OF NATURALLY OCCURRING DTH IN LEPROSY, Robert L. Modlin, Cathleen L. Cooper, Christoph Mueller, Thomas H. Rea & Barry R. Bloom, University of Southern California School of Medicine, Los Angeles, CA 90033 and Albert Einstein College of Medicine, Bronx, NY 10461.
Immunohistologic and molecular analysis of reversal reactions of leprosy was undertaken to study the mechanisms underlying regulation of cell-mediated immunity and delayed-type hypersensitivity (DTH) in man. Reversal reactions are often associated with a reduction of bacilli in lesions and are therefore thought to be a DTH reaction against M. leprae antigens. Immunohistologic analysis of reversal reactions demonstrated a selective increase of phenotypic T-helper and T-cytotoxic cells. Molecular analysis of biopsy specimens for IFN-gamma mRNA expression revealed a ten-fold increase in specific mRNA-containing cells over that observed in unresponsive lepromatous patients. The capability of CTL activity in reversal reactions was substantiated by demonstration of a four-fold increase in huHF serine esterase gene expression above that detected in unresponsive lepromatous individuals. Of interest, the microanatomic location of these serine esterase mRNA containing cells was identical to the distribution of $CD4^+$ cells. The data suggest that reversal reactions represent a hyperimmune DTH response characterized by a selective increase of CD4+ IFN-gamma producing cells and T-cytotoxic cells which result in the clearing of bacilli and concomitant tissue damage.

C 650 ROLE OF COSTIMULATORY SIGNALS IN T CELL PROLIFERATION
Daniel L. Mueller, Marc K. Jenkins and Ronald H. Schwartz, LCMI, NIAID, NIH, Bethesda, MD 20892. Proliferation of murine Type I $CD4^+$ T cell clones requires simultaneous occupancy of the T cell antigen receptor and delivery of an accessory cell-derived costimulatory signal. In contrast, isolated T cell receptor occupancy induces the cell into a state of reduced proliferative responsiveness to antigen. Based on the observation that PKC-activating phorbol esters can at times substitute for the presence of accessory cells in T cell proliferative response to mitogens or anti-CD3 monoclonal antibodies, we investigated the requirement for accessory cells in the antigen- and Con A-induced hydrolysis of PIP_2 and activation of PKC. The presence of normal accessory cells was found to be unnecessary for the development of PKC-dependent phosphorylations and the addition of normal accessory cells had no effect on the activity of PKC.

Given these findings, the ability of phorbol esters to mimic accessory cells in the induction of T cell IL-2 synthesis and proliferation presents a paradox. We have studied the effects of treatment with a calcium ionophore and phorbol ester on T cells and find that increased $[Ca^{2+}]_i$ and PKC activation are in fact insufficient biochemical second messengers in the induction of proliferation. While proliferation was induced at high T cell density in response to these stimuli, incubation of T cells at decreased cell density demonstrated markedly reduced proliferation, and single T cells failed to divide. This suggested that cellular interactions were required in the response. Additions of either IL-2 or normal accessory cells allowed proliferation at low density, consistent with a requirement for an accessory cell-derived costimulatory signal in the induction of IL-2 synthesis, even in the proliferative response to ionomycin and PMA. This result underscores the importance of an accessory cell-derived costimulatory signal, acting independently of T cell receptor-mediated increases in $[Ca^{2+}]_i$ and PKC activation, in the induction of T cell proliferation.

C 651 CHARACTERISTICS OF THYMOCYTES GROWN WITH INTERLEUKIN 4 AND ANTI CD3 ANTIBODIES. Richard Murray, Gregory Frank, and Albert Zlotnik. Department of Immunology, DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, CA 94304. We have previosuly reported that thymocytes proliferate to Interleukin 4 (IL-4) and phorbol myristate actetate (PMA). The subsets that proliferate under these conditions are the CD4-8-, CD4+8- and CD4-8+ thymocytes. We have been interested in identifying the natural stimulant that PMA mimics in this system. Here we report that monoclonal antibodies directed against the ϵ chain of the murine CD3 complex stimulate proliferation of thymocytes in the presence of IL-4. The characteristics of this proliferation are similar between thymocytes grown in IL-4/PMA and thymocytes grown in IL-4/anti-CD3. However, one difference concerns the double positive CD4+8+ subset. This subset disappears within 24 hours of culture in IL-4 and PMA but is present at this time in cells grown with IL-4/anti-CD3. This effect of PMA appears to be specific for CD4+8+ since the single positive subsets (CD4+8- and CD4-8+) and not affected and are expanded by IL-4 and PMA.
In some subsets (CD4+8-) anti-CD3 induces proliferation by itself but this proliferation is dependent on endogenous lymphokine production (IL-2 and IL-4).

C 652 HLA-B27 TRANSGENIC MICE ARE MORE SUSCEPTIBLE TO YERSINIA ENEROCOLITICA INFECTION THAN HLA-B27 NEGATIVE LITTERMATES. Cheryl L. Nickerson, Harvinder S. Luthra, Suresh Savarirayan, and Chella S. David; Departments of Immunology and Rheumatology, Mayo Clinic, Rochester, MN 55905.
HLA-B27 is present in 60-90% of the patients with seronegative spondyloarthropathies. The pathogenesis of these diseases is not known, however, reactive arthritis often occurs in HLA-B27 positive individuals following infection with Yersinia enterocolitica, Salmonella enterides, Salmonella typhimurium, Shigella flexneri, Campylobacter jejuni or Chlamydia trachomatis. In an effort to develop an animal model of reactive arthritis, Yersinia enterocolitica 0:8 WA was injected intravenously into HLA-B27 transgenic mice and their corresponding negative siblings. To date, no reactive arthritis has been observed, however, at a dose of 10^4 or 10^5, several of the mice developed hind limb paralysis. All but one of these mice were HLA-B27 positive as shown in the table below:

	I.V. Dose	Paralysis	I.V. Dose	Paralysis
$B27^+$	10^4	10/20 50%	10^5	4/20 20%
$B27^-$	10^4	1/19 5%	10^5	0/18 0%

Upon gross dissection and histopathologic examination, spinal abscesses were found in the paralyzed animals. In summary, $HLA\text{-}B27^+$ mice develop substantially more spinal abscesses and symptoms of paralysis than their $HLA\text{-}B27^-$ littermates. Consequently, the HLA-B27 gene appears to induce susceptibility in mice to acute infection with Yersinia enterocolitica.
Supported by a grant from the Minnesota Arthritis Foundation.

C 653 ANTIGENIC REQUIREMENTS FOR STIMULATION AND TARGET CELL RECOGNITION BY IMMUNE AND NON-IMMUNE LYMPHOCYTES. Judith A. Owen, Kim T. Grahl, Douglas R. Dorer, Margaret R. Dalesandro, and James B. Skeath, Dept. Biology, Haverford College, Haverford, PA. 19041. The molecular requirements for recognition of antigen-modified cells by cytotoxic T lymphocyte precursors (CTLps) and their activated progeny cytotoxic T lymphocytes (CTLs) have been studied using haptenated stimulator and target cells. We describe experiments designed to determine the molecular requirements for recognition by fluorescein-specific CTLps and CTLs derived both from naive and from immunized mice. We demonstrate that the cell surface hapten concentrations required for recognition of stimulator cells by CTLps and of target cells by their mature daughter CTLs 5-7 days later are indistinguishable. In contrast, CTLs and their precursor CTLps, derived from mice primed *in vivo* with hapten-conjugated cells, require lower cell surface hapten densities for recognition than do naive T cell populations. The mechanism of this apparent affinity maturation in CTL recognition is currently being explored in parallel with ongoing studies of memory generation in the fluorescein-specific B cell response.

C 654 AUTOREGULATION OF INTERLEUKIN-1 IN PULMONARY TUBERCULOSIS PATIENTS

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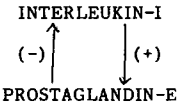
The production of Prostaglandin E, a major immunesuppressor secreted by the macrophages was inhibited by the addition of 0.1 M indomethacin to the cultures of monocytes harvested from patients suffering from pulmonary tuberculosis and those from equal number of normal controls. The IL-1 activity was estimated in the supernatants of these cultures by their ability to proliferate mice thymocytes. It was found that the supernatants from cultures with indomethacin showed a greater IL-I activity than the ones without it(44% P 0.001). This indicates the possibility of PGE offering a negative feedback control over IL-I production. The defective cell mediated immunity in patients with pulmonary tuberculosis may be explained through the inhibition on IL-I production by PGE whose enhanced production is reported in our earlier studies. The results and our hypothesis on the autoregulation of IL-I production will be presented and discussed.



C 655 **SPECIFIC SUPPRESSION TO ALLOANTIGEN, INDUCED BY THE INFUSION OF PHOTOINACTIVATED LEUKOCYTES, IS IN VIVO CELL TRANSFERABLE.**

Maritza Perez, Lori John, Carole Berger, Richard Edelson, Department of Dermatology and Pathology Columbia University, NYC and Department of Dermatology, Yale University, New Haven, CT 06510.

Partial tolerance to skin allotransplantation can be induced by infusion of leukocytes containing effector cells of skin allograft rejection after photoinactivation with 8-methoxypsoralen (8-MOP) in the presence of ultraviolet A light (UVA). CBA/j skin graft persisted viable for 42 days after engraftment onto BALB/c mice which had received repetitive infusions of 8-MOP/UVA inactivated leukocytes from syngeneic BALB/c mice rejecting a CBA/j skin graft. This skin allograft survival was associated with a cell population which transfer specific suppression onto naive syngeneic recipients as demonstrated by inhibition of Delayed Type Hypersensitivity Response (DTH). The phenotype of this transferable inhibitory cell population will be presented.

C 656 IN VIVO GENERATION OF ANTIGENIC VARIANTS IN MURINE RETROVIRUSES. J.M. Pozsgay[1,2] and K.J. Blank[2], [1]Molecular Biology Graduate Group, U. of Pennsylvania School of Medicine, and [2]Dept. of Microbiol. and Immunol., Temple U. School of Medicine, Philadelphia, PA.

Inoculation of a murine leukemia virus (GV) into immunocompetent adult mice results in variant viruses which differ from the parental virus (GV) at specific epitopes recognized by monoclonal antibodies directed against the env gene product, gp70. Biological clones isolated from GV express the GV phenotype suggesting that the loss of specific epitopes is the result of selective de novo processes in the immunocompetent host. Additionally, inoculation of adult mice with a biological clone expressing the GV phenotype also results in similar variant viruses. However, inoculation of GV into neonatal or nonlethally irradiated mice results in a population of viruses expressing only the GV phenotype suggesting that the emergence of antigenic variants may be influenced by neutralizing antibodies and/or cellular host responses.

SDS-PAGE analysis of immunoprecipitates of ^{35}S labelled lysates of fibroblasts infected with clones expressing GV or variant pehnotypes shows a size difference of the gp70 precursor. Additionally, the recognition of a neutralizing epitope (E-55) associated with gp70 by mAb55 is dependent on the appropriate native conformation of the epitope which appears to require glycosylation for expression. Experiments are in progress to further examine the immunogenetic basis for the generation of these variants and to determine the molecular changes in the virus genome responsible for changes in epitope expression.

C 657 DIRECT ACTIVATION OF T CELLS BY Con A AND @CD3 Ig. José Quintáns, A.Yokoyama, B. Evavold and R. D. Mayforth. Dpt. Pathology, U. of Chicago, Chicago, Ill.. We have investigated the capacity of murine splenic T cells depleted of accesory cells (AC) to proliferate in response to stimulation by Con A, @ CD3 ab and activated T cells. The depletion procedures consisted of carbonyl iron treatment, 2x "panning" on anti-Ig coated flasks, 2x anti-Ia cytotoxic treatments and PERCOLL gradient purification of small resting T Ia$^-$ cells. The appropiate concentration of Con A (10 ng/ml) and plastic-bound (pb) @CD3 Ig or its F(ab)' 2 fragments induce proliferation, Il2 r expression and Il2 (but not Il4) secretion in T Ia- cells cultured for 48 h at 5x10^5 cells/well. Responsiveness of Tla$^-$ cells to Con A and @CD3 in low density cultures (5x10^4 cells/well) is restored by the addition of irradiated Th2 cloned cells but not Th1, splenic cells or rIl1 + rIl2 + rIl4. Likewise, responsiveness to non activating doses of Con A (1ng/ml) or soluble @CD3 is restored by the addition of irradiated Th2 costimulatory cells. These experiments demonstrate that the ability of T cells to proliferate in the absence of AC is critically dependent on T-T interactions. T cell subsets prepared by either negative (L3T4$^-$ and Ly2$^-$ cells) or positive selection proliferate in response to pb @CD3 Ig. Although the proliferative responses of both L3T4$^-$ and Ly2$^-$ cells are maximal at 48h, the L3T4$^-$ cells require 10x more pb @CD3 Ig for maximal stimulation and their responses decline much faster than those of Ly2$^-$ cells. In addition, L3T4$^-$ cells are not stimulated by pb @CD3 F(ab)'2 fragments and their responses to @CD3 Ig are inhibitable by anti FcR as well as anti-LFA abs. Reponses of both L3T4$^-$ and Ly2$^-$ cells are inhibitable by @Il2 and @Il2 r abs but not by @L3T4, @Ly2 or @Il4. These experiments document interesting differences in the triggering requirements of L3T4$^-$ and Ly2$^-$ cells. Supported by NIH grants PO1 CA 19266, T 326M-07183 and GM 07281.

C 658 STUDIES ON THE INHIBITION OF CYTOTOXIC T CELL LYSIS BY ADENOVIRUS GP19K. Frances C. Rawle and Linda R. Gooding. Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, GA 30322.
The 19K glycoprotein encoded in the E3 region of Ad2 and Ad5 (gp19K) binds to class I MHC antigens in the endoplasmic reticulum and prevents their translocation to the cell surface. This has been proposed as a mechanism by which virus infected cells can avoid recognition by the host cytotoxic T lymphocyte (CTL) response. We have shown that gp19K can inhibit target cell lysis by adenovirus specific CTL, but the effectiveness of this inhibition varies greatly between different mouse strains. This is due in part to differences in the affinity of gp19K for different MHC class I molecules, but this cannot account for all the variation observed. The polyclonal CTL response to Ad5 in C57BL/10 (H-2^b) mice is exclusively D^b restricted. Gp19K completely inhibits lysis by Ad5 specific D^b restricted CTL of virus infected SVC57C, an SV40 transformed fibroblast cell line of C57BL/10 origin, indicating that D^b does bind to gp19K. However, when a panel of different D^b expressing cell lines were infected with Ad5 and gp19K deletion mutants, and used as targets for Ad5 specific CTL, the degree of inhibition of lysis by gp19K varied widely depending on the target cell. The surface expression of D^b on SVC57C is barely detectable by FACS, although sufficient for CTL recognition. We are presently determining whether the effectiveness of gp19K inhibition of cell lysis is dependent on the level of MHC expression or the rate of turnover, and measuring how much the class I expression must be reduced to inhibit CTL recognition.

C 659 RESPONSE OF CD4$^+$8$^+$ THYMOCYTES TO ACTIVATION SIGNALS: A TUMOR MODEL. Jeff S. Riegel, Wendy L. Havran, and James P. Allison. Dept. Microbiol. and Immunology and Cancer Research Laboratory, University of California at Berkeley, Berkeley, CA. 94720

It has been shown that CD4$^+$8$^+$ immature thymocytes fail to secrete IL-2 or express IL-2 receptors in response to activation signals. Furthermore, they cannot induce IL-2 gene transcription. Several tumor lines have now been characterized which have a CD4$^+$8$^+$ phenotype and fail to secrete IL-2 or express IL-2 receptors in response to stimulation with ionomycin plus PMA. These cells also do not express IL-2 mRNA after stimulation, as determined by Northern blotting and RNase protection. To determine the molecular mechanism for this lack of transcriptional activity, nuclear extracts were analysed for the presence of the DNA binding factor NFAT-1. This nuclear factor is present only in activated T cells and binds specifically to the IL-2 enhancer. Functionally mature mouse and human tumors expressed high levels of NFAT-1 after stimulation. In contrast, all of the CD4$^+$8$^+$ tumors expressed 10-100 fold less of this DNA binding activity, but normal levels of the NF-IL2-A DNA binding activity. This provides one possible explanation for the lack of IL-2 gene expression in these cells and may reflect a molecular switch that characterizes the developmental state of normal CD4$^+$8$^+$ thymocytes.

C 660 MHC Class II gene association with Lyme Arthritis
Giovina Ruberti*, Jayne S. Danska and C. Garrison Fathman, Stanford University Medical School, Stanford, CA. *Fellow Fondazione Cenci Bolognetti-Istituto Pasteur, Rome-Italy

We have undertaken an MHC analysis using the polymerase chain-reaction (PCR) and dot blot analysis of the amplified lyme arthritis patients DNA with allele specific oligonucleotide (ASO) probes. Genomic DNA for the first domain of the DQ beta chain and of the DR/βI from 20 patients with lyme arthritis has been amplified and we are analyzing the distribution of DR4DRβI and DQβalleles in this population to test the hypothesis that the MHC class II genes might be involved in presentation of selected spirochete epitopes whose recognition by T lymphocytes leads to lyme arthritis.

C 661 Antigen-specific Ts cells regulate non-responsiveness in the insulin system. Rubin,B., Kuhlmann,J., Worsaae,A. and Pierres,M. Inst. Exp. Immunology, Nørre Allé 71, 3, DK-2100 Copenhagen Ø, Denmark.

Most inbred strains of mice do not respond to porc insulin (PIns). Experiments were conducted to elucidate the mechanism of the non-responsiveness in H-2^k mice: 1) Purification of CD-4^+ T cells from PIns-immune B10.MBR mice revealed PIns-specific T helper (Th) cells, 2) these PIns specific Th cells could be activated by I-A^k and I-E^k expressing L929-fibroblasts. Therefore, both I-A^k and I-E^k molecules can present PIns in an immunogenic manner and activate PIns-specific Th cells. By means of different cell-fractionation procedures, it was found that antigen-specific T suppressor (Ts) cells regulated the PIns immune response. These Ts cells were of the FcR^-, CD-4^-, CD-8^+, Thy-1^+ phenotype, and they were present in normal mice. We believe that these experiments indicate that antigen-specific Ts cells exist and are important regulators of immune and autoimmune responses.

C 662 HELPER T CELL UNRESPONSIVENESS INDUCED IN VITRO BY SUPPRESSOR T CELLS, Padmini Salgame, Robert Modlin & Barry Bloom, Albert Einstein College of Medicine, Bronx, NY 10461; University of Southern California School of Medicine, Los Angeles, CA 90033.
We have earlier provided evidence that Ts-cells may be important regulatory cells determining Th-cell unresponsiveness in lepromatous leprosy. To analyze the mechanism of suppression, CD8+ Ts-clones were established from lesions and peripheral blood of lepromatous patients. The Ts-clones fail to show any evidence of cytotoxicity of antigen exposed, MHC-matched target cells: i) An ori^--SV40 transformed macrophage line; ii)EBV transformed B-cell line; iii)primary macrophages iv) and M. leprae reactive CD4+ cells. The possibility of functional inactivation of CD4+ clones by Ts-cells was investigated. M leprae-responsive CD4+ clones were preincubated with Ts CD8+ clones, APC and antigen for 16 hours, after which the CD8+ cells were removed from culture. The CD4+ clones were then restimulated with M. leprae and APC. CD4+ clones incubated with CD8+ cells and antigen were unresponsive to restimulation by antigen, although they were not killed and could respond well to IL-2. Addition of IL-2 in the pre- or post-incubation culture neither prevented the induction of unresponsiveness nor reversed it. Earlier models of tolerance have suggested that receptor occupancy in the absence of second signals induces tolerance in B- and T-cells. We would suggest that in the presence of Ts-cells, a second signal may be negated leading to Th-cell unresponsiveness.

C 663 *THE EFFECT OF GRAFT VERSUS HOST DISEASE ON LYMPHOCYTE FUNCTION*,
Kathy L. Schreiber and James Forman, Department of Microbiology,
University of Texas Southwestern Medical School, Dallas, TX 75235
Graft versus host disease (GVHD) poses a serious threat to the survival of patients with bone marrow transplants. The state of immunosuppression established in GVHD results in a variety of immunological abnormalities at the humoral and/or cellular level. We have developed a murine model of chronic GVHD across a minor histocompatibility (mH) barrier. In this model, immunosuppression develops. Spleen cells from mice undergoing this type of GVHD are unable to respond to the polyclonal activators lipopolysaccharide and Concanavalin A. However, the response against the B cell leukemia BCL_1 remains intact. The protective immune response against BCL_1 is directed towards the mH antigen H-40 and is mediated by cytotoxic T lymphocytes. Thus, the specific T cell response against a mH antigen can occur in the presence of chronic GVHD despite the absence of a polyclonal B and T cell response

C 664 IN VITRO AND IN VIVO STUDIES TO DETERMINE THE CYTOTOXIC T-LYMPHOCYTE EPITOPE OF THE NUCLEOPROTEIN OF LYMPHOCYTIC CHORIOMENINGITIS VIRUS,
Manfred Schulz,Stefan Oehen,Manuela Hany,Hans Hengartner and Rolf Zinkernagel,
Institute of Pathology,University Hospital,CH-8091 Zürich,Switzerland

Lymphocytic choriomeningitis virus (LCMV), a member of the arenavirus family, has a bisegmented RNA genome which encodes at least three polypeptides. The smaller RNA segment encodes two virus structural proteins, the glycoprotein (GP) and the nucleoprotein (NP).
Upon infection of mice with LCMV a cytotoxic T cell immune response directed against these proteins is measurable in vitro and in vivo. It can be demonstrated that depending on the haplotype of the mice, one or the other protein may play a major part in the immune response.
In order to define the immunogenic epitope(s) of the nucleoprotein which are recognized specifically by the T cell receptors of cytotoxic T cells, stepwise 3' truncated gene fragments encoding the nucleoprotein were cloned and expressed in vaccinia virus. With these recombinant vaccinia viruses, protection experiments in mice against LCMV infection were performed in parallel with in vitro studies, namely specific recognition of target cells expressing truncated fragments of the nucleoprotein by LCMV primed spleen cells.

C 665 MOLECULAR CHARACTERIZATION OF THE IL-1 RECEPTOR, John E. Sims, Benson M. Curtis, Byron Gallis, Kathryn S. Prickett, Michael B. Widmer, Stephen Lupton, Robert W. Overell, R. Bruce Acres, Carl J. March, Thomas P. Hopp, Steve Gillis, David L. Urdal, David Cosman, and Steven K. Dower.
Immunex Corporation, 51 University Street, Seattle, WA 98101
cDNA clones encoding the mouse and human T cell IL-1 receptors have been isolated and expressed in mammalian cells. The recombinant receptor binds IL-1 indistinguishably from the natural IL-1 receptor, and is functional in signal transduction. Deletion of the cytoplasmic portion of the receptor abolishes its signal transduction abilities. Sequence and secondary structure analysis suggest that the cytoplasmic segment of the IL-1 receptor binds a nucleotide. Experiments designed to test this hypothesis and to examine the mode of signal transduction will be presented. Also to be discussed are the mechanism of triggering of the receptor by IL-1, and the nature of IL-1 receptors expressed in other cell types such as B cells.

C 666 ROLE OF TGF-β IN SUPPRESSOR T CELL FACTOR REGULATION OF LYMPHOKINE GENE EXPRESSION. J.G. Smith, K.J. Hardy, and S.S. Rich, Dept. of Microbiology and Immunology, and Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX 77030.
MLR T suppressor factor (MLR-TsF) is the product of alloantigen primed Thy-1+, CD8+ murine lymphocytes. MLR-TsF suppresses T cell proliferation in response to H-2 alloantigens in MLR and to Con A, and acts at least in part by interfering with IL-2 dependent activation events. Recently we have shown that MLR-TsF also profoundly decreases the amount of steady state mRNA for IFN-γ in Con A treated splenocytes. In contrast, induced message for IL-2 and IL-2R, and constitutive message for β-actin are unaffected. Neither suppression of proliferation nor inhibition of IFN-γ mRNA are reversed by the addition of rIL-2. Since the multipotent cytokine TGF-β has been recently shown to be both antiproliferative to some T and B cells and inhibitory to the production of certain lymphokines, the role of TGF-β as a possible constituent of MLR-TsF was examined. It was found that MLR-TsF contained significantly more TGF-β than control factors. Exogenous TGF-β1, like MLR-TsF, decreased the level of Con A induced IFN-γ but not IL2R or actin mRNA. Pretreatment of either exogenous TGF-β1 or MLR-TsF with anti-TGF-β antibody blocked the suppressive effect on IFN-γ transcripts without increasing levels of other transcripts. Our results suggest that TGF-β1, or a crossreactive product of the TGF-β gene family, is an active component of MLR-TsF and largely accounts for its IFN-γ regulatory effect. Interestingly, addition of anti-TGF-β alone to Con A activated T cells resulted in increased levels of IFN-γ transcripts, implying an autocrine or paracrine role for TGF-β in normal immunoregulatory processes. Supported by NIH AI 21240.

C 667 IL-4 CAN MEDIATE CD8 INDUCTION ON MATURE HUMAN CD4+ T CELLS. H. Spits, X. Parlard, R. de Waal Malefyt and J. E. deVries. DNAX Research Institute, Palo Alto, CA.
IL-4 can act as a growth factor for human CD4+ and CD8+ T cells. Purified CD4+ activated and cultured in the presence of IL-4 acquire the CD8 antigen, whereas single positive CD8+ cells remain single positive when cultured in IL-4. To ensure that IL-4 mediates this effect on single CD4+ T cells, a cloning experiment was performed with IL-4 as growth factor. T cells were activated with the EBV transformed B cell JY in an MLC and cloned 6 days later by limiting dilution in the presence of IL-4 and an anti IL-2 Mab. As comparison the same T cells were also cloned in IL-2 and an anti IL-4 antibody. The cloning efficiencies in IL-4 and IL-2 were comparable. Forty-seven percent of the clones isolated in IL-4 were CD4+CD8+, whereas none of the clones isolated in IL-2 were double positive (DP). Northern blot analysis showed that IL-4 induced CD8 gene expression in CD4+ T cell clones. The antigen specific cytotoxic activity of a DP CTL clone CD506 isolated in IL-4, which was specific for HLA-DR on JY cells was blocked by an anti-CD4 mAb and not by anti-CD8. Clone CD506 could kill Dandi cells but only in the presence of anti-CD8 mAbs. Anti CD8 could block CD506 mediaetd cytolysis against Daudi cells coated with anti CD3 mAbs. In contrast anti CD8 was unable to affect anti CD3 induced cytotoxic activities mediated by clone CD506 cultured in IL-2 which did not express CD8.

C 668 CD4+ MEMORY T CELLS (CD45R/2H4-, CDw29[4B4]+) ARE THE DOMINATING CELLTYPE IN REACTIVE SKIN DISEASES AND CUTANEOUS T CELL LYMPHOMAS, W. Sterry, V. Mielke, Dept. of Dermatology, University of Kiel, FRG. CD4+ helper T cell are functionally and phenotypically heterogenous and include a suppressor/inducer (CD45R/2H4+, CDw29[4B4]-) as well as a helper/inducer subset (CD45R/2H4-/CDw29[4B4]+). Recently, it became evident that these subsets reflect different stages of helper T cell maturation before and after activation. Therefore, these T cell subsets have been designated as naive T cells (CD45R/2H4+, CDw29[4B4]-) and memory T cells (CD45R/2H4-, CDw29[4B4]+). We analysed the expression of these antigens in dermal lymphohistiocytic infiltrates from different benign skin diseases and cutaneous T cell lymphomas (chronic contact dermatitis (n=14), parapsoriasis en plaques (n=5), lymphomatoid papulosis (n=4), mycosis fungoides (n=15), Sezary's syndrome (n=2), pleomorphic T cell lymphoma (n=6) and high grade T cell lymphomas (n=4)). In almost all cutaneous T cell infiltrates memory T cells were preferentially found whereas in the peripheral blood both subsets are equally distributed. This implicates, that T cells infiltrating the skin already have had contact with their respective antigen. Where the switch from naive to memory T cells takes place can not be answerded by our findings, as we have investigated rather longstanding skin diseases. However, these memory T cells, which can be activated more easily, make diseased skin more effective in the amplification of an immune response.

C 669 THE DEVELOPMENT OF LYMPHOKINE-SECRETING HELPER EFFECTORS FROM PRECURSORS AND LONG-LIVED MEMORY CELLS

Susan L. Swain, Andrew Weinberg, Joseph Voland and Michele English. Dept of Biology and the UCSD Cancer Center, University of California San Diego. La Jolla, Ca. 92093.

We have studied the secretion of lymphokines by helper T cells freshly obtained from lymphoid organs and after several days of stimulation with antigen or mitogen and lymphokines. We find that fresh Th synthesize and secerte -IL2,IFNg,IL3 and GMCSF but very little IL4 or IL5 within 24-48 hours. This pattern resembles the pattern of lymphokines secreted by Th1 cell lines. The Th responsible for this secretion are CD4 positive T cells which are long-lived since they disappear very slowly following adult thymectomy. They are also sensitive to the in vivo administration of ATS(antithymocyte serum) and they express high levels of Pgp-1. The kinetics of lymphokine secretion and the phenotype of the cells support the hypothesis that lymphokine secretion from fresh lymphoid cells comes from a population of memory cells. In contrast we find that we can also stimulate a separate, ATS-resistant population to become lymphokine-secreting cells after four days of *in vitro* priming.These primed cultures rapidly synthesize an secrete large amounts of IL4 and IL5 in addition to IFNg , IL3 and GMCSF(A phenotype which could be combination of both Th1 and Th2 helpers), when they are restimulated with Ag or mitogen. The cells whic are responsible are CD4 positive and have a shorter lifespan since they decline considerably after adult thymectomy. We suggest that the lymphokine secreting cells detected after priming come from a population(s) of helper T cell precursors which have differentiated to become effectors. This generation o effectors requires lymphokines, especially IL4 and/or IL2 and APCs. Thus the development of helper Th appears to follow a similar pathway as that of cells of the B cell lineage developing into Ab-secreting cells and CD8 positive T cells which develop into cytotoxic effectors from precursors.

C 670 IL-2 PRODUCTION IN SYPHILITIC RABBITS, Mark A. Tomai, Suzanne M. Warmka, Barbara J. Elmquist and Thomas Fitzgerald, Department of Medical Microbiology and Immunology, University of Minnesota-Duluth, School of Medicine, Duluth, MN 55812

Interleukin-2 production by T cells has been shown to be required for both humoral as well as cell-mediated immune responses. Thus, IL-2 production was measured in syphilitic rabbits as a function of their immune response. Maximal IL-2 production induced by Con A at 10-14 days post-infection was only 1/2 that observed for uninfected rabbits and this correlated well with a decrease in T cell proliferation (< 35% that of normal rabbits) upon stimulation with Con A. This decrease in IL-2 production in infected rabbits was restored upon removal of most of the adherent cells. Furthermore, the IL-2 production by 10-14 day infected spleens was restored above normal levels upon addition of indomethacin. This decrease in IL-2 levels was not due to an increase in the ability of infected spleen cells to adsorb IL-2. Finally, studies assessing IL-2 production at various times postinfection indicated that at 4 days post-infection IL-2 levels were higher than normal, however as early as 10-14 days after infection IL-2 levels decreased below normal levels and continued to be depressed as late as 30 days post-infection. These results may explain why all organisms are not eliminated during primary infection with T. pallidum and why secondary and tertiary phases of the disease may develop.

C 671 MOLECULAR CLONING AND IMMUNOLOGICAL ANALYSIS OF THE MEASLES VIRUS F-GENE CODING FOR THE FUSION PROTEIN.

José P. Versteeg-van Oosten*, Simon A. Langeveld*, Petra de Vries‡. Ad D.M.E. Osterhaus‡, Fons G.C.M. UytdeHaag, Harry O. Voorma, Peter J. Weisbeek*. *State University of Utrecht, Department of Molecular Cell Biology, Utrecht, The Netherlands. ‡National Institute of Public Health and Environmental Hygiene, Bilthoven, The Netherlands.

Our research is focused on the development of a subunit vaccine against measles virus. It involves the immunologic characterisation of the two surface proteins: the fusion (F) and the haemagglutinin (HA) protein. It has been shown that especially the antigenic presentation of the fusion protein is important for eliciting a functional immune response. To study the immunologic properties of the F protein, we expressed the F gene in E.coli as a β-galactosidase-F-fusion protein after insertion in a pEX vector. We constructed deletion mutants with fragments generated with restriction enzymes and with the polymerase chain reaction method. Using a panel of monoclonal antibodies a rough epitope mapping has been performed. Two area's were found on the protein, with one area two monoclonal antibodies react and with an other area four monoclonal antibodies react. Both area's were found in F1, the c-terminal part of the protein. The pepscan method was used to fine map the epitopes of the monoclonal antibodies reacting with the second area on the primary sequence.

C 672 CD4+ T CELLS ARE NEEDED FOR THE INDUCTION OF CD8+ EFFECTOR CELLS AGAINST MALARIA SPOROZOITES, Walter Weiss, Martha Sedegah, and Richard Beaudoin, Naval Medical Research Institute, Bethesda, MD 20814

In at least one viral system, CD8+ effector cells can be induced in animals lacking CD4+ T cells. Since CD8+ effector cells are important in immunity to malaria sporozoites, we wished to know if they,too, could be generated without help from CD4+ cells. We depleted BALB/c mice of their CD4+ T cells by injection of an anti-CD4 monoclonal antibody, and then tried to immunize them with irradiated Plasmodium yoelii sporozoites. When challenged with infectious sporozoites, these mice were not protected against malaria infection. Although they did not make antibodies to sporozoites, passive transfer of hyperimmune serum into these animals still did not protect them against a sporozoite infections. CD8+ T cells from these animals functioned normally in in vitro assays against TNP labelled targets.

It appears that, unlike viral systems, the generation of CD8+ effectors in malaria requires CD4+ helper cells. Thus both CD4 and CD8 epitopes should be included in any synthetic vaccine against malaria sporozoites.

C 673 DIFFERENTIATION OF CLONED PRE T CELLS INTO T CELLS IN VITRO, Katsuyuki Yui, Junji Hamuro, and Mark I. Greene. Department of Pathology & Lab. Med., Univ. Pennsylvania, Philadelphia, PA 19104

Differentiation of T lymphocytes takes place primarily in the thymus. Precursors of T cells migrate from fetal liver or bone marrow, rearrange T cell receptor (TCR) genes, express TCR, undergo thymic selection and finally emerge as mature single positive T lymphocytes. Most studies of thymic T cell development have been performed by using polyclonal populations of T lymphocytes, which have made the interpretation of the results complicated.

In order to clarify the thymic development of T cells, we have established cloned precurssor T cells (C9 clone) from nude mice by culturing nylon wool non-adherent $CD4^-CD8^-$ spleen and lymph node cells in the presence of WEHI3 supernatant and Con A supernatant. The phenotype of the C9 clone was $Thy\text{-}1^-$ $CD3^-CD4^-CD8^-IL2R(IL2\ receptor)^-$ and they have been maintained more than 16 months without changing phenotype. When the C9 clone was stimulated with IL4, IL1/IL2, IL1/IL6, GM-CSF, the cells were induced to express Thy-1, TCR and IL2R proteins. However, culture of the cells with GM-CSF/IL2 did not induce the expression of these molecules. Southern blotting of the DNA isolated from GM-CSF/IL2 culture suggested that they have undergone partial $D\beta1\text{-}J\beta1$ rearrangement. The cultured cells were then recloned twice by limiting dilution. The cloned cells were again shown to induce expression of CD3 complex by the stimulation of IL4 enriched medium. Therefore, we have established a system in which to induce differentiation of cloned pre T cell line into TCR^+ cells in vitro.

C 674 PRODUCTION OF INTERLEUKIN 6 AND TUMOR NECROSIS FACTOR ALPHA BY THYMIC STROMAL CELLS AND IMMATURE THYMOCYTES. Albert Zlotnik, Takashi Suda, and Frank Lee. DNAX Research Institute, Palo Alto, CA 94304. We have previously reported that either interleukin 6 or tumor necrosis factor α induce thymocyte proliferation when used with phytohemagglutinin (PHA). When IL-4 and phorbol ester (PMA) are used to induce thymocyte proliferation, IL-6 induces an marked enhancement of proliferation in CD4+8- thymocytes. These observations indicate that both TNFα and IL-6 are important molecules regulating thymocyte proliferation. We therefore investigated which cells within the developing thymus produce these mediators. Both a thymic epithelial cell clone (3D.1) and a thymic macrophage clone (IG18.LA) produce IL-6 as demonstrated by mRNA analysis and bioactivity. Efforts to find IL-6 in a cDNA library from unstimulated CD4-8- thymocytes were unsuccessful. IG18.LA but not 3D.1 produced TNFα. Supernatants from both adult CD4-8- and fetal day 15 fetal thymocytes contained TNFα following stimulation with calcium ionophore and PMA. These observations indicate that several cell lineages within the developing thymus are able to produce TNFα and IL-6.

C 675 A SECRETED FORM OF THE HUMAN LYMPHOCYTE CELL SURFACE MOLECULE CD8 ARISES FROM ALTERNATIVE SPLICING, P. Giblin*, J.A. Ledbetter, and P. Kavathas*, *Department of Laboratory Medicine, Yale University School of Medicine, New Haven, CT 06510, and Oncogen, Seattle, WA 98121

The human lymphocyte differentiation antigen CD8 is encoded by a single gene which gives rise to a 32 kDa glycoprotein expressed on the cell surface as a dimer, and in higher molecular weight forms. We demonstrate that the mRNA is alternatively spliced such that an exon encoding a transmembrane domain is deleted. This gives rise to a 30 kDa molecule that is secreted and exists primarily as a monomer. Messenger RNA corresponding to both forms is present in peripheral blood lymphocytes (PBL), Con A activated PBL and three CD8+ T cell lines with the membrane form being the major species. However, differences in the ratio of mRNA for membrane CD8 (mCD8) and secreted CD8 (sCD8) exist. In addition, the splicing pattern we observe differs from the pattern found for the mouse CD8 gene. This mRNA is also alternatively spliced, but an exon encoding a cytoplasmic region is deleted giving rise to a cell surface molecule which differs in its cytoplasmic tail from the protein encoded by the longer mRNA. Neither protein is secreted. This is one of the first examples of a different splicing pattern between two homologous mouse and human genes giving rise to very different proteins. This represents one mechanism of generating diversity during speciation.

C 676 SUBSETS OF RAT $CD4^+$ T CELLS DEFINED BY THEIR DIFFERENTIAL EXPRESSION OF VARIANTS OF THE CD45 ANTIGEN: DEVELOPMENTAL RELATIONSHIPS AND IN VITRO AND IN VIVO FUNCTIONS, Fiona M Powrie and Don Mason. MRC Cellular Immunology Unit, Sir William Dunn School of Pathology, University of Oxford, OXFORD OX1 3RE.

$CD4^+$ T cells in the rat can be divided into two non-overlapping subsets by their reactivity with the monoclonal antibody MRC OX-22 which binds some of the high molecular weight forms of the CD45 antigen. Recent work, to be described has shown that the two subsets represent different stages of T cell maturation, with distinct T cell functions. The lymphokine repertoire of the memory T cell pool will be discussed with reference to the antigenic environment from which the cells are obtained.

C 677 THE NATURE OF T-T COLLABORATION IN THE REJECTION OF CLASS I MHC-DISPARATE PANCREATIC ISLET ALLOGRAFTS, Gill, Ronald G. and Lafferty, Kevin J., Barbara Davis Center for Childhood Diabetes / Univ. Colo. Health Sciences Center, 4200 E. 9th Ave, Box B-140, Denver, CO. 80262

We studied the cellular interactions required for the rejection of cultured MHC class I-disparate islet allografts. This model was suitable for studying T-T collaboration in that islet allograft immunity is CD4 dependent but rejection of the cultured islet graft is mediated by the CD8 cell. Recipient C57Bl/6 (B6) mice were grafted with MHC class I-disparate B6.C-H-2bm1 (bm1) islets beneath the renal capsule. Islet grafts were pretreated for 7 days in 95% oxygen culture to reduce immunogenicity. Thirty days after grafting, recipient mice were immunized with 10e6 live spleen cells from the strains indicated below. Rejection of the established graft was not triggered by challenge with donor-type bm1 spleen cells, indicating that the MHC class I stimulus was insufficient to initiate allograft immunity. Further, immunization with a mixture of 10e6 bm1 and 10e6 MHC class II-disparate B6.C-H-2bm12 (bm12) spleen cells failed to trigger host immunity. However, challenge with 10e6 (bm1 x bm12)F1 spleen cells triggered acute rejection of the established bm1 islet grafts. The requirement for linked presentation/recognition of class I and class II allo-antigens to trigger allograft immunity indicates that the antigen-presenting (APC) plays an essential role for T-T collaboration in vivo.

Spleen Cell Challenge	# Grafts Rejected / Total
none	0/3
bm1	0/6
bm12	02
bm1 + bm12	0/5
(bm1 x bm12)F1	5/5

Late Additions

C 700 DIFFERENTIAL EXPRESSION OF HUMAN AND MURINE CLASS I GLYCOPROTEIN IN A HUMAN T-B HYBRID, Jeff Alexander, Alan Payne, Richard Murray, Jeffrey Frelinger and Peter Cresswell, Department of Microbiology and Immunology, Duke University Medical Center, Durham, NC 27710 and Department of Microbiology and Immunology, University of North Carolina, Chapel Hill, NC 27599

Human or murine class I genomic DNA was transfected into a B-lymphoblastoid x T-lymphoblastoid hybrid cell line. This fusion hybrid has lost both T cell derived copies of chromosome six and contains deletions spanning the class II region on both copies of chromosome six derived from the B-cell parent. Previous data have described a trans-acting factor within this region that is responsible for class I antigen expression. HLA-Bw58 and B7 glycoproteins, although synthesized, were not transported to the plasma membrane in the hybrid. Surprisingly, H-2 class I glycoproteins K^b and D^p were surface expressed. These data suggest a fundamental difference between human and mouse histocompatibility antigens in their requirements for intracellular transport. The role of glycans in this transport dicotomy is currently under investigation. In addition, hybrid human-murine genes are being used to identify regions of the class I molecules involved in this transport phenomenon.

C 701 COMMONALITY OF THE B CELL AND T CELL REPERTOIRES FOR INFLUENZA HAEMAGGLUTININ IN THE $H\text{-}2^d$ HAPLOTYPE, Barbara C. Barnett, Christine M. Graham, David S. Burt, John J. Skehel and D. Brian Thomas, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA.

An extensive analysis of the Class II ($I\text{-}A^d$)-restricted T cell repertoire for influenza haemagglutinin (HA) of the H3 subtype, elicited by natural infection, has shown that a majority of $CD4^+$ memory T cell clones focus on an antibody binding region of HA, site B. Synthetic peptides defined two distinct T cell epitopes within the primary sequence of site B, HA1 177-199 and HA1 186-200, and $CD4^+$ clones were sensitive to the same single amino acid substitutions, at residues 193 or 198, in mutant viruses and in synthetic peptide analogues, that abrogated BALB/c antibody recognition of the native HA. Furthermore, competitive inhibition studies together with comparisons of the proliferative responses to mutant viruses and the corresponding peptide analogue showed that these single substitutions within an antibody binding site affected antigen processing, or TCR or Class II interaction, depending on the specificity of the $CD4^+$ clone. Such extensive commonality of the BALB/c B and T cell repertoires for HA suggests that the antigen-specific B memory cell may be instrumental in selection of the peripheral T cell repertoire.

C 702 IDENTIFICATION OF A HUMAN CYTOLYTIC T CELL'S PEPTIDE-MHC STIMULATION COMPLEX, AND CHARACTERIZATION OF ITS INTRACELLULAR GENERATION, Lawrence R. Brown, Vivian L. Braciale and Thomas J. Braciale, Department of Pathology, Washington University School of Medicine, St. Louis, MO 63110

We have defined the components of the antigenic complex recognized by a human, influenza specific, class II MHC restricted, cytolytic T lymphocyte clone. With synthetic peptides and sequencing of viral RNA from a panel of closely related stimulatory and non-stimulatory strains, we have identified the stimulatory sequence between residues 129 and 140 of the A/JAPAN/57 hemagglutinin (HA). Furthermore, we have demonstrated the critical role of certain specific residues between positions 130(Lys) and 139(Gln). The use of DR 11 as the restriction element was shown by differential recognition of a series of HLA typed B lymphoblastoid target cell lines.

We probed the means by which the antigen presenting cell (APC) handles the antigen to generate and present the recognition complex to the T cell by introducing the HA sequence to the APC via a variety of routes. The differences in characteristics and requirements for T cell recognition of APCs exposed to recombinant vaccinia, synthetic peptides, purified protein, or infectious or inactivated influenza virus provided insight into the intracellular pathway used to generate the peptide-MHC antigenic moiety.

C 703 A DELETION IN INFLUENZA HAEMAGGLUTININ THAT AFFECTS ANTIGEN PROCESSING FOR CD4$^+$ T CELLS, David S. Burt, Jacob Hodgson, Kingston H.G. Mills, John J. Skehel and D. Brian Thomas, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA.

The specificity of an I-E^k-restricted T-helper clone, 3F10, was mapped to the sequence HA1 226-245 of X31 virus (H3N2 sub-type) using synthetic peptides. In T cell proliferation assays 3F10 was non-responsive towards a laboratory mutant virus of X31 with a deletion between HA1 residues 224-230. Furthermore, whilst this clone was stimulated optimally by a tryptic cleavage fragment of HA, HA1 28-328, from X31, it failed to recognise an equivalent fragment, HA1 28-328 del., derived from the deletion mutant. However, when HA1 28-328 del. was denatured, reduced and carboxymethylated, recognition by 3F10 was restored, indicating that the epitope seen by this clone was still intact in the deletion mutant. Both the mutant virus and HA1 28-328 del. were optimally recognised by T cell clones specific for other regions of HA1, suggesting that the deletion did not affect the uptake of mutant virus by antigen presenting cells. These results suggest that residues within the sequence HA1 224-230 in X31 are not essential to the epitope seen by 3F10, but may be required for processing and presentation of the epitope seen by this clone from native HA.

C 704 MACROPHAGE-SPECIFIC DIFFERENCES IN IA TURNOVER LINKED TO THE *Bcg* GENE. Bobby J. Cherayil and Shiv Pillai, MGH Cancer Center, Harvard Medical School, Boston, MA 02129. *Bcg (Lsh/Ity)* is a murine gene which determines innate resistance or susceptibility to infection by mycobacteria, leishmania and salmonella and is a useful model for studying the genetic basis of susceptibility to infectious diseases such as tuberculosis and leprosy. The *Bcg* gene is located on chromosome 1 and has pleiotropic effects on macrophage function. Recently, Zwilling *et. al.* (*J.Immunol.*1987;136:1372) have suggested that differences in macrophage Ia protein stability may be linked to this gene. We have examined this possibility by carrying out pulse-chase experiments in primary peritoneal macrophages and splenic lymphocytes of DBA/2 (*Bcg* resistant) and BALB/c (*Bcg* susceptible) mice. In splenic lymphocytes from both strains of mice, and in peritoneal macrophages from DBA/2 mice, the Ia alpha and beta chains had a half-life exceeding 24 hours. In contrast, in macrophages from BALB/c mice they had a half-life of only a few hours. Based on the macrophage-specific instability of the Ia protein, two models for the putative biological function of the *Bcg* gene will be discussed.

C 705 INTERNAL IMAGES AND T-CELLS, W.L. Cleveland, Dept. of Medicine, St. Luke's/Roosevelt Hosp. Center and Dept. of Microbiology, Columbia Univ., New York, NY 10032

The suggestion by Jerne and by Lindenmann that antibody idiotopes may imitate epitopes on conventional antigens and thereby function as internal images has recently been supported by a number of experimental studies. As initially formulated, the internal image concept refers to **native idiotopes and epitopes.** Recent evidence that internal images can induce both B-cell and T-cell immunity raises the issue of internal image recognition by T-cells. Most evidence indicates that T-cells recognize antigen after proteolytic cleavage to produce peptides that bind to MHC-molecules. We propose the existance of a new type of internal image in which immunoglobulin v-region peptides, formed by processing, imitate peptides from conventional antigens. We refer to such denatured internal images as **residue internal images,** since they are associated with the residue of peptides remaining after processing. In some cases, residue internal images may be actual **sequence images,** i.e., the v-region sequence may be identical to the conventional antigen sequence. To be effective, an internal image vaccine may need to bear both native and residue internal images. The residue internal image concept suggests a new dimension of idiotype regulation.

C 706 LACK OF DEMONSTRABLE ENDOCYTOSIS OF B LYMPHOCYTE MHC CLASS II ANTIGENS, Janet E. Davis and Peter Cresswell, Department of Microbiology and Immunology, Duke University Medical Center, Durham, NC 27710

Mature HLA-DR complexes are purported to spontaneously internalize from and recycle to the plasma membrane of B but not T lymphocytes. Using a neuraminidase protection assay, we have radiolabeled surface class II antigens on intact cells and cultured these cells under conditions which permit or prevent endocytosis; subsequently, surface glycoproteins on viable cells were desialylated and class II molecules were analyzed by immunoprecipitation and two-dimensional gel electrophoresis. A panel of human B lymphoblastoid cell lines and activated tonsillar B cell blasts failed to exhibit any internalization of class II complexes; control transferrin receptor molecules were endocytosed as ascertained by insensitivity to neuraminidase digestion. Class II^+ PHA blasts and Sezary cells of the T lineage were also deficient in detectable HLA-DR internalization. Results did not vary regardless of the time allowed for efficient endocytosis (1 h to 16 h), the choice of radiolabel used (^{125}I or ^{35}S-methionine), or the addition of anti-class II monoclonal antibody during the chase period for endocytosis. Therefore, within the limits of sensitivity of this assay, class II complexes do not appear to be internalized, either spontaneously or when cross-linked by antibody. In view of these results, we do not believe that endocytosis and recycling represent a dynamic pathway for regulating surface expression of class II antigens or a means of associating with and presenting foreign antigenic peptides. Supported in part by USPHS grants #5 T32 CA09058-14 and #5 RO1 AI23081-03.

C 707 HELPER T CELL DERIVED BINDING FACTORS INITIATE CONTRASUPPRESSION TO ENHANCE ISOTYPE-SPECIFIC ANTIBODY RESPONSES, Peter B. Ernst, Tim Quinn and Lenore Zettol, Intestinal Disease Research Unit, McMaster University, Hamilton, ONT. Canada, L8N 3Z5

Within 6 hrs of immunization, serum contains an aggregate of antigen, immunoglobulin (Ig) and T cell derived factors which activate the "antisuppressor" T cell circuit that enhances isotype-specific responses by competing with suppression. We have characterized the T cells and factors that mediate this response and examined their relationship to contrasuppression.

To generate the factors in vitro, antigen-pulsed macrophages were co-cultured with T cell preparations. These supernatants were then mixed with a source of Ig and tested for their ability to enhance SRBC-specific PFC in supressed cultures. Supernatants from the co-cultures of antigen-pulsed macrophages and T cells were incapable of initiating contrasuppression unless a source of Ig was provided. T cells from the intestinal Peyer's patches produced factors which enhanced IgA while splenic T cells selected for IgG. By absorbing the supernatants against monoclonal IgG or IgA attached to a solid phase, we showed that isotype-specific binding factors (BF) were necessary for the enhancement of IgG and IgA respectively. Furthermore, we could reconstitute the BF depleted supernatants with the adherent fraction. IL-1 stimulated $CD4^+$ $CD8^-$ T cells provided the BF for this molecular interaction. V. villosa adherent T cells from naive mice could, when activated with this molecular aggregate, enhance antibody responses when added to a suppressed culture.

Thus, upon stimulation with IL-1, $CD4^+$ cells produce isotype-specific BF which interact with antigen and Ig to activate the contrasuppressor inducer cell thereby initiating enhancement of isotype-specific antibody responses. This work was supported by the MRC of Canada.

C 708 DOWNREGULATION OF AN ANTIGEN-SPECIFIC AUTOIMMUNE RESPONSE BY SELECTIVE INHIBITION OF REGULATORY T CELL FUNCTION C. Kelly, Renal Section and Graduate Group in Immunology, Univ. of Penn.,Phila. PA., 19104.

In previous studies of antigen-specific T cell responses in two distinct models of autoimmune tubulointerstitial nephritis (TIN), Vicia villosa lectin binding (VV^+) T cells have been shown to be necessary for effector T cell expression and mediation of TIN. In anti-tubular basement membrane disease, antigen-specific VV^+ T cells direct the phenotypic selection of $CD8^+$ nephritogenic T cells in susceptible mouse strains (J. Immunol. 141, Nov. 1,1988) . This function is mediated by an antigen-binding, $I\text{-}J^{S+}$ soluble protein factor. Current studies investigate the role of the T cell glycoprotein which binds VV lectin in mediating VV^+ T cell function. Using the previously described effector T cell induction assay, we found that N-acetyl-d-galactosamine (GalNac) (at 25 mM but not 2.5 mM) inhibits VV^+ T cell function and $CD8^+$ effector T cell selection. When $CD8^+$ effector T cell differentiation occurs in the presence of soluble factors derived from antigen-primed VV^+ T cells, GalNAc is not inhibitory. These studies suggested that soluble Gal NAc may competitively bind to a soluble protein which stimulates VV^+ T cells, in part by binding to the VV lectin receptor, to synthesize and/or secrete their biologically active soluble factor. As an additional test of this hypothesis, we prepared detergent solubilized membranes from VV^+ T cells and purified VV lectin binding proteins by affinity chromatography. Like GalNAc, these membrane derived lectin binding proteins also inhibit VV^+ T cell function and $CD8^+$ effector T cell selection. Inhibition by soluble "lectin receptors" is dose dependent and is demonstrable with lectin binding glycoproteins derived from 15-20 x 10^6 cells, in an assay utilizing 10 x10^6 VV^+ cells. We are now further characterizing the lectin receptor and its endogenous ligand.

C 709 A METHOD FOR THE IDENTIFICATION OF THE IMMUNODOMINANT EPITOPE OF CHLAMYDIA TRACHOMATIS, W. J. Knowles, E. D. Huguenel, B. Haigh, T. Michaud, A. C. Ohlin, G. Davis and V. T. Marchesi, Molecular Diagnostics, Inc., West Haven, CT 06516.

Elementary bodies and outer membranes of Chlamydia trachomatis produce a high-titered IgG response in rabbits and mice as measured by ELISA and microscopic immunofluorescence assays. Western blot analysis of total elementary body protein identifies a 40kD major outer membrane protein (MOMP) as the predominant antigen. To identify the chemical structure of the epitope, purified MOMP was subjected to chemical and enzymatic fragmentation and the resulting peptides were purified by HPLC and assayed for immunoreactivity. An immunoreactive 6kD cyanogen bromide peptide was amino-terminal sequenced and a series of overlapping synthetic peptides were synthesized and assayed for immunoreactivity. Sequential single amino acid deletions at both the NH_2 and COOH termini allowed us to identify the precise epitope as a 12 amino acid peptide spanning residues 291-302 of MOMP. Two amino acid substitutions at positions 293 (PHE→GLY) and 300 (PRO→GLY) completely eliminated antibody binding. The 12-amino acid synthetic peptide is a potent immunogen producing high-titered antibody responses that are specific for the MOMP molecule.

C 710 **EVIDENCE FOR THE ROLE OF A TRANS-ACTING GENE IN THE TRANSPORT AND EXPRESSION OF HLA CLASS I GLYCOPROTEINS, Carilee A. Lamb and Peter Cresswell, Department of Microbiology and Immunology, Duke University Medical Center, Durham, NC 27710**

Posttranslational regulation of HLA class I antigen expression is being studied in a variant B-LCL x T-LCL somatic cell hybrid which has greatly reduced levels of surface class I. This phenotype has previously been shown to result from loss of an HLA-linked trans-acting gene. Analysis of an independently derived mutant harboring the same defect has shown that this trans-acting gene is not required for transport of class II molecules. Class I heavy chain is synthesized in this cell line and associates with β_2m. Transport of the class I appears to be blocked in the ER or cis-Golgi as the majority of the class I glycoproteins are not processed to the Endo H resistant form. The ability of the cell to significantly increase expression of surface class I when the incubation temperature is lowered from 37°C to 22°C suggests that this gene may function to stabilize a particular conformation of the protein. Consistent with this is the increased sensitivity of class I molecules in the mutant as compared to the parent to degradation when cell lysates are incubated at elevated temperatures. The inability to immunoprecipitate class I antigens in the mutant is possibly due to the action of endogenous proteases present in these lysates. Two complementing approaches are being employed to isolate this gene and further analyze its role in class I biosynthesis. The first involves inactivation of the trans-acting gene by insertion of a retroviral vector and subsequent PCR amplification of regions flanking the vector. In another approach a cDNA library will be introduced into the mutant cell line and the cDNA will be reisolated from cells reexpressing surface class I.

C 711 IMMUNITY TO IMMUNOGENIC TUMOR VARIANTS: ANALYSIS OF VARIANT-SPECIFIC AND PARENTAL CROSS PROTECTIVE IMMUNITY *IN VIVO*. Stephen J. LeGrue and William Simcik. M. D. Anderson Cancer Center, Houston, TX. We have induced a panel of highly immunogenic (Imm+) variants of the murine fibrosarcoma MCA-F using 1-methyl-3-nitro-1-nitrosoguanidine (MNNG), 5-aza-2'-deoxycytidine (5-azaCdR), and UV radiation. These tumors grew in immunosuppressed mice, but were completely rejected by normal syngeneic hosts. Mice that had rejected large numbers of Imm+ also developed a strong, tumor-specific immunity to the parental tumor. Immunization with low numbers of Imm+ engendered only variant-specific immunity. The frequency of Imm+ variant generation was similar for the three induction different protocols (64% to 92%), suggesting that generation of Imm+ was more closely related to the cell line used than to the inducing agent. However, the strength of the Imm+ phenotype was related to the agent used, since MNNG induced clones had the strongest immunogenicities and UV-B the weakest. The strong neoantigens expressed by MNNG induced Imm+ were variant-specific, while UV and 5-azaCdR induced clones displayed significant cross-reactivities not attributable to the parental tumor antigen. Increased or inappropriate expression of class-1 MHC antigens did not correlate with the Imm+ phenotype. We investigated the phenotypes of the spleen cells mediating tumor rejection using the local adoptive transfer assay (LATA). Variant-specific immunity to MNNG, 5-azaCdR and UV induced Imm+ were all mediated by Thy1.2+, L3T4+, Lyt2.1- T cells. After immunization with high numbers of Imm+ to engender both anti-Imm+ and anti-parental immunity, both CD4+ and CD8+ effectors rejected the Imm+ in LATA, while only the CD4+ T cells could transfer resistance to the parent. Immunity to the parental tumor antigen engendered by the Imm+ suggested associative recognition of the parental and neoantigens together on the cell surface. This hypothesis was supported by failure of Imm+ to protect against an antigenically distinct tumor (MCA-D) admixed with it, either at the time of immunization or at challenge. Fusion of the Imm+ variant with MCA-D yielded a unique, hybrid parental tumor antigen that was associatively recognized with the original Imm+ neoantigen, demonstrating the importance of antigen co-expression. Grant RR-5511-23.

C 712 ENHANCED IMMUNE RESPONSE TO BOVINE SERUM ALBUMIN (BSA) IN A LOW RESPONDER STRAIN OF MICE IS INDUCED BY STRUCTURALLY MODIFIED ANTIGEN.

J. Gabriel Michael, Patricia L. Domen, Allen Litwin and Annette Muckerheide, Department of Molecular Genetics, Biochemistry and Microbiology, University of Cincinnati Medical Center, Cincinnati, Ohio 45267.

We have recently demonstrated and reported that substitution of anionic side chain carboxylic groups with aminoethylamide groups on protein antigens exhibits a pattern of enhanced immunogenicity both *in vivo* and *in vitro*. This enhanced immunogenicity was also observed in low responder strains of mice and we investigated the mechanism by which it is achieved. We examined antigen processing and presentation of native (nBSA) and modified BSA (mBSA) to T helper cells isolated from C57/BL low responder mice. A greatly reduced amount of mBSA than nBSA was required to activate both nBSA and mBSA primed Th. Proliferation of nBSA and mBSA primed T cells increased in proportion to the amount of time of exposure of the antigen presenting cells (APC) to nBSA, peaking at 8h. Conversely, APC required less than 30 min exposure to mBSA to achieve optimal activation, indicating rapid uptake of mBSA. Paraformaldehyde fixed APC recognized mBSA without a lag phase processing, indicating that this event also occurred quite rapidly. APC processed nBSA was presented to primed T cells more effectively than the soluble antigen as shown by the increased rate of T cell proliferation. In contrast, mBSA was equally well presented to Th cells by APC as in soluble unprocessed form. Our data demonstrate that the reduced response in low responders is greatly enhanced by a modified antigen which is rapidly taken up and processed by APC.

C 713 DIFFERENCES IN ANTIGEN AND ANTI-Ig PROCESSING BY TRINITROPHENYL ANTIGEN-BINDING CELLS (TNP-ABC). C.D. Myers and E.S. Vitetta. UT Southwestern Med. Ctr., Dallas, TX 75235.

B cells which bear surface immunoglobulin (sIg) receptors specific for a particular antigen are abile to present fragments of that antigen very efficiently to T cells. This is due, in part, to the high affinity of the receptor, which facilitates antigen binding at low concentrations. Using TNP-ABC and specific antigen, we have demonstrated that the TNP-ABC process antigen very effectively. We have compared specific antigen with its polyclonal analog, anti-Ig, and demonstrated differences in the kinetics of degradation of anti-Ig and TNP-antigens by TNP-ABC. Both antigen and anti-Ig bound by TNP-ABC are degraded into small fragments which are released into the supernatant. However, the following differences have been found: 1) The rate of release of small fragments of TNP-antigen parallels the rate at which these cells become able to directly conjugate with T cells (a measure of antigen presentation), reaching a plateau between 4 and 6 hours. In contrast, the degradation of anti-Ig and release of fragments continues for 12 hours. 2) Analysis of initial kinetics demonstrated that release of fragments of TNP-antigen begins 15 minutes after binding; there is no significant release of anti-Ig fragments until about 30 minutes. 3) In contrast to anti-Ig where there is significant accumulation of degradation intermediates within the cells, there is very little intracellular accumulation of intermediate-size fragments of TNP-antigen. Thus, we propose that the processing of antigen bound *via* specific sIg may involve a specific intracellular pathway and that intracellular routing may be determined either by the degree of cross-linking of sIg induced by antigen *vs* anti-Ig or the mode of interaction of the various ligands with sIg.

C 714 ANALYSIS OF THE γ/δ T CELL RECEPTOR OF A NORMAL HUMAN THYMOCYTE CLONE, A. Okada*, I. Bank+, L. Chess+ and F. Alt*, Departments of Biochemistry (*) and Medicine (+), College of Physicians and Surgeons of Columbia Univerity, New York, New York 10032.

We have recently analysed the structure of the γ/δ T cell receptor (TCR) expressed by the normal human thymocyte clone CII. CII expresses a $C_\gamma 2$ constant region that is a polymorphic form lacking a copy of an internal exon; the sequence of this constant region accounts for the size of the γ chain and noncovalent linkage of γ and δ chains in the CII TCR. In order to elucidate its role, this γ/δ TCR will be reconstituted in immortalized T-cell lines. In addition, the productively rearranged human γ/δ receptor will be transgenically introduced into mice in order to assess the effect of the complete receptor on the development of T cells.

C 715 CYTOTOXIC T-CELLS RECOGNIZING RESPIRATORY SYNCYTIAL VIRUS (RSV) FUSION PROTEIN CLEAR VIRUS BUT INCREASE LUNG PATHOLOGY IN RSV-INFECTED MICE, Peter J.M. Openshaw & Martin J. Cannon, National Institute for Medical Research, London, England NW7 1AA.
RSV-induced lung disease is known to be frequent and severe in previously immunized children, but the cause of this reaction is unknown. We have described RSV clearance in mice by a CTL line and clone of unknown antigen specificity, which both increase lung pathology in infected mice (Cannon, Openshaw and Askonas, J.Exp.Med 168:1163). We now report the in vivo effects of H11a, a fusion protein specific $CD8^+$ K^d restricted CTL clone (Cannon and Bangham, J.Gen.Virol. in press). I.V. injection of H11a accelerates RSV clearance from the lungs of infected mice but causes lung haemorrhage, neutrophil efflux and cachexia. Both CTL line and H11a recognize RSV serotypes A and B in vitro, and augment lung disease in A and B infected mice in vivo. Flow cytometric analysis of stained cells from the lungs of infected CTL recipients shows no selective increase in cells of the injected phenotype. We conclude that CTL recognizing diverse RSV antigens can augment lung disease in RSV infected mice, and that simple enumeration of T-cell phenotype does not necessarily identify the cells responsible for disease.

C 716 GENERATION OF AN INTERNAL IMAGE ANTI-IDIOTYPIC ANTIBODY DIRECTED AGAINST HUMAN ANTIBODIES TO HUMAN IMMUNODEFICIENCY VIRUS P24 ANTIGEN, Steven M. Rosen, Candace C. Allen, Harlan W. Waksal, and Samuel D. Waksal, ImClone Systems, Inc. New York, NY 10014

The humoral immune response to human immunodeficiency virus has been shown to contain antibodies which act to mediate the uptake of virus through Fc receptor mediated mechanisms. It is therefore possible that vaccination with the entire envelope polypeptide may present immunologic determinants that enhance infection. One means by which to generate an immune response to HIV that shall possess neutralizing activity in the absence of infection enhancing activity is to generate anti-idiotypic Abs that bear the internal image of neutralizing human antibodies directed against HIV.

We affinity purified human antibodies from HIV^+ patients on a viral lysate column. We have produced 15 monoclonal anti-idiotypic antibodies directed against these Ab_1's. Two of these monoclonals were shown to be Ag inhibitable by their ability to inhibit the binding of polyclonal human antisera to HIV viral lysate on Ortho HIV Ab test wells. One monoclonal, 8B8, when coupled to KLH and used to immunize mice, produced an Ab_3 that bound to viral lysate in an ELISA assay. An affinity column containing 8B8 was used to purify an Ab_1 that was shown to bind to p24 and p17 by Western blot analysis. These data suggest that 8B8 may be a potential vaccine candidate.

C 717 TRANSGENIC ANTI-SELF $CD4^-/CD8^-$ PERIPHERAL CTL EXHIBIT FUNCTIONAL MATURITY WHEN STIMULATED BY THE ANTI-CLONOTYPE John H. Russell, Deborah E. McCulley, Christopher A. Nelson, William C. Sha and Dennis Y. Loh, Departments of Medicine and Pharmacology, Washington University Medical School, St. Louis, MO 63110

We have recently described a transgenic mouse model which co-expresses the TCR α and β chains from the 2C cell line (recognized by the 1B2 anti-clonotype). T cells bearing the transgenic clonotype are positively selected by elements of the $H\text{-}2^b$ MHC for expression on $CD8^+$ cells. Thus in the periphery of $H\text{-}2^b$ animals 40-80% of the T cells are $1B2^+/CD8^+$. The same peripheral expression is observed when the transgenes are expressed in F1 animals bearing a "neutral" MHC haplotype (eg. $H\text{-}2^{b/s}$). However, when the transgene is expressed in F1 animals which also express the $H\text{-}2L^d$ gene product, negative selection occurs by clonal deletion. However, this deletion is functional rather than structural as the 1B2 clonotype is present on 10-40% of peripheral T cells. These cells are unusual in that they express neither of the characteristic peripheral molecules CD4 or CD8. The absence of CD8 expression on the $1B2^+$ cells appears to allow these potentially self-reactive clones to exist without evidence of autoimmunity. The original clone as well as $1B2+/CD8^+$ cells from $H\text{-}2^b$ animals are strongly inhibited by anti-CD8 reagents. In an effort to understand the process of negative selection and self-tolerance we have examined the capacity of these cells to be activated directly by the anti-clonotype rather than antigen ($H\text{-}2L^d$). The results demonstrate that the clonotype is fully functional on these double negative cells, indicating a normal maturation in the thymus. Further examination of their surface phenotype also supports the conclusion that these are fully mature cells which are phenotypically distinct from double negative cells which exist in the thymus of $H\text{-}2^b$ animals.

C 718 CLASS I MHC RESTRICTED CTL AND ANTIBODY RECOGNIZE A CONTINUUM OF SITES LOCATED IN AN IMMUNODOMINANT REGION OF THE INFLUENZA HEMAGGLUTININ, Marianne T. Sweetser, Vivian L. Braciale and Thomas J. Braciale, Department of Pathology, Washington University School of Medicine, St. Louis, MO 63110
Class I H-$2K^d$-restricted cytolytic T lymphocytes (CTL) are directed against two immunodominant sites on the A/JAP/305/57 influenza hemagglutinin (HA) that can be mimicked by synthetic oligopeptides spanning residues 202-221 in the HA1 and 523-545 in the hydrophobic, transmembrane region. Analysis of the fine specificity of HA1-specific CTL clones demonstrated that these CTL clones can be subdivided into at least two group based on their patterns of recognition of closely related influenza H2N2 field strains and a monoclonal antibody derived variant of A/Guiyang/4/57. Using a series of nested synthetic peptides spanning the 202-221 region, the minimal amino acid residues necessary for recognition by the two groups of CTL clones were defined and found to consist of two separate but overlapping sites. Sequence comparison of the HA of the A/JAP/305/57, the influenza field isolates and the monoclonal antibody derived variant has identified two amino acids, Asn at position 207 and Gly at position 215, that are critical for T cell recognition. Thus, animo acid substitutions induced either by antigenic drift or by monoclonal antibody selection can affect class I CTL recognition.

C 719 PROLONGATION OF ORGAN ALLOGRAFT SURVIVAL BY GRAFT PRETREATMENT WITH MONOCLONAL ANTIBODIES (MAb) REACTIVE WITH ANTIGEN-PRESENTING CELLS J. Richard Thistlethwaite, Jr., Robert Kang , Michelle Josephson, Laurence McCahill and David M. Lloyd. Department of Surgery, University of Chicago, Chicago, Illinois 60637.
Pretreatment with antibodies reactive with Class II MHC antigens has previously been reported to be successful in removing antigen-presenting dendritic cells (DC) from rodent tissue grafts. We have extended these experiments to intact whole organ grafts. Intact LDA F_1 ($RT1^{a,1}$) rat pancreases were placed in a normothermic (37°C), oxygenated hemoperfusion circuit and were perfused with anti I-A and/or anti I-E. Following 3 hours of perfusion with MAb and exogenous complement, cells of DC morphology were not depleted; however, 61 ± 14 cells/mm^2 were labeled with the MAb, whereas only 2 ± 2 additional cells/mm^2 of DC morphology could be identified. Islets isolated from MAb perfused pancreases showed a marked reduction in their ability to stimulate DA rat ($RT1^1$) T cells in a mixed islet-lymphocyte culture (81% inhibition). MAb perfused LDA pancreases also demonstrated a prolonged survival (17 ± 2 days) compared to controls (11 ± 1 days) ($P < 0.01$) when transplanted into streprozotocin treated DA recipients. Pancreases perfused for shorter intervals did not show prolonged survival. These results suggest that pretreatment of intact organs with MAb's reactive with antigen-presenting DC's may not eliminate these cells, but appears to functionally inactivate them and allow for prolonged allograft survival in the absence of recipient immunosuppression.

C 720 N-GLYCOSYLATION OF AN ANTIBODY BINDING REGION OF INFLUENZA HAEMAGGLUTININ ABROGATES $CD4^+$ T CELL RECOGNITION, D. Brian Thomas, John J. Skehel, David S. Burt, Jacob Hodgson and Christine M. Graham, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA.
Antigenic variation in the haemagglutinin (HA) of influenza A viruses frequently introduces new oligosaccharide attachment sites (Asn-X-Ser/Threo) and carbohydrate addition prevents antibody recognition by steric hindrance. Here we show that an amino acid substitution in mutant viruses of the H3N2 subtype (HA1 63 Asp → Asn), that introduces an N-glycosylation site ($Asn_{63}Cys_{64}Thr_{65}$), abrogates antibody and $CD4^+$ T cell recognition. I-A^d restricted, HA specific T cell clones from BALB/c mice, previously infected with X31 virus recognise a synthetic peptide corresponding to antigenic site E, HA1 56-76, and are sensitive to a single substitution (HA1 63 Asp → Asn) in mutant viruses. Recognition of mutant viruses is restored however by tunicamycin-treatment of virus infected target cells, thereby confirming that carbohydrate addition prevents $CD4^+$ T cell recognition.

C 721 MONONUCLEAR PHAGOCYTE (Mϕ) DIFFERENTIATION FROM PLURIPOTENT HEMOPOEITIC STEM CELLS.
PHENOTYPICAL AND FUNCTIONAL ANALYSIS OF EARLY COMMITTED PROGENITOR CELLS.

Willem van Ewijk, Pieter J.M. Leenen and Marleen L. Melis, Department of Cell Biology, Immunology and Genetics, Erasmus University, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands.

Phenotypical and functional analysis of Mϕ progenitors is hampered by the low frequency of these cells in normal bone marrow. We have immortalized Mϕ progenitors by somatic cell hybridization of m-CSF cultured mouse bone marrow cells to HAT sensitive myeloid tumor cell lines. Such hybrids show the absence of 'mature' Mϕ markers, and presence of 'immature' Mϕ markers. These hybrids have retained the capacity to differentiate in vitro into functional cells with a mature Mϕ phenotype.

Immunization of rats with such progenitor hybrids has led to a panel of monoclonal antibodies directed to Mϕ precursors in vivo. These antibodies can be used to isolate Mϕ progenitors by electronic cell sorting and to address questions about the heterogeneity of the Mϕ progenitor pool.

C 722 HUMAN T-CELL EPITOPE MAPPING OF THE MENINGOCOCCAL CLASS 1 OUTER MEMBRANE PROTEIN.
Emmanuel J.H.J. Wiertz[1], Jacqueline A.M. van Gaans[1], Peter Hoogerhout[1], Robert C. Seid Jr.[2], John E. Heckels[3], Geziena M.Th. Schreuder[4] and Jan T. Poolman[1].[1]National Institute of Public Health and Environmental Protection P.O.box 1, 3720 BA, Bilthoven, the Netherlands and [4]Dept. of Immunohematology, AZL, Leiden, the Netherlands;[2]Praxis Biologics, Rochester, New York, USA and [3]University of Southampton, UK. Immunity to disease caused by Neisseria meningitidis is associated with the presence of bactericidal and opsonic antibodies to the capsular polysaccharide (CPS), lipopolysaccharide and to outer membrane proteins (OMPs). The CPS of group A and C meningococci are proven efficacious vaccines, although the immunogenicity in infants is poor and the immunity is of short duration. The combination with T-helper epitopes will certainly improve the immunogenic properties of these T-independent (Ti_2) antigens. The group B CPS is poorly immunogenic in humans probably because of tolerance due to structural similarity to host glycopeptides and/or glycolipids. We have focused our research onto the class 1 OMPs which show limited heterogeneity amongst meningococci. Murine monoclonal antibodies to these proteins are highly bactericidal in vitro and will be used to map B-cell epitopes. T-epitopes have been identified by theoretical prediction of immunodominant sites by analysis of the amino acid sequence of the OMP followed by their solid phase synthesis and subsequent testing for polyclonal activation of T-lymphocytes obtained from HLA-typed volunteers immunized with the OMP. In addition human T-cell clones are generated with OMPs and maintained with antigen, EBV-transformed B-cells, fresh feeders and rIL_2. The clones are tested for antigen specificity, in vitro helper function, MHC restriction element, expression of surface markers and recognition of common meningococcal T-cell epitopes.