# SELF REACTIVITY AND ITS REGULATION

*Organizer: Constantin Bona and Eli Sercarz*

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## *Plenary Sessions*



## *Poster/Discussion Sessions*

## Self Reactivity and Its Regulation

*Keynote Address*

**C 001** KEYNOTE ADDRESS: THE CELLULAR BASIS OF IMMUNOLOGIC TOLERANCE, G.J.V. Nossal, The Walter and Eliza Hall Institute of Medical Research, Post Office, Royal Melbourne Hospital, Victoria 3050, Australia.

The capacity of the immune system to discriminate between self and not self remains as the central aspect of immunoregulation, but we now know that the discrimination is not absolute, and that a degree of expressed and/or latent anti-self reactivity is normal. However, the degree of anti-self reactivity is under tight control and this means limits to the numbers and affinity spectrum of anti-self immunocytes that can be tolerated. The linchpin of immunologic tolerance is the censorship of anti-self immunocytes, comprising both T and B cells.

Censorship of these two lymphocyte repertoires can take two forms, namely clonal deletion and clonal anergy. For antigens present in the central lymphoid organs, the deletion is frequently due to clonal abortion, that is death of the anti-self cell before is it mature enough to be exported to the periphery. Many auto-antigens and particularly cell surface macromolecules specific for certain organs and tissues are not present within the thymus, and it is now clear that such antigens also can delete immunocyte T or B cell clones peripherally. Clonal anergy represents an apparently irreversible silencing of anti-self T or B cells without actual lymphocyte killing, and it can occur centrally or peripherally. It is probable that the "decision" between deletion and anergy depends on the strength of the negative signal delivered by antigen.

Two controversial issues requiring discussion are the role of post-immunization censorship of B cells hypermutating towards anti-self reactivity; and the molecular and cellular basis of T cell-mediated suppression of immune responses. Recent experiments from our laboratory dealing with these will be presented.

*Tolerance at the T and B Cell Levels: Positive and Negative Selection*

**C 002** SELF TOLERANCE IN THE T CELL AND B CELL COMPARTMENTS OF HEN EGG LYSOZYME TRANSGENIC MICE **A. Basten, S. Adelstein, G. Gammon*, H. Pritchard-Briscoe, J. Crosbie, E. Sercarz* and C.C. Goodnow, Centenary Institute of Cancer Medicine and Cell Biology, University of Sydney, Australia, *Department of Microbiology, University of California, Los Angeles.**

Transgenic mice provide an opportunity to study tolerance to bone fide self antigens under rigorously controlled conditions. A variety of transgenic lines on a C57BL/6J background were created expressing the gene for the well defined protein antigen, hen egg lysozyme (HEL) in soluble form linked either to the mouse metallothionein-1 or mouse albumin promoters. Self tolerance in the B cell versus T cell compartments was assayed by immunising the progeny with HEL coupled to the foreign carrier, horse red cells, or HEL-FITC respectively. Self tolerance in the B cell compartment was shown to be: (a) dependent on a critical threshold of HEL in the serum and (b) associated with a reduction in average antibody affinity. By contrast T cells were found to be tolerant irrespective of the self antigen level in the serum, confirming that T cells are more sensitive to tolerance induction than B cells. Immunisation with defined peptides from HEL of a line in which the T cell but not B cell compartment was unresponsive resulted in proliferation to a peptide expressing a minor T cell determinant whereas no response was obtained to native HEL or a major determinant peptide. This finding supports the contention that exposure to crossreactive peptides carrying minor self determinants can lead to a breakdown in self tolerance.

**C 003** THE NUMBER OF MECHANISMS FOR GENERATING T CELL TOLERANCE IS GROWING. Lori A. Jones, L. Thomas Chin, Dan L. Longo and Ada M. Kruisbeek, BRMP-NCI, NIH, Bldg. 10, Rm12N226, Bethesda, MD 20892.

Clonal deletion represents a major mechanism used by T cells to acquire tolerance to self-antigens and is thought to occur only in the thymus. When this mechanism fails, additional contributions to the phenomenon of tolerance are made by intra-and extra-thymically induced clonal non-responsiveness. Most likely, the mechanism involved in generating T cell tolerance is determined by the manner of presentation of self-antigens, and perhaps also by the developmental stage at which T cells encounter these antigens. Self-antigens for which these rules have been established include H-Y antigens, products of the minor lymphocyte stimulatory (Mls) loci and largely unknown gene products that are recognized in association with $E_\alpha E_\beta$-class II MHC molecules. In the present study, we investigated whether interference with both the intra-thymic clonal deletion and the clonal anergy pathways would reveal yet another mechanism of self tolerance. This condition can be achieved by blocking $E_\alpha E_\beta$-expression with mAb treatment from birth, affects T cells with a variety of self-reactive receptors, and results in appearance of functional self-reactive peripheral T cells. Strikingly, re-introduction of $E_\alpha E_\beta$ (after termination of mAb treatment) results in clonal disappearance of these cells, demonstrating that clonal deletion can occur not only in immature precursor T cells but also in mature single positive T cells with self-reactive receptors. This process is in part thymus-independent, as it also occurs in thymectomized mice. The molecular parameters distinguishing deletion from activation are currently investigated with in vitro model systems.

**C 004** TRANSGENIC MOUSE MODEL OF POSITIVE AND NEGATIVE SELECTION OF T CELLS, Dennis Y. Loh, Howard Hughes Medical Institute and Departments of Medicine, Genetics, and Molecular Microbiology, Washington University School of Medicine, St. Louis, MO 63110

T cells normally display MHC restriction and self-tolerance. In an attempt to understand the molecular basis of the development of these characteristics, we have developed a T cell receptor transgenic mouse model. Through the use of such a model, we have elucidated the principles of positive and negative selection as it applies to thymocyte development. Most recently, we have concentrated our efforts to understand the molecular basis of recognition that leads to cell death on one hand and survival on the other.

**C 005** B LYMPHOCYTE TOLERANCE AND IgD, David Nemazee, Division of Basic Research, Department of Pediatrics, National Jewish Center, Denver CO 80206, and Kurt Buerki, Preclinical Research, Sandoz, Ltd, Basel, Switzerland, CH-4002.

Autospecific B lymphocytes are subject to negative regulation through the complementary, and, perhaps, partially overlapping mechanisms of deletion and functional inactivation. We have observed deletion of autoreactive B cells specific for class I antigen ($H\text{-}2K^k$) in mice transgenic for genes encoding the heavy and light chains of an IgM anti-$K^k$ antibody, 3-83. Because there is some controversial older data that supported the idea that co-expression of both IgM and IgD on B lymphocytes may in some way alter or negate the tolerance signal, we generated transgenic mice using an extended heavy chain construct that, when introduce with the 3-83 light chain gene, encodes both the IgM and IgD forms of 3-83. Two founder lines have so far been generated that express high levels of the transgenes and transgene-encoded cell surface IgM and IgD. Results of analysis of transgenic mice in which the B cells develop in the presence or absence of antigen will be presented.

**C 006** MOLECULAR ANALYSIS OF THE STATE OF T CELL CLONAL ANERGY, Ronald H. Schwartz, Sang Kang, Bart Beverly, Kurt Brorson, Daniel Mueller and Michael Lenardo,Laboratory of Cellular and Molecular Immunology and Laboratory of Immunology, NIAID, NIH, Bethesda, MD 20892

Interleukin-2 (IL-2) producing T cell clones, activated only through their antigen-specific receptors, are induced into a state of anergy in which the cells produce 10 to 50-fold lower amounts of IL-2 when restimulated with antigen and normal presenting cells. Analysis of anergized cells revealed normal amounts of T cell receptor expression and normal signal transduction as measured by increases in intracellular calcium and phosphorylation of T cell receptor gamma chain after stimulation with antigen and presenting cells. The decrease in IL-2 production was seen at both the protein and mRNA levels. Preliminary nuclear run-on experiments suggest that the decrease is at the level of transcription of the IL-2 gene.

IL-2 gene transcription is controlled by an enhancer/promoter region located in the first 300 base pairs 5′ of the transcription start site including response elements that bind the NF-AT, NF-κB, AP-1 and octamer (Oct) transcription factors. We used gel mobility shift assays to compare nuclear proteins from anergized and normal T cells of the murine clone A.E7 before and after stimulation with antigen and presenting cells. Increases in NF-AT, NF-κB, and Oct 2 were seen 4 hrs after activation in both anergized and normal cells. Increases in AP-1 levels were also found in both groups, but a difference in their kinetics was observed. Normal cells showed maximum levels at 3 hrs, while anergized cells were still less than maximal at 4-1/2 hours. The significance of this difference at the level of transcription is currently under investigation.

*Self-Reactive Repertoires and Lymphocyte Subsets in Self Recognition*

**C 007** CONTROL OF RECOMBINATION EVENTS DURING LYMPHOCYTE DIFFERENTIATION.

Frederick W. Alt, Lori Covey, Gary Rathbun, Paul Rothman, Suzanne Li, Eugene Oltz, Ami Okada, Jianzhu Chen, Beverly Gorham and Monica Mendelsohn. The Howard Hughes Medical Institute and Departments of Biochemistry and Microbiology, College of Physicians and Surgeons of Columbia University, New York, New York. 10032

Ability of VDJ recombinase to assemble particular gene segments in appropriate cell types and stages within lymphoid lineages is effected by modulating accessibility of substrate gene segments to a common VDJ recombinase. Accessibility has been correlated with transcription of targeted unrearranged gene segments. To elucidate controlling elements, we created transgenic mice that carry a hybrid antigen receptor gene mini-loci in which germline TCR or Ig heavy chain variable region gene segments (V, D, and J) were combined with various Ig or TCR receptor transcriptional regulatory elements including downstream Ig heavy chain, Ig light chain, or TCR enhancer elements and upstream TCR of IgH promoter elements. Consistent with the postulates of the accessibility model, we find that such transcriptional control elements can dominantly target rearrangement of the associated gene segments in a lineage specific fashion. We are also using new approaches to further address these issues including the development of novel cell lines with higher (more physiological) levels of VDJ recombinase activity as recipients for transfected recombination substrates and the use of ES cell/somatic chimera technology to assay substrates in an in vivo setting.

We have defined transcription units that initiate upstream of class-switch recombination target sequences of four different germline H chain genes including $\gamma 1$, $\gamma 2b$, $\gamma 3$, and $\epsilon$. Treatment of pre-B cell lines or normal splenic B cells with LPS or LPS plus IL-4 differentially induces transcription from these germline $C_H$ promoters followed by induction of switch recombination to the corresponding genes. These results imply a role for transcription of germline CH genes in heavy chain class-switching. Such a role could include targeting of appropriate class switch sequences for recombination and /or the generation of germline transcripts for substrates in putative trans-splicing mechanisms of class-switching. We have identified DNA regions that are involved in regulating transcription of germline C region genes and are analyzing the effects of dominant germline transcription vectors that were introduced into cells and animals to test postulates of the potential functions described above.

**C 008** SELECTION OF $\gamma\delta$ T CELL CLONOTYPES AND RECOGNITION OF "SELF". Andrei Augustin, Ramanujam Rajasekar, and Gek-Kee Sim*, Department of Medicine, National Jewish Center for Immunology and Respiratory Medicine, and Department of Microbiology and Immunology, University of Colorado Health Sciences Center, Denver, CO and *Basel Institute for Immunology, Basel, Switzerland.

$\gamma\delta^+$ T cells present among resident pulmonary lymphocytes (RPL) constitute an interesting population, with respect to their reactivity and pattern of clonotypes selection. Mice exposed to aerosols containing a Mycobacterium tuberculosis extract (PPD) have an elevated number of activated $\gamma\delta^+$ $CD4^-CD8^-$ T cells in the lung, which can be propagated *in vitro* when restimulated with the same antigenic preparation, or in the presence of IL-2. Furthermore, considering that immunodominant mycobacterial antigens responsible for T cell reactivity in mice and man are heat shock proteins homologs, which are structurally well conserved in phylogeny, we investigated the effects of heat shock on pulmonary T cell induction and selection *in vitro*. Our experiments indicated that $\gamma\delta^+$ RPL, which react to mycobacterial antigens, can also be activated by self cells, in which a heat shock response was induced. To structurally characterize the receptors expressed by $\gamma\delta^+$ RPL we analyzed a large number of nucleotide sequences derived from $\gamma$ and $\delta$ mRNA, obtained from RPL of various mouse strains. Two predominant phenotypes have been detected: BID (a fetal type of rearrangement of the V$\delta$5 gene, in which all gene segments retain intact germ line sequences) and GxYS (characterized by a diversified, but convergent V$\gamma$4/J$\gamma$1 junction). From analyzing the pattern of selection of these phenotypes, we concluded that they are both positively selected in a thymus-independent fashion. Their preferential peripheral expansion in some mouse strains is not driven by structures coded in the classical MHC region. Interestingly, we detected a similar pattern of selection for the $V\beta8.2^+$ $\alpha\beta^+$ $CD4^-CD8^-$ T cell clonotypes present in RPL. These observations lead us to the hypothesis that the homing of distinct $\gamma\delta$ T cell clonotypes to different epithelia is not due to their expressing tissue-specific homing receptors, but to a preferential peripheral expansion, at various anatomical sites, driven by self ligands. Indeed, a recent analysis performed on $\gamma\delta^+$ RPL isolated from mice, at various points in time, in the perinatal period, is compatible with this view.

C 009 EXPRESSION AND PAIRING OF $V_H$ AND $V_K$ FAMILIES IN SELF REACTIVE AUTOANTIBODIES, Constantin Bona, Department of Microbiology, Mount Sinai School of Medicine, New York, NY 10029

The analysis of the expression of $V_H$ and $V_K$ genes in a panel of 300 hybridomas producing self reactive antibodies, with various specifities, showed that the majority of V gene families are expressing corresponding to the size of family, excepting $V_H$ 36-09, $V_K$23, and $V_K$28 under expressed and $V_H$7183, $V_K$1 and $V_K$28 over-expressed.
Comparison of sequence analysis of 16 NZB hybridomas using $V_K$1 gene with NZB $V_K$1 germline gene showed that $V_K$1 expressed genes derived from various $V_K$1 subgroups. Study of pairing of 10 $V_H$ and 12 $V_K$ families appears to be stochastic, since failed tests for independence and deviation from that expected for an unselected population with exception of $V_H$11 and $V_K$9.
A comparison of expressed $V_H$ and $V_K$ gene families obtained from strains prone to autoimmune diseases or normal strains showed that the general rule of random $V_H$ and $V_K$ pairing is not violated by strain differences. Molecular method was used to determine Ly1 origin of hybridomas and it appears than $V_H$ and $V_K$ gene pairing is stochastic in both Ly1+ and Ly1- cells.
The pairing of $V_H$ and $V_K$ in hybridomas producing antibodies with 9 different specificities exhibit some form of bias with the exception of antibodies specific for DNA and Sm which do not significantly differ from the pattern and pairing expected from an unselected population.

C 010 **V GENES AND ANTIBODY SPECIFICITIES EXPRESSED BY CD5 B CELLS.** **Leonore A. Herzenberg, Neelima M. Bhat, Nelson N.H. Teng and Aaron Kantor. Departments of Genetics and Gynecology and Obstetrics, Stanford Unversity Medical School, Stanford, California 94305.** CD5 B cells in mice belong to a separate developmental lineage whose repertoire is largely fixed during the first few weeks of life. These cells produce much of the serum Ig and many of the autoantibodies found in normal mice throughout life. CD5 B cells in man show many of the same characteristics vis a vis repertoire as their murine counterparts. In this presentation, we will contrast the development and repertoire of CD5 B cells in these two species and provide further evidence of the similarities between the murine CD5 B cell lineage in the mouse and the CD5 B subset/lineage in human peripheral blood and spleen. In addition, we will integrate these findings with recent data from our laboratory and elsewhere bearing on how the CD5 B cell repertoire is established.

**C 011** ANTI-$Fc_\gamma R$ AUTOANTIBODY IN AUTOIMMUNE DISEASE, Jay C. Unkeless*, Constantin Bona#, and Peter Boros*, Department of Biochemistry* and Microbiology#, Mount Sinai School of Medicine, New York, NY 10029. Sera of mice prone to autoimmune disease such as NZB, NZB/NZW, TSK, and me$^v$ have high titers of anti-$Fc_\gamma R$ IgM, which for old NZB ♀ mice is 2% of the total IgM [1]. This antibody is probably responsible for the paralysis of macrophage $Fc_\gamma R$ function reported by Russell et. al [2] since ultracentrifuged serum from NZB and TSK mice, but not normal mice, and anti-$Fc_\gamma R$ IgM mAbs inhibit the binding of FITC-IgG1-anti-$DNP_{20}$BSA complexes to macrophages. We now report that the anti-$Fc_\gamma R$ IgM mAbs, and some anti-$Fc_\gamma R$ mAbs isolated by EBV transformation of peripheral leukocytes from patients with scleroderma, efficiently (10-0.1 $\mu$g/ml of mAb) trigger the degranulation of human neutrophils, measured by the release of neutrophil elastase and $\beta$-glucuronidase. Results from transmission electron microscopy are consistent with massive degranulation. The anti-$Fc_\gamma R$ mAbs (at 10 $\mu$g/ml) also trigger the secretion of cytokines from human mononuclear cells that stimulate the proliferation, measured by a crystal violet dye-binding assay, of primary human dermal fibroblasts growing in medium containing 10% serum. The factors responsible for the stimulation of proliferation are potent, showing maximal effect at 1:20 dilution. The mAbs alone had no effect on the proliferation of the fibroblasts, and conditioned medium from mononuclear cells alone was only slightly stimulatory. Preliminary analysis of cytokines present in the conditioned supernatants show elevated levels of IL-1$\beta$, IL-6, but not elevated levels of TNF$\alpha$. These results suggest that anti-$Fc_\gamma R$ autoantibody may play a role in the pathophysiology of autoimmune phenomena.

1. Boros, P., Chen, J., Bona, C., and Unkeless, J.C. (1990) *J. Exp. Med.* **171**, 1581-1595
2. Russell, P.J., and Steinberg, A.D. (1983) *Clin. Immunol. Immunopathol.* **27**, 387-402

## *Genetic and Molecular Mechanisms of Autoimmune Diseases*

**C 012** ROLE OF MHC, TCR, MLS AND ENTEROBACTERIA IN EXPERIMENTAL ARTHRITIS, Chella S. David, Department of Immunology, Mayo Clinic, Rochester, MN 55905.
Mice strains of haplotype H-$2^q$ and H-$2^r$ are susceptible to collagen induced arthritis (CIA) when injected with chick or porcine type II collagens respectively. The genes responsible for the susceptibility have been narrowed down to the class II H-2A genes. Strains SWR and AU (H-$2^q$) are resistant to CIA and found to have a deletion of 50% of their T cell receptor (TCR) $V_\beta$ genes. The possible role of TCR $V_\beta$ genes in CIA was demonstrated in several gene complementation analyses. Another strain, RIII S/J (H-$2^r$) with a large deletion of TCR $V_\beta$ genes was found also to be resistant to CIA induced by porcine type II collagen. In order to confirm the role of a TCR $V_\beta$ gene in CIA in the H-$2^r$ haplotype, backcrosses and $F_2$'s were made between RIII and B10, both of which are resistant. These mice were typed for the TCR $V_\beta$ deletion, H-2, and injected with porcine type II collagen. While there was a complete correlation between TCR $V_\beta$ deletion and resistance to CIA in H-$2^{r/r}$ mice, some of the H-$2^{r/b}$ mice with TCR $V_\beta$ deletion were found to be susceptible to CIA. This indicates that in the H-$2^{r/b}$ mice a hybrid class II molecule ($A^{r/b}$) presents a different epitope on the collagen II molecule and is recognized by a TCR $V_\beta$ chain which is not part of the deletion in the mice. In the B10.RIII arthritic mice, T cells isolated from the joints predominantly express TCR $V_\beta 6$, which is one of the genes deleted in the RIII mice. In vivo studies with anti-TCR $V_\beta$ antibodies are currently underway. Introduction of the Mls-$1^a$ gene into B10.RIII mice reduces the incidence of CIA indicating a role for negative selection of self reactive T cells. These studies indicate the importance of the trimolecular complex of MHC, TCR and collagen type II epitope in the incidence of collagen induced arthritis. The TCR $V_\beta$ receptor involved is dictated by the MHC class II genes on the antigen presenting cells and the epitope of the type II collagen, which is presented to the T cells. There is no one universal TCR $V_\beta$ gene which is involved in the disease process. Enterobacteria have been implicated in the HLA-B27 linked susceptibility of reactive arthritis in humans. Studies in mice using Yersinia enterocolitica indicates a role for MHC class I genes from the H-$2^q$ and H-$2^d$ haplotypes in combination with certain T cell receptor $V_\beta$ genes in the disease process. Preliminary studies indicate that the class I gene maps to H-2D region. Interestingly, the expression of the HLA-B27 transgene in mouse strains is inhibited in the context of H-$2D^d$ molecule. We believe there is a connection between the role of H-$2D^d$ genes in susceptibility to YIA and their role in B27 expression. B27 and $D^d$ may have a structural homology which may be the binding site for the Yersinia 'superantigen' which positively selects certain self reactive T cells with particular $V_\beta$ genes. The same binding site may be important in endogenous peptide binding during processing and transport of class I molecules to the cell surface. Studies are currently underway to confirm these hypotheses.

**C 013** THE ROLE OF SOMATIC MUTATION AND ENVIRONMENTAL ANTIGENS ON THE GENERATION OF ANTI-DNA ANTIBODIES. Matthew D. Scharff, Daniel L. Lustgarten, Sylvie Corbet and Samuel Behar. Department of Cell Biology, Albert Einstein College of Medicine, Bronx, N.Y. 10461.

We have previously shown that a single somatic mutation in the S107 (T15) anti-PC antibody converts it from an antibody that can protect mice from a pneumococcal infection to an anti-dsDNA antibody. Based on this paradigm, we have studied the S107 $V_H$ encoded anti-PC, anti-influenza and anti-DNA responses of NZB/WF1 mice. The initial response to these foreign antigens and its impact on the subsequent utilization of the S107 $V_H$ gene family in anti-DNA antibodies varies enormously from animal to animal. While no consistent pattern was discerned, individual examples of both negative and positive influences occurred. In order to evaluate V gene utilization and the role of somatic mutation, monoclonal antibodies were generated from F1 animals that utilized the S107 $V_H$ family. The IgG $V_H$11 encoded anti-DNA antibodies from older autoimmune animals have large numbers of somatic mutations. In some of these antibodies, there are an unusually large number of replacement substitutions in the framework of the light chains.

**C 014** GERMLINE V GENES, THE IMMUNOGLOBULIN REPERTOIRE, AND PATHOGENIC AUTOANTIBODIES. R. S. Schwartz, L. Tucker, A. Long, F. Young, T. Guillaume, D. Rubinstein, C. Huang, K. Stewart, K. J. Barrett , and B. D. Stollar. Departments of Medicine and Biochemistry, Tufts New England Medical Center, Boston, MA 02111. Molecular studies of human antibodies and their corresponding V genes suggest that pathogenic autoantibodies originate from germline V genes that contribute to the immunoglobulin repertoire in normal persons, and not from "abnormal" V genes that are peculiar to patients with autoimmune diseases. Furthermore, genetic studies of lupus-prone mice have shown that the normal B cell repertoire can generate a population of potentially lethal autoantibodies if it falls under the influence of abnormal "autoimmunity" genes. Evidence that "natural" human autoantibodies can play a role in autoimmune diseases comes from studies of Id-16/6, an idiotypic marker originally detected in an IgM anti-DNA antibody derived from a patient with systemic lupus erythematosus. High levels of IgM and IgG Id-16/6$^+$ antibodies occur in the serum of most patients with active lupus, and Id-16/6$^+$ immunoglobulins have been found in the immune deposits of the skin and renal lesions of the disease. These results indicate that Id-16/6 marks both germline IgM and pathogenic IgG antibodies. $V_H$18/2, an unmutated germline gene (also known as $V_H$ 26 and $V_H$ 30P1) has been found to specify the principal idiotypic determinant of Id-16/6. Id-16/6 was found in unusually high frequency in a large panel of monoclonal antibodies produced by EBV transformed B cell clones. These Id-16/6$^+$ antibodies were encoded either by a gene identical to $V_H$ 18/2, or to a very closely related (at least 98% identical) $V_H$ gene. Populations of resting peripheral blood B cells from normal subjects, studied by *in situ* hybridization with oligonucleotide probes derived from VH 18/2, were found to express genes related to VH 18/2. Additional evidence that germline genes can encode regions of autoantibodies is that an unmutated germline D gene segment (*DXP' 1*) has been identified as the coding element for the CDR3 of five independently derived anti-DNA autoantibodies. These results indicate that germline immunoglobulin V genes are capable of generating autoantibodies without undergoing somatic mutation. The high precursor frequency of anti-DNA antibody producing B cells in humans suggests that autoreactive B cells are not deleted from the repertoire. On the contrary, they may participate in an as yet unknown way in the normal immune response.

**C 015** SINGLE GENE DEFECTS AND AUTOIMMUNITY, Charles L. Sidman*, Ruth D. Allen* and Harald von Boehmer+, *The Jackson Laboratory, Bar Harbor, ME 04609 and +The Basel Institute for Immunology, Basel 4005, Switzerland.

Two classes of autosomal recessive single gene mutations that lead to autoimmunity have been investigated using somatic bone marrow radiation chimeras and germline introduction of T cell receptor transgenes. On the one hand, the allelic "motheaten" (*me*) and "viable motheaten" ($me^v$) mutations identified a gene acting cell-autonomously in most if not all hemopoietic lineages. The other class of genes, represented by the non-allelic "lymphoproliferation" (*lpr*) and "generalized lymphoproliferative disease" (*gld*) mutations, influenced cells both internally and through extracellular environmental interactions. Although similar phenotypically, cells homozygous for *lpr* or *gld* acted completely differently on both the donor and recipient sides of bone marrow transplantation. These differences lead to the hypothesis that the *lpr* and *gld* mutations affect one or the other of a ligand:receptor pair of molecules relevant in T cell development and activation. By combining either of these mutations with T cell receptor *alpha* plus *beta* transgenes coding for reactivity for male antigen plus H-2D$^b$, the influence of positive and negative selection on the aberrant T cell subset overrepresented in *lpr* and *gld* mice, and the effect of these mutations on such selection processes, was investigated. The phenotype of the proliferating cells in *lpr* and *gld* mice was found to depend on the interaction of developing T cells with self antigens. Morover, these mutations altered the normal processes of positive and negative selection operative during thymic maturation of T cells. Together, these data suggest that the *lpr* and *gld* mutations may provide clues regarding the molecular and genetic mechanisms controlling T cell development and tolerance versus activation against autoantigens.

**C 016** T CELL ANTIGEN RECEPTOR GENES IN AUTOIMMUNITY, Argyrios N. Theofilopoulos, Department of Immunology, Scripps Clinic and Research Foundation, La Jolla, California 92037

The role of T cell antigen receptor genes (TCR) in autoimmune diseases is actively investigated at the genomic and expression levels. To more completely and reproducibly characterize TCR V$\beta$ repertoire, define intrathymic positive and negative selection processes and study autoimmune disease correlations, we developed an accurate and sensitive "multiprobe V$\beta$ RNase protection assay". Several self-superantigen-dependent V$\beta$ clonal deletions were identified with this system in mice. Further, broad analysis in separated CD4$^+$ and CD8$^+$ murine subsets gave improved resolution of V$\beta$ repertoire selection as a whole and revealed significant strain- and/or subset-specific expression skewing for several V$\beta$s. The influence of background ligands in this process was documented.

Using the multiprobe RNase protection assay, the entire TCR V$\beta$ gene repertoire of pre-diseased and diseaded murine strains with spontaneous lupus was analyzed. Depending on their H-2/*Mls* haplotypes, all relevant self-superantigen-related V$\beta$ clonal deletions were detectable. Moreover, no "leakage" of such self-superantigen-related clones with age, thymic degeneration or disease appearance was observed. Importantly, however, with advanced age and disease, autoimmune mice generally showed a less stable non-deleted V$\beta$ repertoire than normal mice, with often large relative changes in expression levels of several V$\beta$s.

A multiprobe RNase protection assay was also developed and used to characterize the effects of thymic selection on human expressed TCR V$\beta$ gene repertoires. The relative abundance of transcripts for each of the analyzed 22 V$\beta$ genes (encompassing 17 of the 20 human V$\beta$ gene subfamilies) within a thymus, and among 17 thymuses, was highly variable. Based on the presence of corresponding mRNAs, no genomic deletions were detected, but several coding region polymorphisms were identified. Analysis of mature T cell subsets revealed the absence of complete superantigen-mediated V$\beta$ deletions, suggesting that this phenomenon is uncommon in humans. However, several V$\beta$ genes were over- or underexpressed in one or both mature single-positive (CD4$^+$8$^-$, CD8$^+$4$^-$) thymocyte subsets compared to syngeneic total, mostly immature, thymocytes. Whether these changes are induced by relatively weak superantigens or conventional MHC-bound peptides, and whether the downshifts are caused by negative selection or lack of positive selection, remains to be determined.

## Self Reactivity and Its Regulation

*Plenary Lecture I*

**C 017** THYMIC AND POST-THYMIC SELECTION OF THE T CELL REPERTOIRE, Harald von Boehmer, Basel Institute for Immunology, CH-4005 Basel, Switzerland. The antigen-receptor on αβ T cells controls T cell development at various stages. On pre-T cells the β TCR chain is incorporated into the membrane without any other known TCR chain and components of the CD3 complex. As a consequence, the cell starts to express CD4 and CD8 genes and the α TCR locus becomes transcriptionally active

On immature $CD4^+8^+$ thymocytes the αβ $TCR^-$ CD3 complex needs to bind to thymus MHC molecules in epithelial cells in order to rescue these cells form programmed cell death. Binding to class I or class II MHC molecules instructs the cell to switch off expression of the CD4 and CD8 genes, respectively, and to assume the pre-T killer and pre-T helper phenotype, respectively. These differentiation events are accompanied by an increase in αβ $TCR^-$ CD3 complex expression on the cell surface and occur in noncycling thymocytes. If the receptor binds to the specific peptide plus the MHC molecule, premature cell death is induced.

Mature T cells can expand considerably in secondary lymphoid organs. This depends entirely on the binding of the TCR to specific peptides and MHC molecules. If specific peptides are continuously present and at a high concentration, the cells will stop to divide and disappear from lymphoid tissue. Some of the cells will, however, remain and become refractory to antigenic stimulation which is accompanied by down regulation of TCR and co-receptors. Thus, with regard to specificity and mechanism, clonal selection in primary lymphoid organs is entirely different from clonal selection in secondary lymphoid organs.

*Molecular Basis of Network Recognition and Mimicry*

**C 018** EARLY EXPRESSION OF HUMAN IMMUNOGLOBULIN REPERTOIRE. Michel Fougereau, David Bossy, Anne-Marie Cuisinier, Francis Fumoux, Michèle Milili, Claudine Schiff, and Cécile Tonnelle. Centre d'Immunologie de Marseille-Luminy, Case 906, 13288 Marseille Cédex 9, France.

Early steps of B cell differentiation involve sequential rearrangements of the H and L loci, which seem induced by membrane molecules involving the lambda-like and V-pre B gene products, associated to μ or μ-like chains. These events take place in the fetal liver and in the bone marrow The occurence of μ and L chains correlates with the selection of the V repertoire, which is organized in 6 VH, 4 $V_\kappa$ and 7 $V_\lambda$ families in humans. Early in ontogeny, VH5 and VH6, the most 3' of the VH locus, are expressed first in the fetal liver, as soon as the 7th week of gestation, in the absence of L chains. The expression of complete IgM molecules can be detected in the bone marrow as early as the 8th week of gestation. From this stage on, the VH and VL repertoires rapidly expand, to closely resemble the adult pattern by the 11th week, as appreciated from the utilization pattern of V gene families, analyzed by in situ hybridization with cDNA probes, on normal PBL or on EBV transformed clones. The expression level correlates with the estimated gene number for the VH families but diverges noticeably for $V_\kappa$ suggesting that the H and L repertoires do not obey the same selective process.

As the initial repertoire may be characterized by a high degree of connectivity, with the presence of polyspecific antibodies, EBV clones secreting such Ig were screened for their VH and $V_\kappa$ expression pattern. It was first observed that whatever the cell origin (embryonic, fetal or adult), a constant proportion of clones (11-16%) expressed polyspecific antibodies, tested on a panel of 8 autoantigens. The pattern of family usage did not significantly differ from non polyspecific clones, suggesting that polyspecificity -and presumably connectivity- is not linked to a restricted repertoire. Sequence data rather suggest that the structural basis for polyspecificity might be due to a direct utilization of germline genes.

**C 019** SELF-REACTIVITY IN VIRAL AND TRANSGENIC ANIMAL MODEL SYTEMS, Mark I. Greene, Richard M. Siegel, Makoto Katsumata, Shinji Komori, and Katsuyuki Yui. Department of Pathology and Laboratory Medicine and the Center for Receptor Biology, University of Pennsylvania School of Medicine, Philadelphia PA, 19104 We have investigated a number of animal model systems in which autoimmune phenomena can occur despite clonal deletion of potentially autoreactive T cells. After infection with Reovirus type III, we have isolated hybridomas representing autoimmune anti-idiotypic B cells reactive against the cellular reovirus receptor. There is significant sequence homology between the $V_H$ region of anti-idiotypic antibodies and the reovirus hemagglutinin antigen which allows reovirus-specific T cells to recognize $V_H$-derived peptides presented in association with class II molecules by autoreactive B cells. This mechanism may allow non-autoreactive T cells to provide help for autoantibody production. T cells which have low affinity for self-antigens may also contribute to autoimmunity. To study the fate of T cells bearing receptors with a range of affinities for self in more detail, we have created transgenic mice which express a Vß8.1-containing T cell receptor on >98% of peripheral T cells. Vß8.1 is known to confer reactivity to Mls-$1^a$. When these mice were backcrossed to Mls-$1^a$, most T cells were found to be deleted in the thymus, but a significant proportion of $CD4^+$Vß $8.1^+$ T cells remained in the periphery. While there was no Mls$1^a$ reactivity in this population, T cell clones derived from these cells displayed significant, yet reduced reactivity to Mls-$1^a$ compared to T cell clones derived from mls-$1^b$ Vß8.1 TG mice. Experiments with anti-CD4 blocking support the idea that cells with low affinity for self Mls-$1^a$ escape clonal deletion in the thymus and are present in the periphery, where they may become autoreactive after stimulation with cross-reactive foreign antigens. The mechanisms for and significance of these findings for the pathogenesis of autoimmunity will be discussed.

**C 020 IMPLICATIONS OF THE SELECTIVE EXPANSION OF RESTRICTED TCR BEARING LYMPHOCYTES AND IMMUNOGLOBULINS IN THE DEVELOPMENT OF AUTOIMMUNE DISEASES,** Carlos Martinez-A. , Jose C. Gutierrez-Ramos, Jose L. Andreu, Jose I. Moreno de Alboran, Maria L. Gaspar and Miguel A.R. Marcos, Centro de Biologia Molecular, CSIC, Universidad Autonoma, Campus de Cantoblanco, 28049 Madrid, and Institute Pasteur, Unite d'Immunologie, Paris.

We have investigated multiple, different strategies for the etiological therapy of autoimmune disease using two murine models of systemic lupus erythematosous (SLE) . One of the models, the MRL/lpr mouse, has thymic atrophy ,abnormal expansion of CD3+CD4-CD8- T cells displaying a restricted repertoire with the preferential expansion of the Vβ8 Tcr gene products leading to a generalized lymphoadenopathy and a wide array of autoantibodies and RF. The other represents a chronic GVH induced in CBA/N mice submitted to autologous bone marrow reconstitution after lethal irradiation and simultaneous CSA treatment, that develops autoantibodies and a selective expansion of Vβ8 bearing T cells together with a marked increase in the concentration of serum immunoglobulins that specifically binds to $F(ab)_2$ fragments of anti-Vβ8 antibodies ( Vβ8-like IgV regions).

Using monoclonal antibodies against the Vβ8 variable region of the Tcr, very low number of T cell vaccination with the population of cells expanded in the process of the autoimmune diseases development as well as administration of human IL 2 by means of life recombinant vaccinia virus infection, had a long term benefitial effect on life expectancy, the development of autoantibodies and expansion and accumulation of the lymphocyte with the abnormal phenotype.

This data together with current knowledge implying polyetiological basis for autoimmune diseases development shows that autoimmune manifestations probably constitutes the final result of distinct partially overlapping combinations of predisposing factors and/or external stimuli and therefore it suffices to terapeutically intervine in one of the mechanisms to prevent or cure the disease.

C 021 IDIOTYPE MIMICRY AND IMMUNE FUNCTION, Maurizio Zanetti and Rosario Billetta, Dept. of Medicine, Univ. of California San Diego, San Diego, CA 92103.
Antibodies (Abs) and their idiotopes can mimic exogenous antigens and under appropriate experimental conditions elicit immunity of predetermined specificity similar to that induced by the nominal antigen. This implies the possibility that structurally-similar determinants are shared between Ab variable (V) regions and epitopes on antigens, be this the case of exogenous or endogenous antigens, including receptors on somatic cells. Little is known, however, about the structural requirements for molecular mimicry by antibody V regions and what the basis mighth be for their immunogenicity through antigen-mimicking idiotopes. In an attempt to directly address this issue we used protein engineering techniques to construct an antibody molecule modified at the DNA level to encode and express in the third hypervariable loop of the heavy chain three copies of the repetitive tetrapeptide Asn-Ala-Asn-Pro (NANP) of a parasite, *Plasmodium falciparum* a causative agent of malaria in humans. The engineered Ab expressed the foreing epitope in a stereochemical configuration immunologically similar to the native antigen. A monoclonal Ab to the native peptide epitope bound to the engineered V region and a synthetic NANP peptide blocked this interaction. Immunization of rabbits and mice with the engineered antibody resulted in the elicitation of a humoral response to both the synthetic peptide and the native antigen. In mice, humoral immunity of the appropriate specificity was induced in strains of different H-2 haplotypes. Collectively, this study indicates that antibody V regions can be engineered to express peptide epitopes of unrelated antigens as part of their primary structure, and be used to induce immunity to the antigen in its native configuration. Because idiotypic determinants map to hypervariable loops of the V regions, the loop we engineered to express the $(NANP)_3$ amino acid sequence can be considered an idiotypic determinant of known molecular structure. In conclusion, Abs as the one described in this communication appear to be a faithful molecular mimic of antigens and can, therefore, be used to study the molecular events involved in activation of the immune system by Ab's idiotypes.
Supported by Council for Tobacco Research, American Institute for Biological Science, NIH AI23871 and NSF DCB 850201.

*Plenary Lecture II*

C 022 **SELECTION OF LYMPHOCYTE REPERTOIRES : THE PHYSIOLOGY OF AUTOREACTIVITY**, Antonio COUTINHO, Unité d'Immunobiologie, Institut Pasteur, 28 rue du Dr Roux, 75724 PARIS CEDEX 15, France.

Embryonic and neonatal V-region are characterized by developmentally controled preferences in the expression of some V-genes and preservation of germ-line sequences (low junctional diversity), encoding a highly connected network (K/N = 5-10, for antibodies) and productive autoreactivities. These conditions lead to central and peripheral "positive selection" of "self-related and connected" lymphocyte repertoires, with expansion in cell numbers and acquisition of a state of "dominant tolerande" towards "self" (the molecular environment available in the first few weeks of life in the mouse). The development of effector functions (antibody production, help and suppression) in connected lymphocytes gradually limits post-natal expansion and establishes the adult levels of network connectivity and size (around 10 % of all lymphocytes), through "negative selection" and elimination of lymphocytes with high values of receptor occupancy mediated by self ligands and V-regions. The network organization of autoreactive cells escaping deletion seems to impose oscilatory, equilibrium dynamics with little clonal expansion and limited possibilities for antigen-driven selection, and may provide for dominant tolerance by "self-assertion" (recruitment of newly-formed autoreactivities). In adults, extensive junctional diversity in lymphocytes produced at high rates from uncommitted precursors, and the consequent turnover rates within a fixed population size, allow for the selection of functionally "disconnected" resting lymphocytes which constitute available repertoires (90 % of all lymphocytes) that are essentially nonself-reactive. Two basic types of organization thus coexist in the immune system : a self-directed network that ensures tolerance, and a large set of independent clones that account for immune responses to nonself.

*MHC Interaction with Self Antigen and Idiotypes*

**C 023** GENETIC AND MOLECULAR MECHANISMS OF AUTOIMMUNE DISEASE, Hugh O. McDevitt, Department of Microbiology & Immunology, Stanford School of Medicine, Stanford, CA 94305. 415/723-5893. The role of Class II MHC molecules in presentation of self-peptides in the initiation of the autoimmune process will be compared and contrasted in (PL x SJL)$F_1$ mice immunized with myelin basic protein, in NOD mice spontaneously developing insulin-dependent diabetes mellitus (IDDM) and in patients with IDDM. Experiments designed to validate the role of specific residues in mediating susceptibility using transgenic NOD mice will also be discussed. MHC-blocking peptides have been successfully used to prevent the induction of EAE in (PL x SJL)$F_1$ mice. The effect of these blocking peptides on EAE and on the immune response to other antigens is currently under study and experiments designed to utilize MHC-blocking peptides to treat already established autoimmune disease are currently in progress.

**C 024** The Problem of TCR Processing and Presentation

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Clone-specific interactions between T cells have been thought to play an important role in immunoregulation. It is reasonable to assume that these are mediated by the recognition of the TCR of one cell by the TCR of another (regulatory) cell. This would conceivably necessitate the processing of the TCR of the target cell into peptides and its presentation by Class I MHC or Class I MHC-like molecules. Since it has been shown that the spectrum of endogenous proteins that are presented by a given cell is quite limited, and since the clonotypic T cell interaction system should instead be a general one, it is conceivable that special mechanisms exist for TCR processing and presentation in activated T cells. We consider that these mechanisms might be related to the co-recycling of TCR and Class I MHC in endosomes of activated T cells. TCR processing and presentation in T cells might follow a route quite different from that of other endogenous proteins of viral antigens because of its special functional role.

**C 025** THE DOMINANT SELF: TOLERANCE AND ITS RELATIONSHIP TO DETERMINANT DOMINANCE.
Gilles Benichou, Guy Gammon, Peter A. Takizawa, Philip T. Ho, Clifford A. Olson, Catherine C. Killion*, Minnie McMillan* and Eli Sercarz. Department of Microbiology & Molecular Genetics, 5304 Life Sciences Bldg., UCLA, 405 Hilgard Ave., Los Angeles, CA 90024-1489. *Department of Microbiology, USC, 1441 Eastlake Ave., Los Angeles, CA 90033.
After immunization with a typical globular protein antigen, such as hen lysozyme, HEL, T cells become activated to only those determinants which are dominant in that haplotype. The very characteristic pattern of dominance is a result of many factors, both at and distant from the site of T cell recognition on the antigen, and includes the relative availability of the determinant, which will be the major focus here. Would minor determinants that could not activate T cells upon HEL injection nevertheless induce T cell tolerance? In fact, experiments (G. Gammon et al, Nature 342:183-185) show that in general, only dominant determinants induce adult tolerance. It can be predicted that there might be many self-directed T cells which escape tolerance induction since they do not achieve a threshold level of concentration on the cell surface in the context of self-MHC molecules. One of the most interesting classes of self-proteins to examine in this respect are the class I and II MHC proteins themselves, which exist in the thymus and are intricately associated with the induction of tolerance and the shaping of the T cell repertoire. In fact, we found that in both class I and II molecules, there exist determinants which can bind to self class II and thereby induce a proliferative T cell response. Moreover, there are other determinants which can bind to self class II, but do not activate a response and can be assumed to have induced neonatal tolerance. The affinity of the immunogenic self-MHC peptides is as high as any strong extrinsic determinant, but it is likely that there is only limited availability of such immunogenic determinants at the time that tolerance is induced. Therefore, the "dominant self" is that assemblage of self determinants which have induced tolerance and are not immunogenic. The remainder of the determinants on self molecules, that are poorly processed and presented, exist as a reservoir of targets for autoimmune attack. Since our conclusion is that T cells directed against determinants from the "cryptic self" have not been tolerized, even relatively high affinity T cells against these determinants may coexist in the individual. Under conditions in which extrinsic determinants activate these untolerized anti-self cells, and the display of the otherwise cryptic determinants increases significantly, autoimmunity may result. Supported by NIH grant AI-28419 and a grant from the Multiple Sclerosis Society RG 1755-B-2.

**C 026** **Presentation of light chain idiotype by B lymphoma cells,** Siegfried Weiß, Bjarne Bogen, GBF, D-3300 Braunschweig, Germany and Institute for Rheumatology and Immunology, N-0172 Oslo, Norway.

Since B cells can function as APC's the question arises whether B cells can also present the antibody they produce to MHC-restricted T cells. To obtain a clonal population of idiotype bearing B cells, we have transfected genes encoding the light chain of MOPC315 and derivatives of it into the B cell lymphoma A20. These B lymphomas display this light chain on their cell surface, and at the same time, they present it constitutively to MHC class I restricted T cells. Thus, two idiotypes exist on the surface of these cells and by extrapolation on the surface of B cells in general: the 'classical' one borne by the native immunoglobulin and a processed form of it allowing regulatory contact with MHC-restricted T cells. The fact that an endogenous protein is recognized in association with class II molecules challenges the current rule that endogenous antigen is only presented by class I. Since minor amounts of the transfected light chain were secreted, it was not excluded that the presentation of this endogenous antigen in the context of class II was due to reuptake or due to recycling of the receptor. We therefore modified the λ light chain by site-directed mutagenesis so that it could no longer be exported from the cell. Nevertheless, the idiotypic epitope was presented. Introducing the ER retension signal KDEL also did not interfere with the presentation of the idiotypic epitope to T cells. This suggests that the ER is a processing compartment. Transport into the ER was found to be essential. No presentation of proteins expressed in the nucleus or the cytoplasm was found. Thus, we suggest that endogenous light chain may be presented in the context of class II molecules as long as it is transported into the ER. We therefore suggest that endogenous proteins that are transported into the ER can be presented in association with class II. This could be the source of class II restricted minor antigens.

*Regulation and Therapy in Autoimmune Diseases*

C 027 *IN VIVO* INHIBITION OF T CELL ACTIVATION BY MHC BLOCKADE, Luciano Adorini, Preclinical Research, Sandoz Pharma Ltd., CH-4002 Basel, Switzerland

A striking characteristic of human autoimmune diseases is the increased frequency of certain HLA class II alleles in affected individuals. Since alleles positively associated with autoimmune diseases share amino acid residues in the hypervariable HLA regions involved in peptide binding, it is likely that disease-associated class II molecules have the capacity to bind the autoantigen and present it to T cells, thereby inducing and maintaining the autoimmune disease. Blocking the antigen-presenting activity of disease-associated MHC class II molecules could thus interfere with disease. A synthetic peptide corresponding to residues 46 to 62 of mouse lysozyme (ML 46-62), binding to I-$A^k$ molecules but not immunogenic in mice, inhibits the priming for T cell responses when co-injected into H-$2^k$ mice together with foreign antigens, such as hen egg-white lysozyme (HEL), or HEL peptides 46-61 and 112-129. The inhibition correlates with the capacity of the competitor to bind to the I-$A^k$ molecule presenting the foreign antigens tested. *In vivo* MHC blockade by peptide competitors binding to class II molecules inhibits not only T cell proliferation but also T cell-dependent antibody responses, even when mice had already been primed with antigen. The I-$A^k$-binding HEL peptide 53-61, appropriately protected from proteolysis, can also inhibit the induction of I-$A^k$-restricted T cell responses when applied in soluble form via osmotic pump remote from the site of priming. Although effective in inhibiting T cell priming, the soluble peptide competitor does not induce a T cell response against itself, indicating a possibility for immunomanipulation of antigen presentation potentially applicable to the treatment of MHC-associated autoimmune diseases. The exogenous competitor ML 46-62 inhibits equally well the I-$A^k$-restricted T cell activation induced by peptide antigens of exogenous or endogenous cellular origin. The capacity of an exogenous competitor to inhibit *in vitro* presentation to T cells of endogenous, as well as exogenous, antigens suggests that *in vivo* MHC blockade based on the administration of exogenous peptide competitors could inhibit presentation to class II-restricted T cells of endogenous cellular antigens, likely the most relevant in the induction of autoreactive T cells leading to HLA-associated autoimmune diseases.

C 028 IMMUNOTHERAPEUTIC APPROACHES FOR DIABETES, Jean-François Bach, INSERM U 25, Hôpital Necker, 161 rue de Sèvres, 75743 Paris Cedex 15, France.

Type 1 diabetes is a genetically controlled autoimmune disease whose clinical manifestations (insulin dependency) only appear several years after the onset of the Langerhans islet β cell aggression. Ideally, one would like to apply immunointervention methods at the prediabetic stage as can be detected by the study of genetic markers (at the genomic level) and immunologic tests (humoral and cellular). Experiments performed in NOD mice and BB rats indicate that, using this approach, complete and long term prevention of the disease can be obtained with cyclosporine, total lymphoid irradiation or monoclonal anti-αβTCR, anti-CD3, anti-CD4, anti MHC class II and anti-γIFN antibodies. Intervention in human prediabetics has not yet been performed at a large scale and should be prepared by the search for efficacious therapeutic maneuvers in recent onset overtly diabetic patients of easier access. Several studies including our extensive clinical trials have indeed shown that cyclosporine and azathioprine were efficient in this setting. Other drugs are presently being tested.

This strategy should be associated with the study of new approaches attempting to induce β cell antigen specific immune paralysis. This is difficult because the target β cell autoantigen is not known (there are several candidates, including a 64 K protein, but no proof has been presented of their pathogenic responsibility). One may however try to circumvent this difficulty by acting at the T cell receptor Vβ level inasmuch there is a restriction of Vβ gene usage in type 1 diabetes as suggested by studies performed in our laboratory. One may passively administer anti-Vβ antibodies (we have shown that anti-Vβ6 monoclonal antibodies are protective in NOD mice), or imunize the prediabetic individuals against the pathogenic T cells (T cell vaccination) or synthetic Vβ fragment (as performed in EAE). Peptide therapy will represent another important approach but this will need the final determination of the target autoantigen(s).

NETWORKS OF ANTI-IDIOTYPIC AND ANTIGEN-SPECIFIC T CELLS REGULATING AUTOIMMUNE REACTIVITY. Irun R. Cohen, Department of Cell Biology, Weizmann Institute of Science, Rehovot, Israel. Adjuvant arthritis (AA) is an autoimmune disease inducible in certain rat strains (Lewis) by immunization with killed *Mycobacterium tuberculosis* (MT) organisms in oil. A T cell response to the 65kDa heat shock protein (hsp65) of MT is a key element in AA. Analysis of factors regulating the expression of AA has revealed the concerted action of two regulatory elements: anti-idiotypic T cells that recognize other T cells and suppressor T cells that recognize MT. The transition of the anti-hsp65 T cell response from benign autoimmunity to clinical AA appears to be controlled by the behavior of the regulatory T cells in a complex manner: sever AA is associated with marked suppression and decreased anti-idiotypic responsiveness; in contrast, benign autoimmunity is associated with decreased suppression and marked anti-idiotypic responsiveness. Anti-idiotypic regulation was also detected in the spontaneous expression of diabetes by NOD mice. Thus, the appearance of disease is influenced by the manner in which existing autoimmunity T cells are controlled.

**C 030** IDIOTYPIC (ID) REGULATION OF AUTOANTIBODIES AS A MECHANISM CAUSING NEPHRITIS IN MURINE MODELS OF SLE, Bevra H. Hahn, Betty P. Tsao, Fanny M. Ebling, Katsunori Ohnishi. Division of Rheumatology, UCLA School of Medicine, Los Angeles, CA 90024-1670
SLE nephritis is caused by deposition of anti-DNA in glomeruli in susceptible mice and humans. One characteristic of pathogenic subsets of anti-DNA is enrichment in certain Ids. We hypothesize that interactions between appropriate MHC, peptides derived from the Id+ regions of IgG, and TcR on helper T cells (Th) cause sustained production of pathogenic antibodies which result in lupus nephritis. One cross-reactive Id, IdGN2, dominates glomerular Ig deposits in human lupus and is found in glomeruli of NZB/W (H-2$^{z}$) mice. Most Mab anti-DNA from NZB/W which are IgG2a or 2b and express IdGN2, when administered daily or implanted as B cell hybridomas, can induce nephritis in healthy BALB/c mice, but not C57Bl/6 mice. Expression of IdGN2 depends upon the H chain of Id+ Ig. Sequencing the $V_H$, D and J regions of several IdGN2+ and IdGN2- Mab anti-DNA from NZB/W showed that most Id+ Mabs contained two discontinuous 3 basic amino acids separated by 1 or 2 uncharged AA sequences (KFKGK most frequently) in the CDR1-FR2 and CDR2-FR3 regions of $V_H$. Sette has noted such sequences can bind to I-E$^{z}$, which is expressed by NZB/W mice. A construct containing the $V_HDJ_H$ (+ promoters/enhancers) of the H chain of A6.1 linked to the IgG2a C region of BALB/c mice was inoculated into fertilized ova of C57 x DBA females mated with C57 males. Resultant transgenic founders expressed anti-DNA transiently. They were mated with BALB/c; the H-2$^{bz}$ offspring developed high serum levels of IgG2a anti-dsDNA which were sustained for 32 wks. They also developed nephritis manifested by proteinuria and azotemia; the nephritis was transient. Matings between transgenic founders and C57 yielded H-2$^{z}$ offspring which did not show sustained expression of anti-DNA or develop nephritis. We conclude that interaction between MHC that contains H-2$^{z}$ (probably in mice that express I-E$^{z}$), the proper TcR on Th, and certain peptides containing 3 basic AA sequences on Ig that could be idiopeptides may account for sustained upregulation of pathogenic anti-DNA and resultant lupus nephritis.

**C 031** FURTHER STUDIES ON THE V-REGION DISEASE HYPOTHESIS,
Ellen Heber-Katz, The Wistar Institute, Philadelphia, PA 19104

Restricted T cell receptor gene usage is now an accepted phenomenon when T cells recognize an antigenic peptide determinant in association with a given MHC molecule. Recently, we have further noted that the same combination of V regions ($V_\alpha 2 V_\beta 8$) is used in a number of experimentally-induced autoimmune diseases (EAE, encephalitis; EAN, neuritis; EAU, uveoretinitis; and AA, adjuvant arthritis) in several species. We feel that these results are inconsistent with the current immunological thinking (the usual epitope model) by which the molecular lesion of autoimmune disease has its locus within the epitope associated with an MHC molecule on the putative target tissue. We have previously proposed the V-region disease hypothesis which places the locus of the autoimmune molecular lesion at the TcR itself. Thus, the finding that similar $V_\alpha V_\beta$ combinations are used irrespective of the antigen specificity or the MHC specificity is a natural outcome of this model.

Recent results indicate the possibility that TcR peptides derived from single alpha or beta chains of the TcR may play a regulatory role in the EAE autoimmune response. This raises an interesting paradox, how to reconcile the observed two chain specificity ($V_\alpha V_\beta$ combination) with a regulatory role for single chains or parts thereof.

*Plenary Lecture III*

**C 032** IMMUNOINHIBITORY ACTIVITY OF CLASS II MHC GENES, Avrion Mitchison, Deutsches Rheuma Forschungszentrum Berlin, Am Kleinen Wannsee 5, D-1000 Berlin 39

In the most frequently used panel of H-2 recombinant strains, B10.A, B10.A(4R), B10.A(5R) and B10, inhibition of the immune response has thitherto mapped to H-2E. Inhibition of the responses to Thy1 antigen and to F liver protein antigen, as described here, maps in a novel pattern to H-2b. The evolutionary significance of this new pattern, and of immunoinhibition in general, will be discussed.

Immunoinhibitory mechanisms mediated by class II MHC molecules can be classified into four groups: (i) intracellular, intramolecular, (ii) intracellular but intermolecular (iii) intercellular, dependent on negative selection, and (iv) intercellular, dependent on positive selection within the thymus (this includes the old category of "suppressor T cells"). Methods of sorting out these mechanisms, and their usage among the inhibitory effects known in mouse and man, will be discussed.

Inhibitory effects of these types can provide guidance for the design of therapeutic strategies in autoimmune and othe immunological diseases. Strategies at present worth considering include (i) local administration of physiological doses of cytokines, (ii) post diease vaccination, as currently applied in leishmaniasis and leprosy, and (iii) gene implantation ("gene therapy).

*Plenary Lecture IV*

**C 033** DRIVING FORCES IN AUTOIMMUNITY, Ivan M. Roitt, Department of Immunology, University College & Middlesex School of Medicine, London W1P9PG, U.K.
It is useful to distinguish organ-specific from non-organ specific or systemic autoimmunity since it is unlikely that similar tolerance mechanisms exist to the two types of antigen involved. At the B-cell level, the existence of somatic mutations and high affinity antibodies strongly suggest antigen drive, a conclusion supported by the existence of antibodies to specific clusters of antigens in particular diseases, such as thyroiditis, pernicious anaemia and SLE. Furthermore, elimination of the antigen in organ-specific disorders involving the thyroid and pancreas, grossly inhibits the autoimmune process. Antigen-specific T-cells are pivotal in both experimental and spontaneous organ-specific disease. Experiments defining thyroglobulin T-cell epitopes in experimental thyroiditis will be described and their exploitation for therapeutic purposes will be discussed. The existence of class-switching and of somatic mutations in the antibodies in systemic autoimmunity strongly implicate the involvement of T-cells. The glaring question concerns their specificity: are they anti-heat shock protein, self-class II or anti-idiotype expressed on the surface of the collaborating B cell? In the latter case, processed Ig receptor could be the T-cell target. T-cell help of this nature could provide an element of polyclonal stimulation which might synergise with signals due to cross-linking of receptors by multivalent antigen.
Initiation or priming of autoimmunity is probably more difficult than boosting established responses. The concentration of available processed autoantigen or help from anti-idiotype T-cells may break through the priming barrier. Primed T-cells of higher affinity responding to cross-reacting antigens, can be boosted by natural autoantigen which might have too low affinity for activationg naive cells. In some cases an abnormality of antigen may be a prime factor and evidence for abnormality of thyroid cells and beta-cells of the pancreas in spontaneous autoimmunity have been recognised. Studies on abnormal glycosylation of IgG in Rheumatoid Arthritis where autoimmunity to this protein is central, will be discussed in relationship to pathogenesis of the disease.

*Late Abstract*

IMMUNE RECOGNITION AND AUTOIMMUNE DISEASE, Leroy Hood, Steven Beall, K. C. Cheng, William Funkhouser, Joan Goverman, Gamal Osman and Dennis Zaller, Division of Biology, California Institute of Technology, Pasadena, CA 91125. Myelin basic protein (MBP) can induce a chronic, remitting, demyelinating disease in mice, experimental allergic encephalomyelitis (EAE). In some ways, EAE resembles the human autoimmune disease, multiple sclerosis. We and others have demonstrated that the N-terminal epitope appears to play a dominant role in inducing the disease, and that the T-cell receptors on the CD4+ T-cells are highly restricted. We have also demonstrated that antibodies against the two dominant types of Vβ chains can block or prevent the disease. We will discuss the behavior of MBP-specific T-cell responses in closely related mice capable of EAE responses and the nature of tolerance in mice transgenic for the predominant MBP-specific T-cell receptor genes. We will also discuss our genetic mapping results with regard to T-cell receptor haplotypes in families where one or more members have multiple sclerosis.

## *T Cell Tolerance Mechanisms I*

**C 100** MTS 35 : A NOVEL MARKER DISCRIMINATING IMMATURE FROM MATURE (IMMEDIATE POST-NEGATIVE SELECTION) SUBSETS.

Richard Boyd*, Dale Godfrey*, Carolyn Tucek* and Patrice Hugo*#.
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As a basis for determining the nature of the thymic microenvironment controlling T cell differentiation and associated selection processes, we recently developed an extensive panel of mAbs to thymic stromal elements. As part of their characterisation we identified a novel mAb (MTS 35) reacting with a plasma membrane antigen expressed both on cortical thymocytes and a subset of isolated thymic medullary epithelial cells. It was negative on peripheral T cells and recent thymic migrants but it did stain B cells. In view of its selective T lymphocyte reactivity with cortical thymocytes, the relevance of MTS 35 as a marker of immature T cells was investigated in detail by 2, 3 and 5 colour flow cytometric analysis in comparison with an extensive panel of standard reagents. MTS 35 was distinct from ThB, HSA, Ly6C, Ly6A/E, H-2K, Thy 1, PNA, CD5, IL2R, CD44, and LFA 1. MTS 35 was present on approximately 85% of adult thymocytes; this included all immature subsets (CD4-CD8-CD3-, CD4+CD8-CD3-, CD4-CD8+CD3-, CD4+CD8+CD3-, and CD4+CD8+CD3low), but most interestingly it was negative on cells with high expression of the TcR/CD3 complex, a feature of mature thymocytes and peripheral T cells. Accordingly, CD4+CD8+CD3high cells,a post-selection subset which convert into mature thymocytes were MTS 35-. In fact, there was a direct inverse relationship between the expression of MTS 35 and CD3: CD4-CD8-CD3- cells had the highest level of MTS 35 expression , CD3low cells were MTS 35low and CD3high cells were MTS 35 negative. This marker is expressed on >95% of thymocytes at day 14-16 of embryogenesis and >95% of SCID mice thymocytes. More refined gating and analysis of the normal adult CD4+CD8+CD3high cells into CD3int and CD3high revealed that MTS 35, while negative on the latter, was present on most but not all of the former cells; we propose that these MTS 35- cells are the immediate precursors of mature T cells. Since in a parallel study we have shown that in the I-E/Mls-1a model, negative selection begins at the CD4+CD8+CD3int stage, we further propose that down regulation of MTS 35 occurs post-positive selection and is complete by the stage at which T cells would have encountered negative selection processes. MTS 35 is thus a unique marker which exquisitely separates mature from immature thymocytes and should prove invaluable for defining selection events occurring during thymopoiesis.

**C 101** CONTRIBUTION OF THYMIC EPITHELIUM IN LIMITING THE DEVELOPMENT OF CLASS I MHC-RESTRICTED SELF-REACTIVE T-CELLS, Douglas A. Carlow, Nicolai van Oers, Soo-Jeet Teh, and Hung-Sia Teh,Department of Microbiology, University of British Columbia, Vancouver, Canada V6T 1W5

The extent to which thymic epithelium (TE) contributes to negative selection against self-reactive T-cells is a subject of current controversy. TE induced deletion/anergy of autoreactive class II restricted $CD4^+8^-$ T-cells has been demonstrated but its influence on the maturation of class I restricted $CD4^-8^+$ autoreactive T-cells is not clear. We have used transgenic mice bearing an αβ T cell receptor (TCR) specific for the male (H-Y) antigen to follow the consequences of H-Y expressed by TE or extrathymic tissues on the maturation of class I restricted $CD4^-8^+$ H-Y specific T-cells. Results obtained with allogeneic bone marrow radiation chimeras and recipients of deoxyguanosine treated fetal thymic implants indicate that male TE can induce deletion of H-Y reactive T-cells. The deletion is not complete however, and anergic $CD4^-8^+$ T-cells bearing TCR with specificity for H-Y can accumulate in significant numbers in peripheral lymphoid organs. We also find no evidence for H-Y antigen transfer from TE, or the extrathymic environment, for intrathymic deletion of H-Y reactive T-cells.

**C 102** THE THYMUS HAS TWO FUNCTIONALLY DISTINCT POPULATIONS OF IMMATURE αβ+ T CELLS: II. EVIDENCE THAT SELECTION PRECEDES TOLERANCE IN T CELL DEVELOPMENT, T.H. Finkel, J.C. Cambier, P.C. Marrack, and J.W. Kappler. National Jewish Center for Immunology and Respiratory Medicine, Denver, CO 80206.

The ordering of selection and tolerance remains one of the hotly debated questions of T cell development. Data which indirectly supports the order selection, then tolerance, comes from studies in chimeric mice and in mice transgenic for promoter deletions which determine the localization of class II MHC expression, which argue that positive selection requires MHC expression in the thymic cortex. However, there is no direct evidence to support this contention and data to the contrary comes from our own work in which we have shown that deletion may occur in the cortex. Our preliminary data suggests that the non-deletable cortical population is the target of intrathymic selection. We had previously reported a direct demonstration of positive selection in the mature cells of the thymic medulla, using mouse strains expressing the 17a β-chain variable domain (Vβ17a) of the T cell receptor. In recent work, we have analyzed Vβ17 expression in H-2q and H-2b fetal animals after thymic organ culture with or without anti-αβTCR. It was hoped that by eliminating the deletable population, selection would become apparent in the thymic cortex. The expected enrichment of Vβ17high cells was found in the thymus of adult H-2q mice compared to H-2b mice (9.1 vs. 3.8%). A slight enrichment of Vβ17low cells was seen in the untreated thymic lobes from H-2q mice compared to H-2b mice (9.7 vs. 7.2%), while the relative number of Vβ3low cells remained constant (6.4 vs. 6.5%). Notably, the relative number of Vβ17low cells increased markedly in the thymic lobes from H-2q treated with anti-αβTCR antibody (13.1%), while the relative number in lobes from H-2b remained constant (7.0%). Again, the relative number of Vβ3low cells remained constant between H-2q and H-2b after antibody treatment (11.9 vs. 11.1%), suggesting that the variation in Vβ17a levels was due to a specific selective event. The results suggest that selection of cells bearing Vβ17a occurs in the non-deletable population of the thymic cortex and is detectable in the absence of the large number of cortical cells which have failed selection and are awaiting deletion. If, as predicted, the non-deletable population precedes the deletable population in ontogeny, then it should be possible to identify a point in ontogeny at which only non-deletable cells are present, i.e. incubation with anti-αβTCR antibody should result in no deletion. If, on the other hand, the deletable and non-deletable populations appear in ontogeny simultaneously, ~50% of immature αβTCR+ cells should be deletable at all gestational ages. Our data suggest that a window exists in ontogeny from ~day 16-day 17 of gestation when all immature αβTCR+ thymocytes are resistant to deletion by ligation of αβTCR, though not to deletion by ligation of CD3 i.e. when the deletable population has not yet developed. In addition, an increase in percentage and absolute numbers of αβ+ immature T cells in cultures treated with anti-αβTCR, suggests that while ligation of αβTCR results in deletion late in ontogeny, ligation of αβTCR may result in selection early in ontogeny.

In summary, we have shown that the non-deletable population of thymocytes precedes the deletable population in ontogeny, that cells within this non-deletable population undergo selection, and therefore that selection precedes tolerance in T cell development.

**C 103** **I-E CO-TOLEROGEN 1 DISPLAYS A TISSUE DISTRIBUTION SIMILAR TO MLS $1^a$, AND A SECOND I-E CO-TOLEROGEN IS ENCODED BY A SINGLE GENE.**

Kenneth J. Gollob and Ed Palmer. National Jewish Cntr., Denver, CO 80206.

The I-E co-tolerogen 1 (Etc-1) leads to the deletion of Vβ5, and $CD4^+$ Vβ11 expressing T-cells in I-E expressing mice (Woodland et al, Sci.1990). The Etc-1 reactive T-cell hybrid 1BVB11.40 was used to examine the tissue distribution of Etc-1. These studies determined that Etc-1 is expressed on B-cells, but not macrophages nor dendritic cells. Furthermore, the ability of B-cells treated with LPS and IL-4 to stimulate 1BVB11.40 was 30-50 times greater than cells treated with LPS or IL-4 alone. This synergy was not due to differential levels of I-E expression. Taken together, Etc-1 displays a tissue distribution similar to that seen for Mls $1^a$, but unlike Mls $1^a$, an MLR is not generated between Etc-1 disparate strains.

A second co-tolerogen (Etc-2) deletes Vβ5 T-cells but not Vβ11 T-cells. This phenotype is different from Etc-1 which acts on both populations of T-cells. The gene or genes encoding for Etc-2 are being mapped by analyzing backcross mice between an $Etc\text{-}2^+$ strain and an $Etc\text{-}2^-$ strain. At this point it has been determined that a single locus encodes Etc-2. This is based on the fact that 24 of 52 backcross mice deleted $V\beta5^+$ T-cells, consistent with the segregation of one gene. Further analysis by genomic southerns will allow for the mapping of Etc-2.

**C 104** THE SPECIFICITY OF POSITIVE AND NEGATIVE SELECTION ANALYZED IN TRANGENIC MICE BEARING CYTOCHROME C-SPECIFIC T CELL RECEPTORS, Stephen M. Hedrick, Nicki McRoberts, Jonathan Kaye*, Department of Biology and the Cancer Center, UC San Diego, La Jolla, CA 92093-0063, *Research Institute of Scripps Clinic, La Jolla, CA 92037.

The genes encoding two different T cell receptors specific for pigeon cytochrome c in association with $IE^R$ were used to generate transgenic mice. The sequences of the two receptors differ by a single amino acid in VDJ junction of the α-chain. The specificity of positive selection differs for these two receptors in the ability of the cells to mature in the presence of an unexpected specificity $IA^b$. The positive selection process appears to involve the progressive loss of CD8, and concomitant increase in the expression of both CD4 and the transgenic TCR. Negative selection occurs in these mice in the presence of $IA^S$ although the T cells from which the genes were cloned do not respond to $IA^S$. The stage of negative selection varies with the age of the mice: neonates show no apparent clonal deletion, young mice (<5 weeks) appear to delete self-reactive T cells late in the CD4+CD8+ stage, and older mice appear to delete the self-reactive cells early in the double positive stage; these mice have very small thymuses and almost no $CD4^+CD8^+$ cells. Experiments in progress are designed to mimic postive and negative selection processes in culture.

**C 105** GENERATION OF MATURE RAT CD8 T-CELLS FROM DEFINED IMMATURE PRECURSORS IN SHORT TERM SUSPENSION CULTURE, Thomas Hünig, Rita Mitnacht, Institut für Virologie und Immunbiologie, Universität Würzburg, Versbacher Str. 7, D-8700 Würzburg, F.R.G.

CD4,8 double positive (DP) thymocytes isolated ex vivo are heterogenous regarding their stage within thymic selection, some of them having received positive, others negative, and most of them no selection signals at all. In order to obtain a homogenous population of double positive thymocytes that would still have the potential to be positively selected, we therefore prepared "fresh" rat DP-cells by allowing highly purified CD8 immature thymocytes to differentiate over night in cell culture. The resulting DP-cells (>95% pure) were then cultured in the presence or absence of immobilized mAb to a constant determinant of the TCR with or without Il-2. Within 2 days, the combination of TCR stimulation + Il-2 led to the complete loss of the CD4 antigen and acquisition of CD53 in about 70% of these cells. Further over night culture without anti-TCR mAb resulted in high (peripheral) level of TCR expression. These cells could then efficiently be restimulated through the TCR, resulting in highly lytic cytotoxic T-cells. Thus, functionally mature CD8 cells with an unselected repertoire were generated. The properties of this repertoire are currently being studied.

**C 106** T-CELL SUBSET SPECIFIC EXPRESSION OF ANTIGEN RECEPTOR β-CHAINS IN α-CHAIN TRANSGENIC MICE, Fredrik Ivars, Unit for Applied Cell and Molecular Biology, Umeå University, S-901 85 Umeå, Sweden.
Earlier studies using T-cell receptor (TcR) transgenic mouse models have demonstrated that the MHC-specificity of the transgenic TcR directs positive selection of the $CD4^+$ $CD8^+$ precursor cells into the mature $CD4^+$ and $CD8^+$ T-cell subsets. We have now extended these studies by analysing the TcR β-chain repertoire in $CD4^+$ and $CD8^+$ T-cell subsets in TcR α-chain transgenic mice, in which the vast majority of the T cells express the same TcR α-chain. A large number of Vβ8 gene-encoded cDNAs were analysed and a very limited repertoire of TcR β-chains was found to be co-expressed with the transgenic α-chain. Most importantly, certain Vβ8-Jβ combinations were found exclusively in one of the subsets and in some cases subset specific differences were localized to the VDJ junctional region of the β-chain genes. In contrast, $CD4^-8^-$ transgenic T-cells, as well as $CD4^+$ and $CD8^+$ T-cells from normal littermate controls, were found to express diverse β-chain repertoires. The present study suggests that very few β-chains with distinct structural characteristics enable positive selection of transgenic T-cells into either the $CD4^+$ or $CD8^+$ subsets. Moreover, the data suggest that the same structural constraints do not apply to the population of $CD4^-8^-$ transgenic T-cells.

**C 107** REQUIREMENT OF DENDRITIC CELLS AND B CELLS IN THE CLONAL DELETION OF Mls-REACTIVE T CELLS IN THE THYMUS, Yoshimoto Katsura, Osam Mazda, Yoshihiro Watanabe and Jun-Ichiro Gyotoku, Department of Immunology, Chest Desease Research Institute, Kyoto University, Kyoto 606, Japan
The present study was performed to identify the cells responsible for the elimination of T cells reactive with minor lymphocyte stimulating (Mls) antigens during T cell development. Experiments were carried out in a fetal thymus organ culture (FTOC) system. To examine the tolerance inducing activity, various populations of cells from adult CBA/J ($Mls\text{-}1^a$) mice were injected into deoxyguanosine (dGuo)-treated FTOC of C3H/He ($Mls\text{-}1^b$) mice with a microinjector, and 2 days later the thymus lobes were injected with fetal thymus cells from C3H/He mice as T cell precursors. After 14 days of cultivation, cells were harvested and assayed for the expression of T cell receptor Vβ6 element.

T cell-depleted populations of thymic as well as splenic cells from CBA/J mice were able to induce clonal deletion. Further characterization of the effector cells was carried out by fractionating the spleen cells before injecting them into dGuo-FTOC. None of the dish-adherent population, dish-nonadherent population or purified B cells alone were able to induce clonal deletion, whereas the addition of purified B cells to adherent cells restored tolerance inducibility. It was further shown that a combination of CBA/J B cells and C3H/He dendritic cells (DC) was effective in eliminating Mls-reactive clones.

**C 108** TH1-CELLS CAN BE SUBDEVIDED ON THE BASIS OF THEIR RESPONSE TO SOLID-PHASE COUPLED ANTI-CD3 ANTIBODIES. Michael Lohoff and Martin Röllinghoff, Institute for clinical Microbiology, Wasserturmstr.3 852 Erlangen, FRG.
Different TH1- and TH2- cell clones were stimulated with solid-phase coupled anti-CD3 antibodies. Lymphokine production (IL-2 and IFNγ in TH1-cells, IL-4 and IL-5 in TH2-cells) as well as the influence of CD3 triggering on the IL-2 induced T-cell proliferation were tested. While all clones tested produced lymphokines in response to anti-CD3, there was a difference in the proliferative characteristic of TH1-cells compared to TH2-cells and within the TH1-cell subset. The IL-2-induced proliferation of all TH2-cells tested was only marginally affected by solid-phase coupled anti-CD3. In contrast, the proliferation of most of the TH1 cells tested was completely blocked. However, a small percentage of TH1 cells was also unaffected. The block of proliferation of most TH1-cells could possibly represent a correlate of the well known phenomenon of peripheral tolerance. Currently, investigations are in progress to test for the notion that concomitant triggering of other cell surface molecules (e.g.CD4, LFA1) together with CD3 reverses the block in cell proliferation obtained with anti-CD3 antibodies alone and to test, if the differentially behaving TH1-cells differ in the expression of these molecules.

**C 109** ANALYSIS OF TCR Vβ 5.2 TRANSGENIC, MICE, *Mark W. Moore, †Michael Bevan, and ‡Francis Carbone, *Department of Microbiology, USC-Cancer Center, Los Angeles, CA 90033, †Department of Immunology University of Washington, Seattle, WA 98195, ‡Monash University, Melbourne, Australia

We have recently cloned the TCR β-chain gene from an anti-ova CTL clone and produced transgenic mice. The β-chain (Vβ 5.2) is expressed at high levels on nearly all T-cells. We intend to use these animals to study T-cell selection by breeding to animals that express the I-E molecule (reported to delete Vβ 5.2 expressing T-cells). We have also made transgenic mice that express ovalbumin and intend to mate these with our TCR transgenic mice to study tolerance to specific antigens. To complete these studies we are currently cloning the TCR α-chain to make transgenic animals with the complete receptor.

**C 110** TCR AND CD4 COENGAGEMENT DRIVES DOUBLE POSITIVE THYMOCYTES TO BECOME CD4 SINGLE POSITIVE T CELLS. Jennifer Punt and Yasuhiro Hashimoto. Department of Pathology, University of Pennsylvania, Philadelphia, PA 19104

The events which determine whether a CD4+/CD8+ (double positive or DP) thymocyte ultimately becomes a CD4 or a CD8 single positive (SP) T cell are unknown. Recent studies, however, have led to the speculation that coengagement of the T cell receptor (TcR) with either CD4 or CD8, as a consequence of intrathymic MHC protein/peptide binding, drives the DP to SP phenotypic shift. We have direct evidence for this from our studies of CD4/CD8 and TcR expression of fetal thymocytes harvested from thymic organs which have been exposed, in vitro, to a combination of anti-TcR and anti-CD4 antibodies. While, separately, these antibodies inhibit development of the CD4+/CD8-/TcR+ thymocyte subpopulation, treatment of the fetal thymus with a combination of the two antibodies results in a dramatic shift from the DP to the CD4 SP, TcR+ phenotypes. A combination of anti-CD3 and anti-CD4 antibodies, however, has no such effect. We will discuss the implications of our observations particularly as they relate to the current understanding of positive selection.

**C 111** TRANSFER OF PURIFIED $CD4^+$ T-CELLS INTO CONGENIC SCID MICE, Jörg Reimann[1] and Mogens Claessons[2], [1]Department of Medical Microbiology and Immunology, University of Ulm, Ulm, Germany and [2]Department of Anatomy, University of Copenhagen, Copenhagen, Denmark

It is a long-standing observation that autoreactive $CD4^+$ T-cells can be grown from different peripheral immunocompetent lymphocyte populations *in vitro* in various human and murine culture systems. These *in vitro* data seem to correspond to *in vivo* experiments in which transfer of limiting numbers of purified $CD4^+$ T-cells into congenic *nude* mice induces organ-specific autoimmune diseases. We have established a similar system in *scid* mice. Low numbers (1 to 5 $x10^5$) of cell sorter-purified splenic $CD4^+$ T-cells were transferred into congenic *scid* mice. Donor-derived $CD4^+$ T-cell populations were engrafted in the spleens of all recipient mice for >12 weeks. The splenic $CD4^+$ T-cell populations from transplanted *scid* mice were transferred into culture. The $CD4^+$ T-cell populations displayed a vigorous proliferative response to syngeneic stimulator cells. Some T-cell clones derived from these lines were exclusively autoreactive, but no alloreactive. In the serum of transplanted *scid* mice, host-derived autoantibodies were detectable. None of the transplanted *scid* mice (n=32) showed clinical or histological evidence of autoimmune disease (thyroid, adrenals, gonads, and gut mucosa were examined histologically). Autoreactive T-cell lines have been propagated *in vivo* by serial passage through *scid* recipients for >8 months. It is concluded that in addition to selfreactive $CD4^+$ T-cells, some undefined effector mechanism (which is missing in *scid* mice) must operate *in vivo* in *nude* mice to induce autoimmune lesions. Attempts to etablish $CD8^+$ T-cell lines *in vivo* in congenic *scid* mice according to this protocol were unsuccessful.

**C 112** THYMIC EPITHELIAL CELLS POSSESS STRONG VETO ACTIVITY, Carsten Röpke, and Mogens H. Claësson, Laboratory of Cellular Immunology, Department of Medical Anatomy, The Panum Institute, University of Copenhagen, DK 2200 Copenhagen N, Denmark.

Negative selection leading to tolerance towards self molecules is believed to occur intrathymically. In the present study, we have investigated whether thymic epithelial cells (TEC), which show high expression of MHC class I, can act as veto cells in vitro and down-regulate alloreactive cytotoxic precursor T cells with specificity for the class I antigens of the TEC. TEC were cultured in a growth-factor defined serum-free medium, which only allows growth of TEC. Veto assay cultures were one-way mixed lymphocyte culture (MLC). TEC being tested for veto suppression were of the same haplotype as the stimulator cells in the veto assay MLC. C3H TEC were titrated into a C57/6 anti-C3H + BALB/c MLC. A 10 times higher sensitivity to TEC-induced down-regulatory signals of CTLp anti-H-$2^k$ as compared with CTLp anti-H-$2^d$ indicates that the suppression observed at low TEC numbers is due to a specific cellular interaction between CTLp anti-H-$2^k$ and the co-cultured H-$2^k$ positive TEC. Limiting dilution analysis showed that the veto suppression was due to clonal delition of CTLp. Thus, these experiments are the first demonstration of veto activity in a non-lymphoid cell compartment.

**C 113** POSITIVE AND NEGATIVE SELECTION FOR Mls AND I-E COINCIDE WITH UPREGULATION OF CD3 ON $CD4^+8^+$ THYMOCYTES, Roland Scollay, Patrice Hugo and Ken Shortman, The Walter and Eliza Hall Institute of Medical Research, Melbourne 3050, Australia.

TCR-CD3 expression on adult murine $CD4^+8^+$ thymocytes is essentially trimodal, with almost 50% of cells being negative or very low, about 50% being low and about 3% being high for CD3 expression. When assessed for expression of a variety of antigens, including CD5, MEL-14, H-2K, LFA-1 and HSA, the $CD3^{hi}$ population was phenotypically intermediate between $CD3^{lo}CD4^+8^+$ cells and mature $CD3^{hi}$ single positive thymocytes. The timing of negative selection for $Mls^a$ (Vβ6) and I-E (Vβ17a) and positive selection for I-E (Vβ6) was analysed by comparing the TCR expression in the CD3 determined $CD4^+8^+$ subpopulations. Negative selection occurred just at the transition from $CD3^{lo}$ to $CD3^{hi}$ and was complete in $CD3^{hi}$ cells. Positive selection effects were much less marked in this system but also appeared to be manifest at the transition from $CD3^{lo}$ to $CD3^{hi}$. Furthermore, resistance to ionomycin induced apoptosis was increased in the $CD3^{hi}$ double positives. Thus the $CD3^{hi}CD4^+8^+$ population seems to represent a post-selection population in transition to being single positive. Kinetic studies indicate that essentially all of these cells go on to become mature single positives, as would be expected of a selected population. This is quite distinct from the $CD3^{lo}$ double positives, most of which die without further differentiation.

**C 114** INDUCTION OF THYMIC MEDULLARY EPITHELIAL CELLS BY T CELL RECEPTOR-POSITIVE THYMOCYTES. Elizabeth Shores, Willem Van Ewijk, and Alfred Singer. Experimental Immunology Branch, National Institutes of Health, Bethesda MD 20892 and Department of Immunology, Erasmus University, Rotterdam, The Netherlands.

In order to study the role of $TCR^+$ cells in the development of the thymus, we have analysed thymi from immunodeficient C.B-17/scid mice which due to a genetic defect, fail to express T cell receptor genes. Immunohistologic analysis reveals that the SCID thymus is largely cortical in nature such that the majority of stromal cells express an antigen defined by the ER-TR4 mAb, which is specific for cortical epithelial cells. Medullary epithelial cells, which express an antigen defined by the ER-TR5 mAb, are rare and scattered randomly throughout the thymus. In contrast, the thymi of SCID mice into which exogenous $TCR^+$ cells have been transferred, possess normal levels of ER-$TR5^+$ medullary epithelial cells which have become aggregated into defined medullary epithelial regions Thus, these results indicate that $TCR^+$ thymocytes are required for normal development of thymic medullary epithelium. This study demonstrates the symbiotic relationship that exists in the thymus between thymic epithelial cells and immature T cells whose development they promote.

**C 115** THE KINETICS OF TCR EXPRESSION BY DEVELOPING THYMOCYTES: $CD4^+8^+3^{hi}$ CELLS AS POST-SELECTION PRECURSORS OF MATURE T CELLS, Ken Shortman, Mark Egerton and Roland Scollay, The Walter and Eliza Hall Institute, Melbourne, 3050, Australia.

Cortical $CD4^+8^+PNA^+$ thymocytes were subdivided on the basis of size, cell cycle status and level of TCR-CD3 expression. Cell turnover rate and precursor-product relationships were determined using continuous in vivo $^3$H-TdR labeling and radioautography on sorted subpopulations. TCR expression, and the possibility of specificity selection, generally occurred soon after division ceased, and most immediate precursors of mature thymocytes were non-dividing cells. A minor population of $CD4^+8^+PNA^+$ TCR-$CD3^{hi}$ thymocytes showed the labeling pattern expected of intermediates between small cortical thymocytes and mature T cells. Only 3.5% of cortical thymocytes acquired such high levels of TCR, but once formed all of these appeared to progress to maturity; this indicated that cell loss due to selection occurred prior to the $CD4^+8^+CD3^{hi}$ stage. Separate studies on the incidence of cells expressing Vß6 and Vß17a have confirmed the post-selection status of these intermediate thymocytes.

**C 116** A DEVELOPMENTAL DEFECT IN THE CLONAL DELETION OF T CELLS REACTIVE TO MLS-$1^a$ Richard M. Siegel, Avinash Bhandoola, Katsuyuki Yui, and Mark I. Greene, Department of Pathology, University of Pennsylvania School of Medicine, Philadelphia PA 19104

We have been investigating the development and deletion of T cells in mice expressing a transgenic T cell receptor Vß8.1 chain highly reactive to Mls-$1^a$. We previously reported that the majority of $CD4^+$Vß$8.1^+$ T cells are deleted from the peripheral immune system when these mice are bred onto an Mls-$1^a$ background. In the thymus, both $CD4^+CD8^-$ and $CD4^-CD8^+$ thymocytes are selectively decreased in Mls-$1^a$ animals. However, developmental analysis of thymocyte ontogeny has shown that clonal deletion of Mls-$1^a$-reactive cells is not constant throughout development. In Mls-$1^a$ transgenic animals less than one week of age, no evidence of clonal deletion was detected. In organ cultures of day 16 fetal thymic lobes from Vß8.1 transgenic mice, we found only marginal effects of the Mls genotype on the development of $CD4^+CD8^-$ cells. We are now determining if these effects are due to a lack of the Mls-$1^a$ ligand in the fetal environment, or perhaps a more fundamental defect in the mechanism of clonal deletion during the fetal and neonatal period. These studies suggest the presence of a developmental window during which potentially autoreactive T cells can escape clonal deletion and migrate to the periphery.

**C 117** THE IMMUNOLOGICAL STATUS OF TRANSGENIC MICE CARRYING RECOMBINANT CLASS I MHC GENES. Stephen J. Simpson , Peter Tomlinson and Andrew L. Mellor, Division of Immunology, National Institute for Medical Research, Mill Hill, London, NW7 1AA, U.K.

Maintanance of T-cell tolerance towards self antigens of the Major Histocompatibility Complex is thought to be achieved in the main, through the deletion of self reactive clones during their ontogeny within the thymus. However, the precise cell types able to fulfill the role of presenting antigen to developing T-cells to ensure clonal deletion remains unclear. We are interested in restricting the expression of a class I MHC antigen to T-cells only in order to determine whether or not this is sufficient for complete tolerance to this antigen to be achieved *in vivo*.

A promotorless H-$2K^b$ gene has been placed under the control of transcriptional promoter and enhancer elements of the T-cell specific human CD2 gene. T-cell specific expression of the H-$2K^b$ gene has been demonstrated by FACS analysis of lymphocytes from the transgenic mice. Transcriptional analysis of tissues from these mice also reveals restricted expression of the antigen. All the lineages of CD2/H-$2K^b$ transgenic mice have been found to be tolerant of the H-$2K^b$ antigen by skin grafting from transgenic H-$2K^b$ control mice. Further investigations to determine the cytotoxic and proliferative responses of T-cells from these mice to the H-$2K^b$ antigen *in vitro* are underway.

**C 118** Vβ8 OVEREXPRESSION IN $CD4^-CD8^-TCR\alpha\beta^+$ THYMOCYTES IS NOT A CONSEQUENCE OF SELF-TOLERANCE. Yousuke Takahama and Alfred Singer. Experimental Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892. In the present study, we have attempted to understand the basis for the increased Vβ8 usage in $CD4^-CD8^-TCR\alpha\beta^+$ thymocytes. We found that the appearance of Vβ8 overexpression in $CD4^-CD8^-TCR\alpha\beta^+$ thymocytes from $V\beta8^+$ strains occured very late, after most of their other repertoire had been established. In addition, there was no compensation in $V\beta8^-$ strains for the absence of $CD4^-CD8^-TCR\alpha\beta^+V\beta8^+$ thymocytes, even though $V\beta8^+$ cells are the predominant population of $CD4^-CD8^-TCR\alpha\beta^+$ cells in $V\beta8^+$ strains. Thus, the Vβ8 overexpression appeared to be generated by a process that is distinct from that responsible for generating the rest of the $CD4^-CD8^-TCR\alpha\beta^+$ thymocyte repertoire. Furthermore, introduction into the neonatal differentiation environment of the super-antigen SEB, against which $V\beta8^+$ cells react, markedly diminished (rather than promoted) the frequency of $CD4^-CD8^-TCR\alpha\beta^+V\beta8^+$ thymocytes, demonstrating that $CD4^-CD8^-TCR\alpha\beta^+V\beta8^+$ cells undergo clonal deletion when their self-ligand is present. We therefore conclude that overexpression of $V\beta8^+$ cells in the $CD4^-CD8^-TCR\alpha\beta^+$ population is not a consequence of tolerance to self antigen.

**C 119** ANALYSIS OF SELF LIGANDS INVOLVED IN NEGATIVE SELECTION OF THE T CELL REPERTOIRE. Melanie S. Vacchio, John J. Ryan* and Richard J. Hodes. Experimental Immunology Branch, NCI, NIH, Bethesda, MD and *Immunobiology and Transplantation Department, Naval Medical Research Institute, Bethesda, MD.

We have recently demonstrated that both $V\beta11^+$ and $V\beta12^+$ T cells are negatively selected in $H\text{-}2^{a,k}$ or $^d$ strains of mice, with the exception of the $H\text{-}2^k$ strain C58/J. C58/J mice express the appropriate MHC haplotype ($H\text{-}2^k$) yet lack expression of a non-MHC antigen, the presence of which is required for deletion of T cells expressing Vβ11 or Vβ12. Further analysis with backcross animals demonstrated that this non-MHC ligand is encoded by more than one gene and that the gene products recognized by $V\beta11^+$ and $V\beta12^+$ T cells are shared though not necessarily identical. It was of interest to determine whether this non-MHC ligand behaved as a minor lymphocyte stimulatory (Mls) antigen with the ability to cause widespread activation of T cells expressing specific Vβ's when presented by H-2 matched APCs. C58/J T cells responded to $H\text{-}2^k$ APCs from animals that do delete $V\beta11^+$ and $V\beta12^+$ T cells. Furthermore, in (CBA/CaxC58/J)xC58/J backcross animals, there was a strong correlation between responsiveness to CBA/Ca APCs and lack of deletion of $V\beta11^+$ and $V\beta12^+$ T cells. Establishment of long term T cell lines of C58/J origin showed enrichment of $V\beta11^+$ T cells in lines specific for the H-2-matched CBA/Ca APC's, whereas T cell lines specific for MHC allogeneic APCs showed no such enrichment. This data suggests the involvement of a novel Mls antigen, $Mls^f$, in formation of the T cell repertoire.

**C 120** REGULATION OF THYMIC STROMA IN SCID MICE, W. van Ewijk*, E. Shores** and A. Singer**, *Department of Immunology and Immunohistology, Erasmus University, Rotterdam, The Netherlands, **Immunology Branch, NIH, Bethesda MD, USA.

Positive and negative selection of differentiating thymocytes is influenced by different thymic stromal cell types, i.e. thymic epithelial-reticular cells (positive selection) and/or interdigitating cells (negative selection). In the SCID thymus prothymocytes are not able to differentiate into the major thymocyte subsets. This inability relates to changes in thymic microenvironments. Thus, the stroma of the SCID thymus is mainly cortical-type (ER-TR4+ve) with only a few scattered medullary (ER-TR5+ve) epithelial cells. Inoculation of SCID mice with allogeneic bone marrow cells restores T cell differentiation in these mice, at least to the level of CD4+8+ thymocytes. Surprisingly, this procedure also leads to a complete restoration of medullary (ER-TR5) microenvironments.

Thus, (a) in SCID mice T lymphocytes cannot differentiate due to a lack in the endogenous recombinase system; (b) this impairment in lymphoid cells influences thymic microenvironmental development; (c) inoculation of allogeneic bone marrow "repairs" the defect in the SCID thymic stroma.

The exact nature of the cells or factors which influence thymic microenvironments is under study.

**C 121** THYMIC NURSE CELLS: A SITE FOR POSITIVE SELECTION AND DIFFERENTIATION OF T CELLS, Wick G. and Josef Penninger,
Immunoendocrinology Research Unit of the Austrian Academy of Sciences and Institute for General and Experimental Pathology, University of Innsbruck, Medical School, A-6020 Innsbruck, Austria

Thymic nurse cells (TNC) are large complexes consisting of thymic epithelial cells (TEC) that contain completely intact T cells. TNC express MHC class I and class II antigens in high density. They have been described both in mammals and in chickens. We have determined the phenotypic characteristics of chicken TNC-L using a panel of monoclonal antibodies against T cell receptor (γ/δ) TCR 1, (α/ß) TCR 2, the chicken analogues for CD3, CD4, CD8 and the interleukin-2 receptor (IL-2R). Furthermore, functional assays for the possible graft-versus-host reactivity (GVHR) of chicken TNC-L were performed in the so called chorionallantoic membrane (CAM) assay. Our results can be summarized as follows:

1. TNC contain T cells expressing either TCR-1 or TCR-2 thus speaking against a clonal origin of TNC-L.
2. TNC-L are at different stages of differentiation showing a significantly higher proportion of single positive (CD4 or CD8) cells as compared to extra-TNC T cells. This suggests that TNC may be a site for T cell differentiation.
3. TNC-L show a surprisingly high GVHR in an allogeneic system with a pock forming efficiency of 1/15 as compared to 1/400 for thymocytes and 1/100 for peripheral blood lymphocytes.
4. TNC-L also react on syngeneic hosts with an efficiency o 1/50.
5. Serial transfer experiments showed that both the allogeneic and syngeneic reactivity possess specificity and thus can be classified as real immunologic phenomena.
6. It is concluded that TNC are sites for positive selection for self MHC reactive T cells.

Supported by a grant from the Jubiläumsfonds of the Österreichische Nationalbank (project No. 3724).

**C 122** DELETION OF Vβ5.1, Vβ5.2 AND Vβ11 BEARING T CELLS IS MEDIATED BY I-E AND A SINGLE NON-MHC GENE PRODUCT. David Woodland[1], Mary Pat Happ[2], Ken Gollub[2], and Ed Palmer[2], [1]Department of Immunology, St. Jude Children's Research Hospital, Memphis, TN 38105, and [2]Basic Sciences Division, Department of Pediatrics, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO 80206.

Previous analysis has shown that T cells bearing Vβ5.2 T cell receptors are clonally deleted in mice which express both the major histocompatibility complex (MHC) molecule, I-E, and a non-MHC gene product (co-tolerogen) closely linked to the mammary tumour virus integrant on chromosome 12 (Mtv-9). We analysed a backcross between MHC-matched, I-E bearing strains of mice which express the Vβ5.2 deleted and Vβ5.2 non-deleted phenotypes, [B10.D2 (Vβ5.2 lo, Mtv-9$^+$) x BxD28 (Vβ5.2 hi, Mtv-9$^-$)]$F_1$ x BxD28 (Vβ5.2 hi, Mtv-9$^-$). Approximately half of the backcross animals inherited the phenotype of Vβ5.2 deletion characteristic of the B10.D2 parent, and there was a perfect correlation between Vβ5.2 deletion and the inheritance of the Mtv-9 locus. There was also a correlation between the deletion of Vβ5.1- and Vβ11- bearing T cells and the presence of the Mtv-9 locus. These results suggest that 1) Vβ5.1- and Vβ11-bearing T cells are also deleted by the Mtv-9 linked co-tolerogen, and 2) this co-tolerogen is either very tightly linked to the Mtv-9 locus on chromosome 12 (<1cM) or is encoded by the viral genome itself. We are currently using transfection to distinguish between these possibilities.

**C 123** TOLERANCE TO CLASS I MHC MOLECULES IN TRANSGENIC MICE.
Helen Yeoman and Andrew L. Mellor, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA. UK.

Classic experiments with chimeric mice demonstrate that bone-marrow cells are the cells within the thymus that tolerise an animal to self MHC (negative selection). However the identity of the cell-type responsible for tolerance remains unresolved.

To investigate mechanisms of tolerance induction further, transgenic mice have been generated which carry a novel Class I MHC molecule (H-2k$^b$) under the control of a promoter that restricts its expression to cells of the erythroid lineage. Flow cytometric analysis of peripheral blood shows that these mice express the transgene on red blood cells but not lymphocytes, and skin grafting from KBD mice onto non-transgenic littermates demonstates the absence of H-2k$^b$ on KBD skin. However, further transcriptional and translational analyses of the transgene awaits investigation.

KBD mice accept skin grafts from mice that express H-2k$^b$ under the control of its own promoter, and are thus tolerant to H-2k$^b$ in vivo. Preliminary in vitro data indicates that KBD T cells can proliferate, but are not cytotoxic to cells that express H-2k$^b$. Thus it appears that restricting the expression of class I MHC molecules to erythroid cells is sufficient for tolerance induction, but not complete clonal deletion.

**C 124** LOW AVIDITY OF AUTOREACTIVE T CELL RECEPTORS FOR THE SELF ANTIGEN IN T CELLS THAT ESCAPE CLONAL DELETION IN THE THYMUS OF VB8.1 TRANSGENIC MICE. Katsuyuki Yui, Makoto Katsumata, Shinji Komori, Linda Gill-Morse, and Mark I. Greene. Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA 19104-6082.

In Mls-$1^b$ Vβ8.1 transgenic mice (Vβ8.1-TG), the Vβ8.1 T cell receptor (TCR) was expressed on >98% of mature T cells. In Mls-$1^a$ Vβ8.1-TG, $CD4^+CD8^-$ T cells were severely decreased among peripheral T cells as well as thymocytes. However, the deletion was not complete, and residual $CD4^+CD8^-$ T cells expressed normal densities of the Vβ8.1 TCR. The T cells did not respond to Mls-$1^a$ but were able to proliferate in response to the occupancy of the TCR by alloantigens. We have isolated several T cell clones from the population of $CD4^+CD8^-$ T cells by stimulation with allogeneic C57BL/6 slpleen cells. Interestingly, $CD4^+CD8^-$Vβ8.1+ T cell clones isolated from Mls-$1^a$ Vβ8.1-TG responded to Mls-$1^a$. The series of experiments suggested that the avidity of the TCR on $CD4^+$Vβ8.1+ T cells in Mls-$1^a$ Vβ8.1-TG to Mls-$1^a$ antigen is reduced when compared to that of Mls-$1^b$ Vβ8.1-TG. T cell clones from Mls-$1^a$ mice required more Mls-$1^a$ antigen for their activation and were more susceptible to the inhibitory effects of anti-CD4 mAb on their proliferative response to Mls-$1^a$ than T cell clones from Mls-$1^b$ mice. Based on these observations, a possible mechanism to account for the generation of autoreactive T cells will be discussed.

*Processing and Presentation of Self-AG/ID*

**C 125** INVARIANT CHAIN AFFECTS THE BIOSYNTHESIS OF CLASS II MOLECULES Mark Anderson and Jim Miller,
*Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL 60637*

The intracellular association of class II molecules with invariant chain (Ii) has been implicated in the association and presentation of antigens with the class II molecule. The effects that Ii may have on the biosynthesis and intracellular sorting of class II could have an important role in the association of class II with endogenous and/or exogenous antigens. Therefore, we sought to determine the effect Ii has on the biosynthesis of class II in EL4 cells transfected with IAd with and without Ii. In these studies, we found that Ii had a profound effect on the biosynthesis of IAd. In the absence of Ii, class II could form dimers efficiently, but these dimers appeared to be misfolded and this altered conformation resulted in the loss of some mAb epitopes and inefficient transport from the RER to the golgi. In addition, class II that was transported through the golgi accumulated an abnormally increased molecular weight associated with N-linked glycosylation. Subsequent transfection of Ii into these cells resulted in recovery of normal class II conformation, causing a restoration of mAb epitopes, efficient intracellular transport, and normal glycosylation. This suggests that Ii has a profound effect on the post-translational transport and modification of class II molecules. These effects could play an important role in the processing and association of antigens with class II in these cells, and studies are currently underway that examine presentation of antigens in both the endogenous and exogenous pathways.

**C 126** SPECIFIC KILLING OF T CELLS PROCESSING AND PRESENTING HBenvAg BY CD4+ CYTOTOXIC T CELL CLONES, V. Barnaba, A. Franco, M. Paroli, F. Balsano. "Fondazione Andrea Cesalpino", I Clinica Medica, Università "La Sapienza", 00161, Roma, Italy.

Human T cells expressing class II molecules can process and present soluble antigens, only if they bear membrane molecules binding antigens with high affinity. We demonstrate that both CD4+ and CD8+ T cells can process and present HBenvAg with high efficiency to class II-restricted CTLs, which in turn kill the presenting T cells. These CTLs reactive to HBenvAg can lyse themselves or other HLA-compatible CD4+ or CD8+ CTLs only in the presence of very low concentration of antigen, while they are made unresponsive by high dose of antigen, when it is presented by T cells. This tolerance seems not to be induced by anergy of HBenvAg-specific T clones. Indeed, the specific T cells retained their ability to kill 51-Cr labeled T clones, pulsed with low antigen concentration, in the presence of non-labeled high antigen-pulsed T cells. Moreover, pulsing with high Ag concentration neither affected the killing of target T cells, that had been previously pulsed with low dose, nor interferred with the recognition of specific HBenvAg peptide. Thus, our data indicate that the inefficiency of HBenvAg-specific T cells to respond to high Ag dose presented by T cells, might reside either in uptake or in processing defects by presenting T cells. This phenomenon may represent a novel mechanism of down-regulation of T cell response to some viral Ag, as HBenvAg, because the specific CTLs must be preserved by suppression in the presence of high Ag concentrations, in order to kill the infected cells.

**C 127** T CELL RECOGNITION PROCESSED IDIOTYPE, Bjarne Bogen[*], Siegfried Weiss[#], Ralph Snodgrass[α], Zlatko Dembic[§], and Anton Berns[○], [*]Department of Immunology and Rheumatology, University of Oslo. [#]GBF, Braunschweig. [α]Lineberger Cancer Research Center, NC. [○]The Netherlands Cancer Institute, Amsterdam. [§]Hoffman La-Roche, Basel.

It has previously been shown that B lymphoma cells spontaneously process BALB/c $\lambda 2^{315}$ Ig light chains and present fragments on I-$E^d$ molecules to cloned BALB/c $CD4^+$ cells. By use of various $\lambda 2^{315}$ gene constructs, we now show that the processing occurs in the endoplasmic reticulum. Cytosolic $\lambda 2^{315}$ fusion proteins do not have access in a processed form to I-$E^d$ molecules. The α/β Tcrs of 7 independent 91-101$_{\lambda 2^{315}}$/I-$E^d$ specific T cell clones have presently been sequenced. The $Phe^{94}$->$Tyr^{94}$ crossreactive clones use an extremely conserved receptor (Vα3 Jα1; Vβ6 Jβ1.2). Also the N-regions are conserved. A heteroclitic response can be linked to an Ile -> Met exchange in the Nα-region. Our finding implies a very limited Tcr repertoire for an idiotope. To be able to study interaction of naive Id-specific T cells with naive Id-expressing B cells, we have produced both Tcr-and $\lambda 2^{315}$- transgenic mice. The characterization of these mice is not yet finished, but experiments so far show that they express the correct proteins and harbor functional Id-specific T cells and Id-expressing B cells in high frequencies.

**C 128** B LYMPHOCYTES AS ANTIGEN PRESENTING CELLS IN THE INDUCTION OF TOLERANCE TO SOLUBLE PROTEIN ANTIGENS, Elizabeth E. Eynon and David C. Parker, Dept. of Mol. Gen. and Micro., Univ. of Mass. Med. School, Worcester, MA. 01655.
We are investigating the ability of resting B cells, acting as antigen presenting cells, to induce tolerance to soluble protein antigens in mice. Using an antigen targeted specifically to B cells, Fab rabbit anti-mouse IgD, we can look at the effect of antigen presentation by B cells alone. We use monovalent Fab fragments of the rabbit anti-IgD to avoid activating the B cells. We know from studies with continuous helper T cell lines that small B cells can process and present Fab anti-IgD very efficiently. Our question is what happens to naive T cells when they encounter antigen for the first time on small B cells. We find that treatment of mice with Fab rabbit anti-mouse IgD results in profound tolerance to challenge seven days later with Fab nonimmune rabbit immunoglobulin precipitated in alum adjuvant. Tolerance is specific for Fab rabbit immunoglobulin since the mice make normal antibody responses to a control antigen given at the same time. Divalent F(ab)'2 anti-IgD does not induce tolerance implying that the B cells must remain in the resting state to induce tolerance. The antigen specific helper T cells in these mice have been affected since they provide poor help for an anti-hapten response when challenged with hapten coupled to rabbit immunoglobulin. Additionally, T cells from anti-IgD treated mice will not help normal, untreated B cells produce anti-rabbit immunoglobulin when transfered into SCID mice.

**C 129** **THE TLA REGION ENCODED 37 PROTEIN EXPRESSED BY TERATOCARCINOMA CELLS MAY FUNCTION AS A RESTRICTION ELEMENT.** *Elizabeth P. Garcia[1], Madeleine Cochet[2], and Suzanne Ostrand-Rosenberg[1].* [1]Biological Sciences, University of Maryland, Baltimore, MD 21228, [2]Unite de Biologie Moleculaire du Gene, Pasteur Institute, Paris, France.

Although the 402AX teratocarcinoma (*H-2$^b$, T1a$^f$*) does not express classical H-2K and H-2D antigens, rejection is T cell mediated and cellular studies indicate that 402AX cells express a functional MHC class I restriction element. To identify this molecule we prepared cDNA libraries from 402AX mRNA. Sequence analysis of the class I clones from this library demonstrated that the only class I transcript expressed is encoded by the TLA region 37 gene. Immunoprecipitation of metabolically labelled 402AX cell lysates with either a β-2-microglobulin-specific antibody or antiserum specific for the C-terminus of the 37 protein precipitates a 43KD protein from 402AX and from murine fibroblasts transfected with the 37 cDNA clone. Immunoprecipitation of surface labeled 402AX cells with 37-specific antiserum demonstrates 402AX expresses multiple forms of the 37 protein which vary in size and glycosylation patterns. To examine the ability of the 37 protein to function as a restriction element or allo-transplantation molecule, 402AX-specific T cell lines have been generated. These T cell lines will be used to determine the role of the 37 protein in T cell recognition of the 402AX tumor.

**C 130** HLA-DR ALLELES DIFFER IN THEIR ABILITY TO PRESENT STAPHYLOCOCCAL ENTEROTOXINS TO T CELLS. Andrew Herman, Gilbert Croteau[#], Rafick-Pierre Sekaly[#], John Kappler, and Philippa Marrack, Howard Hughes Medical Institute, Division of Basic Immunology, Department of Medicine, National Jewish Center for Immunology and Respiratory Medicine, Denver, Colorado 80206.[#] Institute de Recherche Clinique de Montreal, Quebec, Canada. Staphylococcal enterotoxins (SE) have been shown to bind to MHC class II proteins and stimulate T cells in a Vβ specific manner, and these Vβ specificities for various SEs have been well documented in mice and humans. This study has been undertaken in order to examine the ability of human class II molecules to present SEs to human and murine T cell hybridomas. Employing a panel of transfectants expressing individual HLA class II antigens, we have shown that HLA-DR alleles differ in their ability to bind and present SEs. Since the HLA-DR proteins share a common α chain, these results indicate that the polymorphic β chain plays an important role in SE binding and presentation to T cells. Antibody mapping techniques and in vitro mutagenesis are being applied to try to localize the region(s) of MHC class II that are important for SE binding. The results of this study should provide information on the region of MHC class II molecules that interact with foreign, and perhaps self, superantigens.

**C 131** PEPTIDES IN GAMMA, DELTA CYTOTOXIC T LYMPHOCYTE RECOGNITION OF LYMPHOMA, Alan M. Krensky, Hubert Kim, and Carol Clayberger, Department of Pediatrics, Stanford University School of Medicine, Stanford, CA 94305

We have previously described autologous tumor specific cytotoxic T lymphocytes (CTL) which express the gamma, delta T cell receptor and are not inhibitable by anti-MHC monoclonal antibodies, but are inhibitable by anti-immunoglobulin and tumor specific anti-idiotypic monoclonal antibodies (Wright, et al., J. Exp. Med., 169:1557). We synthesized peptides corresponding to the tumor immunoglobulin hypervariable regions and tested their effect on tumor specific cytolysis. Peptides corresponding to the CDR1 of the specific tumor immunoglobulin heavy chain (mu) inhibit tumor specific cytolysis, while peptides corresponding to the other CDRs have no effect. Unexpectedly, this inhibitory effect by the specific peptide is generalizable to any CTL-Burkitt's lymphoma interaction. Inhibition occurs by binding to the target and is specific for Burkitt's lymphoma targets. The peptide sequence is not homologous to any known sequences in the database. Most recently, we generated monoclonal antibodies which inhibit cytolysis by binding to the specific tumor target in this system and are in the process of characterizing the proteins that they recognize.

**C 132** **MOLECULAR INTERACTIONS BETWEEN ANTIGENIC PEPTIDES AND MAJOR HISTOCOMPATIBILITY COMPLEX MOLECULES.** Søren Mouritsen, Ole Werdelin, and Søren Buus. Institute for Experimental Immunology, University of Copenhagen, Denmark.

The phenomenons of antigen presentation and MHC restriction of T cell responses have, though well established, been poorly understood at the molecular level. Recently developed techniques have directly demonstrated and measured the interaction between antigenic peptides and MHC molecules. Each MHC molecule possesses a single binding site that through the specific recognition of "peptide motifs" is capable of binding many seemingly different peptides. MHC molecules are unable to distinguish between self and non-self, and are to a large extent preoccupied with self-peptides. The ability to measure peptide-MHC binding is currently used to probe the nature of both antigen processing and presentation. In all peptide-MHC combinations tested so far the conditions for optimal binding is observed at an acidic pH. The rate of association at pH 5, compared to at pH 7, is about 15 fold faster. The affinity is similarly increased. As a consequence of this binding the processed peptide is protected against further proteolytic degradation. The findings would suggest that MHC molecules bind antigen in a late endosome/lysosome compartment. The core region of the bound antigen is protected against further proteolytic degradation while the remaining part of the antigen can be cleaved off and the resulting peptide/MHC complex returned to the cell surface for presentation to T cells.

**C 133** **EXPRESSION OF HLA-B27 IN TRANSGENIC MICE IS DEPENDENT ON TIIE α2-α3 DOMAINS OF THE H-2D GENES**, Cheryl L. Nickerson-Nutter, Kristine L. Hogen, and Chella S. David, Department of Immunology, Mayo Clinic, Rochester, MN 55905.

The level of HLA-B27 expressed in transgenic mice varies with different H-2 haplotypes. Decreased expression was observed in mice of the H-$2^d$, H-$2^q$, and H-$2^v$ haploytpes. Interestingly, these three haplotypes have multiple D region genes, including H-2D and H-2L, whereas, the other haplotypes contain only a single "D/L" gene which is more homologous to H-2L than to H-2D. Minimal expression of B27 was observed in B10.RKDB mice, mapping decreased expression to the H-2D-region genes. Introduction of B27 into B10.D2-dm1 and BALB/c-dm2 mice further narrowed down the region responsible for inhibiting expression of B27. Expression of B27 was decreased in the dm2 mice demonstrating that the H-$2D^d$ molecule is responsible for the "low expression effect". High levels of expression were observed in the dm1 mice which has a hybrid D/L gene. The recombination point occurs in the α2 region. The sequence specific for the $L^d$ gene begins at residue 155. Therefore, decreased expression of B27 maps to the region 3' of residue 155 in the H-$2D^d$ molecule.

| | MHC | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | K | A | E | C4 | D | D2 | D3 | D4 | L | B27 Expression |
| B10 | b | b | b | b | - | - | - | - | b | high |
| B10.K | k | k | k | k | - | - | - | - | k | high |
| B10.RKDB | k | k | k | k | **d** | **d** | **d** | **d** | b | low |
| B10.D2 | d | d | d | d | **d** | **d** | **d** | **d** | **d** | low |
| BALB/c-dm2 | d | d | d | d | **d** | - | - | - | - | low |
| B10.D2-dm1 | d | d | d | d | d/- | - | - | - | -/d | high |

**C 134** **DETECTION OF PEPTIDE-MHC BINDING BY FACS ANALYSIS**
A.A. Sinha, C. Lock, T. Mietzner, A. Black, P. Jones, H.O. McDevitt

**The binding of peptide fragments derived from protien antigens to class II MHC molecules is a prerequiste for the activation of CD4+ T helper cells. However, peptide-MHC association has been demonstrated experimentally only relatively recently. Although several elegant methods have been used, they often involve the use of radioactive materials, the biochemical purification of class II, and involve some amount of time and expertise. We have screened for the binding of biotinylated ovalbumin 323-339 and hen egg lysozyme 46-61 to live cells by FACS analysis (using avidin-texas red). Binding can be demonstrated on spleen cells, fibroblasts, and B cell lines and can be specifically inhibited by anti-class II MAb or an excess of native peptide. We are assessing the binding of HEL 46-61 to several I-Ak beta chain mutant fibroblast lines to better understand structure-function relationships within class II molecules.**

**C 135** SELECTIVE INTERACTION OF Ni WITH A MHC-BOUND PEPTIDE: A GENERAL MODEL FOR HAPTEN RECOGNITION BY T CELLS, F. Sinigaglia, and P. Romagnoli, Central Research Units, F. Hoffmann-La Roche & Co. Ltd., 4002 Basel, Switzerland

T cells generally recognize foreign antigens as peptides associated with self-molecules encoded by genes of the MHC. However, in the case of non-peptidic haptens, the molecular structure of the antigenic complex is still undefined. Previously, we isolated MHC class II-restricted nickel (Ni)-specific T cell clones from patients with Ni-allergy (Sinigaglia et al. 1985 J. Immunol. 135:3929). By using a competition assay for peptide binding to MHC molecules (Kilgus et al. 1989, Proc. Natl. Acad. Sci. USA 86:1629) we ask whether these clones recognise Ni-modified MHC antigen directly, or whether Ni-peptide-MHC complexes are the target of recognition. We find that Ni creates a new antigenic determinant by interacting with a peptide bound to the MHC molecule. These findings suggest a general model of hapten recognition by T cells.

**C 136** CRITICAL AMINO ACID RESIDUES OF AN IMMUNODOMINANT EPITOPE OF LAMBDA REPRESSOR: A STUDY USING CASSETTE MUTAGENESIS, Alex Szabo, A.D. Strosberg and Jean-Gérard Guillet, Unité d'Immuno-Pharmacologie Moléculaire (CNRS et Université de Paris VII), Institut Cochin de Génétique Moléculaire, 22, rue Méchain, 75014, Paris, France

Immunization of BALB.C or CBA mice with the protein lambda-repressor (cI) results in the production of helper T cells specific for residues 12-26. We describe here the use of cassette mutagenesis to study the specific amino acid residues of this immunodominant epitope interacting with the T cell receptor or MHC or both. Several hundred variant proteins were generated in which residues were replaced by random mixtures of all twenty amino acids. Crude lysates of E. coli expressing these proteins were then tested for their ability to stimulate T cell hybrids originally derived by immunization with the wild-type protein. The changes in the variants of interest were determined by sequencing the corresponding gene. A panel of T cell hybrids containing members of both I-$A^d$ and I-$E^k$ restriction were tested against proteins randomized at positions 8,9,10,11,12,13. The T cells accepted virtually all substitutions at these positions These findings indicate that these residues play no critical role in either interaction with MHC or T cell receptor, or in antigen processing or presentation. By contrast, among proteins mutagenized at positions 17,18, the only ones recognized were those containing the wild-type lysine or arginine at position 17 and the wild-type leucine at position 18. These results are in agreement with those we obtained using peptides indicating positions that 17, 18 are critical for recognition.

**C 137** INTERACTIONS BETWEEN THE T-CELL RECEPTOR, MHC CLASS II ANTIGEN AND STAPHLOCOCCAL ENTEROTOXINS. Gary M. Winslow, Philippa Marrack, and John W. Kappler. Howard Hughes Medical Institute, Department of Basic Immunology, National Jewish Center for Immunology and Respiratory Medicine, 1400 Jackson St., Denver, Colorado 80206.

Staphlococcal enterotoxins are powerful stimulators of T-cells that bear T-cell receptors (TCR) composed of specific Vβ elements. Several lines of evidence suggest that the enterotoxins mediate interaction between the T-cell receptor (TCR) and the Class II MHC antigen on the presenting cell: an absolute requirement for the Class II antigen, the binding of intact enterotoxins to Class II molecules in vitro, and the identification of residues of the TCR that are required for enterotoxin-mediated stimulation. Although the binding of the enterotoxin to the Class II molecule appears to be different than the binding of conventional antigen, the molecular details of the interaction are incompletely understood. We will report progress of studies of the interactions between the TCR, Class II antigen, and Staphlococcal enterotoxins A and B (SEB), and the effect of altered forms of SEB on T-cell stimulation both in vitro and in vivo.

**C 138** TOLERANCE INDUCTION TO A PROTEIN ANTIGEN USING B CELLS FROM A TRANSGENIC MOUSE, Victoria N. Yuschenkoff, Elizabeth E. Eynon and David C. Parker, Department of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester, MA 01655

Since acquired tolerance in the T cell compartment is antigen-specific and MHC-restricted, it must require the participation of an antigen presenting cell. To test the hypothesis that small B cells are the antigen presenting cells in acquired tolerance to protein antigens, we transfused lymphocytes from transgenic mice expressing the membrane-bound form of human u chain on their B cells into syngeneic, non-transgenic sibling mice, and then challenged the mice with human μ chain (Fc fragment) in alum adjuvant and measured serum antibody responses. We find that adult mice transfused with transgenic spleen or lymph node cells are unresponsive to challenge with human μ chain as compared to non-transfused control animals. When challenged with a control protein antigen, chicken gamma globulin, transfused and non-transfused animals make similar antibody responses indicating that the tolerance is specific for human μ chain. We propose that the resting B cells fail to deliver accessory signals required for naive T cell activation and instead induce T cell tolerance for the antigen.

*Pathogenic Autoantibodies*

**C 139** MOLECULAR CHARACTERIZATION OF A PATHOGENIC ANTI-ERYTHROCYTE AUTOANTIBODY. Michael J. Caulfield and Deborah Stanko, Section of Immunology, Research Institute of the Cleveland Clinic Foundation, Cleveland, OH 44195

NZB mice are genetically predisposed to developing anti-erythrocyte autoantibodies resulting in the development of Coombs-positive hemolytic anemia. We now have evidence that this spontaneous autoantibody response consists of antibodies that are similar in specificity and idiotype expression to a pathogenic mAb (G8) that was "cloned" from an autoimmune NZB mouse. The G8 autoantibody recognizes native erythrocytes from mice but not those from other species and appears to have the same specificity as naturally occurring pathogenic autoantibodies from NZB mice. Although G8 is an IgM antibody, it is clearly pathogenic and causes anemia in a normal mouse strain. Furthermore, an anti-idiotypic mAb (E8) prepared against the G8 autoantibody inhibits ~ 60% of anti-erythrocyte AFC from aged Coombs-positive NZB mice indicating that the G8 mAb expresses a recurrent idiotype characteristic of spontaneously arising Coombs autoantibodies. Northern blot analysis revealed strong hybridization with the J558 VH gene probe. Thus, G8 is clearly distinct from antibodies that recognize bromelain-treated MRBCs and which belong to the VH 11 gene family. Sequence analysis of the V region genes encoding G8 indicate that they are closely related to germline VH and VL genes. Interestingly, the VH region of the G8 (anti-MRBC) mAb is nearly identical to that of an anti-DNA mAb obtained from the MRL strain as well as to a germline gene (VH6) derived from C57BL/6 mice that encodes anti-NP antibodies. To address the possibility that the G8 autoantibody is derived from unmutated germline genes, we have used PCR to amplify V genes from germline DNA from NZB and BALB/c mice using one oligonucleotide primer complementary to the CDR2 region with the other primer corresponding to the conserved N-terminal sequence. The resulting amplified DNA was shown to hybridize with the corresponding CDR2 oligos or with the J558 probe (in the case of VH amplification). The results suggest that a pathogenic autoantibody may be encoded by unmutated germline VH and VL genes.

**C 140** **POLYREACTIVITY IN THE BrMRBC-SPECIFIC REPERTOIRE IS A PROPERTY OF B CELLS THAT ARISE LATE IN ONTOGENY.** Ronald B. Corley, Harvey J. Sage, and John D. Conger, Division of Immunology, Duke Medical Center, Durham, NC 27710

The nonimmune B cell repertoire is characterized by B cells expressing IgM molecules of low affinity, high connectivity, and polyreactivity. Polyreactivity is particularly evident in the early immune system. Because $CD5^+$ B cells comprise a high percentage of the developing repertoire, it is possible that polyreactivity is a property of this B cell subset, particularly of those cells that arise early in ontogeny. Because BrMRBC-specific B cells are derived from the $CD5^+$ B cell subset, we have been studying hybridomas of this specificity for the secretion of polyreactive antibodies. Polyreactivity was not found in any of the antibodies that utilize the V region families most characteristic of the BrMRBC-specific response, those expressing the $V_H11/V\kappa9$ and $V_H12/V\kappa4$ gene families. Instead, polyreactivity was observed only in a set of three antibodies which expressed members of the $V_H558$ family. Of these, two expressed a light chain that used the same $V\kappa10$ V gene and the other expressed the $V\kappa21E$ gene. All three antibodies expressed very complex CDR3s in their H chain that encoded 9-14 amino acid residues. These had extensive N nucleotide insertions and one of the antibodies showed evidence of a D-D fusion. This type of CDR3 is characteristic of B cells which arise late in ontogeny. The structure of these antibodies contrasts sharply with those expressing the $V_H11/V\kappa9$ and $V_H12/V\kappa4$ combinations, which have small V-D-J junctions (3 amino acids) with no N insertions, characteristic of B cells that arise early in ontogeny. Whether or not the BrMRBC-specific repertoire is unique in terms of the profiles of polyreactivity is not known, but these results clearly indicate that not all B cells that arise early in ontogeny are polyreactive. The structural basis for the polyreactivity exhibited by the $V_H558$ expressing antibodies is currently under investigation.

**C 141** POSITIVELY CHARGED REGIONS ON NATIVE ANTIBODIES ENHANCE THE DEPOSITION OF IMMUNE COMPLEXES IN GLOMERULI. V. Joyce Gauthier and Mart Mannik. Department of Medicine, University of Washington, Seattle WA 98195

The presence of cationic charge on proteins enhances their deposition in the anionic glomerular basement membrane (GBM). Goat IgG possesses a charge spectrum similar to human IgG. The ability of goat antibodies to mediate the deposition of preformed immune complexes in mouse glomeruli has been shown to be greater than with more anionic rabbit antibodies (Arthritis Rheum 32:S25, 1989). To determine if interaction of these antibodies with a polyanionic matrix as an in vitro analog for the GBM would enrich the population of antibodies responsible for glomerular deposition, monomeric affinity purified goat antibodies($Ab_G$) to human serum albumin (HSA) were separated by ion exchange chromatography. The $Ab_G$ that bound to carboxymethyl Sepharose (Pool 2, 34.7%) were separated from those that did not bind (Pool 1, 65.3%) at pH 9 in 0.01 M borate buffer. By isoelectric focusing these represent overlapping populations with pI ranges of 5.2-8.2 for Pool 1 and 6.6-9.1 for Pool 2. Soluble, preformed immune complexes prepared with these pools of antibodies showed identical size distribution on ultracentrifugation and disappearance kinetics from circulation of C57Bl/6J mice. Pool 2, containing antibodies more capable of electrostatic interactions with anionic surfaces, demonstrated a two-fold increase in immune complex deposition over complexes prepared from Pool 1 (83.4±0.1 vs 40.6±8.7 ng/kidney, p=0.007). Immunofluorescence microscopy demonstrated more deposits in animals receiving immune complexes prepared with Pool 2. These results demonstrate that the deposition at the GBM of immune complexes with native goat antibodies is enhanced in populations capable of charge-charge interactions with anionic surfaces. The cationic regions in these molecules have not yet been identified, but sequence analyses of antibodies to DNA from MRL/lpr mice have revealed unusually cationic residues in the variable regions (Shlomchik et al, JEM 171:26S, 1990). Even small populations of antibodies bearing cationic charge are sufficient to mediate deposition of non-cationic antibodies (J Immunol, in press 1990). Therefore, the charge-charge interactions in the glomerular localization of autoantibodies and immune complexes in lupus warrant further inquiry.

**C 142** CHARACTERIZATION OF ACETYLCHOLINE RECEPTOR-REACTIVE ANTIBODY CLONOTYPES WITH DISEASE-CAUSING POTENTIAL: IDENTIFICATION AND PURIFICATION BY PREPARATIVE ISOELECTRIC FOCUSING. K.A. Krolick and P.A. Thompson. Department of Microbiology, University of Texas Health Science Center at San Antonio, San Antonio, Texas 78284.

Lewis rats immunized with purified acetylcholine receptor (AChR) produce polyclonal anti-AChR antibodies capable of interfering with neuromuscular transmission. The resulting symptoms of Experimental Autoimmune Myasthenia Gravis are likely caused by a number of antibody effector activities, including AChR modulation from the surface of muscle fibers, steric interference with the binding of acetylcholine to its receptor, and complement mediated membrane damage and recruitment of inflammatory elements. Previous studies from this and other laboratories have suggested that the exact nature (e.g., fine-specificity, isotype) of the anti-AChR antibodies produced may determine the severity of resulting AChR-dependent neuromuscular impairment. In other words, not all AChR-reactive antibodies are equally effective at perturbing AChR function. Therefore, we have investigated, more directly, the possibility that only a particular clonotypic subset of the total expressed anti-AChR antibody repertoire is directly responsible for subsequent disease symptoms. The strategy used combines fractionation and purification techniques based on antibody separations according to their isoelectric point (i.e., isoelectric focusing), with passive antibody transfers into healthy recipient rats. Results indicate that, indeed, of the anti-AChR antibodies produced in this clonotypically heterogeneous response, a small identifiable subset appears to be most prominently responsible for the neuromuscular dysfunction observed in this autoimmune disease model.

**C 143** SCID MICE RECONSTITUTED WITH PERIPHERAL BLOOD MONONUCLEAR CELLS FROM TYPE 1 DIABETIC PATIENTS PRODUCE AUTOANTIBODIES THAT RECOGNIZE β CELL AUTOANTIGENS. Jacob Petersen*, Kim Hejnæs*, Michael Marshall and Thomas Dyrberg. *Hagedorn Research Laboratory, Niels Steensens Vej 6, DK-2820 Gentofte. Novo Nordisk, Novo Alle, DK-2880 Bagsværd, Denmark.

To investigate the autoimmune phenomena associated with Type 1 diabetes we injected SCID mice intraperitoneally with peripheral blood mononuclear cells (PMNC) from resent onset diabetic patients. All mice (n=11) produced human immunoglobulins but in variable concentrations (IgG: 1-2816 µg/ml, IgM: 0-344 µg/ml) reaching the highest levels 8-11 weeks after reconstitution. Serum from one mouse (human IgG concentration 512 µg/ml) injected with PMNC from an islet cell cytoplasmic antibody (ICA) and 64kD antibody positive patient, contained ICA staining human islets with an intensity equal to that induced by the patient serum in a dilution of approximately 1:64. In immunoprecipitation analysis of $^{35}$S-methionine labelled rat islets, the mouse serum bound the 64 kD islet cell protein with the same intensity as serum from the patient. An IgG producing cell line (carrying the B cell surface molecules IgG, CD19, and CD21) was established by EBV-transformation of cells from the spleen of this mouse. Further, preliminary results suggest the generation of a specific islet cell immunerespons upon immunization of reconstituted SCID mice with rat islets. However, none of the mice showed elevated blood sugar levels or impaired glucose tolerance. In conclusion, our results suggest that PMNC reconstituted SCID mice may be useful to obtain specific autoantibodies and to dissect the molecular mechanisms of the pathogenesis of autoimmune diseases.

**C 144** TOLERANCE AND EXPANSION IN B CELL CLONES: BIVALENT RESPONSE TO SURFACE IGM CROSSLINKING, Peter van Endert #* and Gerd Moldenhauer #; # Dept. of Immunol., German Cancer Research Center, Heidelberg, FRG, and * Dept. of Microbiol. and Immunol., School of Medicine, Stanford, CA 94305. Both B- and T-lymphocytes can receive positive as well as negative signals supporting or suppressing growth and differentiation through their antigen receptor, thus being able to maintain specific tolerance or immune responsiveness towards appropriate antigens. In B-cells, the type of response is thought to depend on the stage of maturation. Here we show that both types of response can be elicited in individual B-lymphocyte clones, depending on the physical form of antigen. While soluble bivalent mAb to idiotype or IgM completely inhibited spontaneous growth of 3 malignant B cell clones, the same reagents and mAb to IgD were mitogenic when coupled to insoluble compounds. Growth inhibition could also be obtained by direct elevation of intracellular calcium levels, while activation of protein kinase C rendered ionophores as well as mAb to IgM or idiotype mitogenic. In the presence of mitogenic insoluble antibodies to surface IgD, soluble mAb to IgM increased cellular DNA synthesis. These results indicate that i) individual B-cells may be capable of a negative and a positive response to antigen at the same stage of maturation, ii) the physical form of the antigenic ligand alone may determine the type of response iii) a quantitative signaling threshold between negative and positive growth regulation may exist.

*Mechanisms of Escaping Immune Recognition of Suppressive/Network Regulation: Tumors, Viruses (AIDS), Parasites*

**C 145 INBRED MOUSE STRAINS DIFFER IN THEIR ABILITY TO RESPOND TO A HEAT SHOCK PROTEIN, hsp70, DURING INFECTION WITH TRICHINELLA SPIRALIS,** Thomas G. Beito, Christopher J. Krco, Susan Kost, David O. Toft, Donald L. Wassom and Chella S. David, Departments of Immunology and Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN 55905, and Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin, Madison, WI 53702.

Parasite induced chronic inflammatory conditions such as filiariasis, schistomiasis and malaria are associated with immune responses to the 70kD family of heat shock proteins (hsp70). The high degree of phylogeneic conservation of amino acid sequences among families of heat shock proteins may account in the occurrences of autoimmunity to self heat shock proteins following infection. Presently, there is a paucity of information concerning the genetic control of immune responses to heat shock proteins following administration of antigens or infection with pathogens. We report here the existence of non-major histocompatibility complex (non-MHC) genetic control of immune responses to T. spiralis hsp70 among inbred strains of mice. Inbred mouse strains were infected with 150 T. spiralis larvae. Thirty days post infection antisera were collected and assayed for antibodies to purified T. spiralis hsp70 by Western blotting. Mice bearing a B10 genetic background (B10, B10.D2, B10.K and B10.Q) were uniformly responsive to T. spiralis hsp70 while corresponding MHC-matched, BALB/c, AKR, C3H, DBA/1, AU and SWR were unresponsive to hsp70. Analysis of one hybrid cross (B10.Q x C3H.Q)$F_1$ indicated that nonresponsiveness to hsp70 was a dominant trait. The data implies that non-MHC genes, possibly those affecting T cell repertoire (e.g. Mls) may affect immunity to heat shock proteins and may act to protect against autoimmunity to self stress proteins.

**C 146** NEONATAL EXPOSURE TO THYMOTROPIC GROSS MURINE LEUKEMIA VIRUS INDUCES VIRUS SPECIFIC IMMUNOLOGIC NONRESPONSIVENESS. Jonathan M. Korostoff*, Donna Murasko#, Kenneth J. Blank and Glen N. Gaulton*. *Department of Pathology and Laboratory Medicine, University of Pennsylvania, School of Medicine, Philadelphia, PA, 19104, #Department of Microbiology and Immunology, Medical College of Pennsylvania, Philadelphia, PA, 19129, and Department of Immunology and Microbiology, Temple University School of Medicine, Philadelphia, PA, 19140. Neonatal exposure to Gross murine leukemia virus results in a profound inhibition of the virus specific T and B cell responses of adult animals. Animals exposed to virus as neonates exhibit a marked depression in virus specific T cell function as measured by the virtual absence of *in vivo* delayed type hypersensitivity responses and *in vitro* proliferative responses to virally infected stimulator cells. Further, serum obtained from neonatally treated mice failed to either immunoprecipitate viral proteins or neutralize virus in an *in vitro* plaque assay, suggesting the concurrent induction of a state of B cell hyporesponsiveness. The specificity of this effect at the levels of both T and B cells was demonstrated by the ability of neonatally treated mice to respond normally following adult challenge with either irrelevant reovirus or influenza virus. The replication of Gross virus within both stromal and lymphocytic compartments of the neonatal thymus suggests that thymic education plays a key role in the induction of immunologic nonresponsiveness to viruses.

**C 147** REGULATION OF IMMUNE ACTIVATION/RETROVIRAL REPLICATION BY $CD8^+$ T CELLS. Jonathan D. Powell, Daniel P. Bednarik, Tamar Jehuda-Cohen, Francois Villinger, Thomas M. Folks, and A.A. Ansari. Department of Pathology, Emory University School of Medicine and Centers for Disease Control, Atlanta, GA 30322.

Previously, we have shown that $CD8^+$ T cells from naturally simian immunodeficiency virus (SIV) infected sooty mangabey monkeys have the ability to inhibit viral replication in vitro. In light of the fact that replication of lentiviruses is intimately linked to immunologic activation of infected cells, we hypothesized that $CD8^+$ T cells inhibit viral replication at the level of cellular activation. To test this hypothesis, an EBV-transformed cell line from an SIV seropositive sooty mangabey monkey which stably expresses the human CD4 molecule and is replication competent for SIV, HIV-1, and HIV-2 was utilized. Autologous lymphocytes markedly inhibited SIV, HIV-1, and HIV-2 replication in the cell line in the absence of significant cytotoxic activity. Next, these cells were transiently transfected with an LTR-driven CAT reporter gene. It was found that autologous $CD8^+$ T cells markedly inhibited CAT activity, suggesting that the inhibition was at the level of transcription. Experiments utilizing a dual-chamber culture vessel separated by a semipermeable membrane implicate a role for a soluble factor(s) in mediating this effect. Furthermore, co-transfection of these cells with an LTR-driven tat plasmid and LTR-CAT was able to quantitatively mitigate the suppressive effect. Thus, transcriptional inhibition appears to be directed at cellular mechanisms of viral transcription. Using an LTR-driven CAT plasmid with a mutation at the NFkB binding site completely inhibited CAT activity in these cells. Indeed, gel retardation assays confirm the presence of NFkB binding proteins. Taken together, these data demonstrate the ability of $CD8^+$ T cells to inhibit NFkB-driven viral replication. This model accentuates the ability of the virus to usurp the activation machinery of immunologically relevant cells and the potential role for $CD8^+$ T cells in regulating this axis.

**C 148** MACROPHAGES AS ACCESSORY CELLS FOR CYTOTOXIC T LYMPHOCYTES

Uwe D. Staerz, Department of Medicine, National Jewsih Center for Immunology and Respiratory Medicine, Department of Microbiology and Immunology, University of Colorado Health Sciences Center, Denver, CO 80206

Based on our previous observation and based on findings reported in the literature we hypothesized that macrophages played an important role during the initiation of primary cytotoxic T lymphocyte (CTL) responses in vivo. Therefore, we examined class I MHC restricted immune responses in mice whose macrophages had been deleted by injection of silica or carrageenan. Under these conditions animals completely failed to raise CTL to an influenza virus infection. However, a vigorous class I MHC restricted response could be restored by the transfer of bone-marrow or peritoneal macrophages.Since a macrophage-hybridoma was as effective we concluded that macrophages but not minor contaminats were important for the induction of T lymphoctes. In further experiments we could show that CTL precursors have to directly interact with macrophages. F1 mice that had been injected with silica were immunized with influenza and provide with macrophages of either parental haplotype. We could only detect CTL that were restricted to the MHC haplotype carried on the transferred macrophages.
These experiments demonstrate the importance of an accessory cell, most likely a macrophage-type cell, for the induction of class I MHC restricted T lymphocyte responses.

**C 149** CHARACTERISATION OF T CELL LINES AND CLONES TO A RECOMBINANT ONCHOCERCA VOLVULUS ANTIGEN, George Strang, Maurice Southworth, Francine B. Perler. New England Biolabs, 32 Tozer Rd, Beverly, MA 01915.
We are interested in the immune response to Onchocerca volvulus and are using a murine system to investigate the immunogenicity of O. volvulus recombinant proteins. Murine T cell lines and clones have been established to an O.volvulus recombinant antigen, OI3, which has been cloned and expressed as a fusion protein to the maltose binding protein (MBP). OI3 was selected using sera from putatively immune individuals who had relatively high levels of antibody to this protein. Less than 20% of chronically infected individuals had detectable levels of specific antibody to OI3. The phenotype of these T cells has been determined and experiments are underway to examine the pattern of lymphokine secretion by these T cells. Monoclonal antibodies to OI3 also have been produced. The purified protein, T cell lines and the antibodies will allow us to examine the various aspects of immunity to this protein. Experiments are planned to assay the protective ability of each of these with respect to their ability to reduce the lifespan of L3/L4 larvae in mice.

## Self Reactivity and Its Regulation

*Allo vs. Self-Recognition*

**C 200** DIRECT EVIDENCE FOR ALLO-MHC PEPTIDE RECOGNITION DURING IN-VIVO ALLORESPONSES. Gilles Benichou, Peter A. Takizawa, Minnie McMillan* and Eli Sercarz. Department of Microbiology and Molecular Genetics, UCLA, 405 Hilgard Ave., Los Angeles, CA 90024-1489. *USC School of Medicine, Department of Microbiology, 1441 Eastlake Ave., Los Angeles, CA 90033.

Three models for the target structure which is recognized by alloreactive T lymphocytes have been proposed: (i) Alloreactive T cells recognize polymorphic motifs present on the intact allo-MHC molecule, regardless of peptides bound to them, (ii) Host-derived peptides interact with the native allo-MHC molecules to create a series of new determinants recognized by T cells, (iii) The allo-MHC molecules are processed into peptides and presented by self MHC molecules to allo-peptide specific T cells. In this report, we provide evidence for this third model. We show that following priming with $A^k$-bearing allogeneic splenocytes or a skin allograft, primed T cells from the recipient (BALB/c, SJL) proliferate in response to certain peptides derived from polymorphic regions of the $\alpha$ and $\beta$ chains of the donor (B10.A) class II MHC molecules ($A^k$). The ability to recall responses to peptides in vitro following in vivo priming with intact cells or skin grafting clearly indicates that allo-MHC class II peptide processing and presentation occurs during the course of an in vivo allogeneic response. Several peptides which were stimulatory in SJL and BALB/c were shown in previous work to be neither immunogenic in donor type B10.A mice nor capable of binding to $A^k$ and $E^k$ molecules. This shows that the in vivo alloresponse to these allo-MHC peptides is restricted to the recipient's own MHC molecules. Thus, self-restricted T cell recognition of processed alloantigens may play a critical role in transplantation.

**C 201** THE INVOLVEMENT OF NON-MHC ENCODED GENES IN CLASS 2 ALLOSTIMULATION, Jerome Bill, Department of Pediatrics, National Jewish Center, for Immunology and Respiratory Medicine, Denver, CO 80206.

Forty murine T cell hybrids reactive with the mutant Class 2 molecule I-$A^{bm12}$ were studied for their ability to produce IL-2 in response to I-$A^{bm12}$ transfected L cells. Six hybrids were unable to respond to the transfected L cells despite continued response to spleen cells from B6.C-H-$2^{bm12}$ mice. To determine the defect in the I-$A^{bm12}$ transfected L cells, these cells were supertransfected with pSV2-neo and B6 genomic DNA and G418 resistant supertransfectants were screened for their ability to stimulate one of the non-responsive T cell hybrids (17BBM151). In this way H3.1, an L cell transfected with both I-$A^{bm12}$ and an as yet unidentified B6 gene, was obtained which stimulates the T cell hybrid 17BBM 151. Efforts are underway to clone the B6 gene transfected into H3.1 and to determine why this non-MHC encoded gene is required for allostimulation.

**C 202** CD1-SPECIFIC T CELLS RECOGNIZE MINIMALLY POLYMORPHIC CD1, Paul A. Bleicher and Cheryl A.Vibbard, Department of Dermatology, Massachusetts General Hospital and Harvard University School of Medicine, Boston, MA 02114.

Recognition of class-1b proteins by $\gamma\delta$ and $\alpha\beta$ double-negative (DN) T cells has been demonstrated with both mouse and human T cells. The limited tissue distribution and polymorphism of class-1b proteins has led to the proposal that $\gamma\delta$ T cells may recognize and eliminate damaged and transformed autologous cells by specific recognition of these proteins. We have shown that $\gamma\delta$ and $\alpha\beta$ DN T cell clones can recognize the class 1b proteins, CD1c and CD1a, respectively. To examine the polymorphism of the CD1a product recognized by the DN $\alpha\beta$ T cells, we have used the PCR to isolate cDNA for the $\alpha$1 and $\alpha$2 domains of CD1a from 10 thymuses and have compared the sequence of these to CD1a from HPB-ALL and MOLT 4. Nine of the sequences were identical to MOLT 4, but differed from HPB-ALL at two nucleotides, generating a substitution of threonine for isoleucine at position 14 and a cysteine for tryptophan at position 51. One cDNA had an additional leucine substitution for lysine at position 5 which differed from both HPB-ALL and MOLT 4 sequences. Although the $\alpha\beta$ DN cell line recognizes MOLT 4, JURKAT, HPB-ALL and transfected CD1 derived from HPB-ALL, analysis of the recognition of transfected CD1 with the polymorphic sequences is in progress. These results indicate that CD1a is indeed minimally polymorphic, and CD1a recognition likely represents autoreactivity rather than alloreactivity.

C 203 COMPARISON OF ALLO VERSUS PEPTIDE + SELF SPECIFIC CTL RECOGNITION OF HLA-B27. Carol Clayberger, Sarah Buxton, Seza Ozen, Richard Benjamin, Peter Parham, and Alan M. Krensky, Departments of Cardiovascular Surgery, Cell Biology, and Pediatrics, Stanford University School of Medicine, Stanford, CA 94305

The MHC class I antigens are polymorphic cell surface glycoproteins which present endogenously synthesized viral or tumor antigens to CD8+ T cells. They also serve as the major targets of alloreactive cytotoxic T lymphocytes (CTL) which mediate organ transplant rejection. In order to investigate the sites on MHC molecules which interact with T cells, we have derived two panels of CTL lines and clones specific for HLA-B27. The first group recognizes a peptide fragment from the influenza A nucleoprotein presented in the context of HLA-B27. The second group recognizes HLA-B27 as an alloantigen. We have compared these two types of CTL for lysis using the following: 1) transfectants expressing HLA-B27 genes mutated at various regions of the alpha 1 and 2 domains, 2) peptides corresponding to the polymorphic regions of HLA-B27, and 3) transfectants expressing HLA-B27 genes mutated in the CD8 binding region. Our findings indicate that allospecific versus self + peptide restricted CTL interact differently with class I molecules.

C 204 BIOCHEMICAL CHARACTERIZATION OF THE LIGANDS RECOGNIZED BY MHC CLASS II-RESTRICTED ALLO-REACTIVE T CELLS.

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Allo-reactive T cells recognize MHC class II molecules without exogenously added foreign antigen. It is still unknown whether a peptide component is involved in allo-reactive T cell recognition.

We are currently developing a method for the isolation of pure complexes between HEL peptide 107-116 and I-E$^d$. This reagent should allow us to determine whether allo-reactive T cell hybridomas are specific for self-peptide/I-E$^d$ complexes or only I-E$^d$, regardless of the peptides bound. The strategy followed consists of producing complexes between I-E$^d$ and an analog of HEL peptide 107-116 to which a biotin moiety is linked through a disulfide bond. The I-E$^d$/biotinylated HEL peptide 107-116 complexes are specifically retained on an avidin-agarose column and then eluted by treatment with a thiol-containing buffer. In preliminary experiments, preparations of pure HEL 107-116/I-E$^d$ complexes ( > 90% pure) were obtained. These I-E$^d$ preparations are currently being tested for their ability to stimulate HEL 107-116-specific, I-E$^d$-restricted T cell hybridomas and I-E$^d$-allo-reactive T cells hybridomas.

C 205 THE Q7 α3 DOMAIN ALTERS T CELL RECOGNITION OF CLASS I ANTIGEN. J. Forman*, L.C. Lowen*, D. Mann+, I. Stroynowski+, N. Nishimura+, L. Hood+, R.E. Hammer**, C.J. Aldrich*.
Dept. of Microbiology*, HHMI**, Dept. Biochem.**, UT Southwestern Medical Center*, 5323 Harry Hines Blvd., Dallas, TX 75235-9048, and Dept. Biol.+, Cal. Tech.+, Pasadena, CA.

Using the hybrid class I molecules LLQQ (L$^d$ in α1/α2, Q7$^d$ α3, TM & CY) and LLQL (L$^d$ α1/α2,TM & CY, Q7$^b$ α3) we have analyzed the role of the α3 domain of class I molecules in T cell recognition. We show that these hybrid molecules are not recognized by unprimed, CD8 dependent CTL. Although an L$^d$ restricted MCMV derived peptide binds to LLQL, causing unregulation of cell surface expression, primary L$^d$-restricted peptide-specific CTL do not recognize MCMV pusled LLQL target cells. In contrast, LLQL and LLQQ targets are lysed by most alloreactive anti-L$^d$ CTL clones from primed mice. These CLT are resistant to CD8 blocking at the effector stage, and are shown to express relatively low membrane CD8 as compared to clones from unprimed mice. Further, by culturing unprimed CTLp in the presence of CD8 mAb we generated CD8 independent CTL that recognize LLQL. Taken together, these data indicate that the α3 domain of Q7 (Qa-2) prevents CD8 dependent L$^d$ specific CTL from recognizing LLQL. This defect is likely due to an alteration iin the ability of CD8 to interact with the Q7 α3 domain and could account for why Q7 molecules do not serve as restricting molecules for antigen. We have introduced the L$^d$, LLQQ and LLQL genes into C3H/HeJ(H-2$^k$) mice and currently have transgenic lines which will allow us to investigate Q7 α3 altered responses in greater detail.

**C 206** THE PRESENCE OF "AUTOLOGOUS" MHC CLASS II (HLA-DR) ANTIGENS ON ALLOGENEIC LEUCOCYTES DETERMINES WHETHER BLOOD TRANSFUSIONS IMMUNISE OR SUPPRESS. Emma L. Lagaay, Dept. of Immunohematology, University Hospital, Leiden, The Netherlands. Administration of blood transfusions prior to allograft transplantation can lead to immunisation as well as to prolonged graft survival. The mechanism underlying the beneficial effect of pretransplant blood transfusions has never been completely understood. We have recently observed that compatibility of a single MHC class II antigen (HLA-DR) between transfusion recipient and blood donor is required to induce prolonged graft survival in man (Lagaay et al, N Eng J Med 1989;321:701). In the present study we investigated the effect of one-HLA-DR-antigen matched and completely-DR-mismatched blood on cellular immune responsivenes. We found that matching for a single HLA-DR antigen between blood donor and transfusion recipient prevented activation of cytotoxic (N=56) and proliferative responses (N=60) by blood transfusion. Completely-DR-mismatched transfusions significantly enhanced cytotoxic ($P<0.001$) and proliferative ($P<0.001$) responses. The absence of activation of cellular responses after HLA-DR-matched-transfusions was accompanied by a decrease in the absolute number of peripheral CD4+ cells ($P=0.015$, N=79). Sharing of MHC-class I antigens between blood donor and transfusion recipient did not have similar effects. These data suggest that recognition of 'autologous' MHC class II antigens on allogenic leucocytes prevents activation of cellular immune responses.

**C 207** FINE MAPPING OF A DRw52-RELATED B AND T CELL ALLO-EPITOPE BY SITE-DIRECTED MUTAGENESIS. David Maurer and Jack Gorski. The Blood Center of Southeastern Wisconsin. Milwaukee, WI 53233.

The DR3-B1 gene of the DRw52 family is the product of a gene conversion event in which sequence elements were donated from a linked DR-B3 allele, DRw52a. We investigated the contribution of polymorphic amino acids acquired in this event to the DR3 and DRw52a allospecificities. We used site-directed mutagenesis to transfer three DR beta chain first domain alpha-helical residues, G73, R74 and N77, normally present in DR3a and DRw52a, to a DR4 beta chain. This mutant DR molecule was recognized by a DR3-specific monoclonal antibody and one DRw52a-specific alloproliferative T cell clone. Analysis of residues shared among reactive constructs defines a dominant epitope controlled by residues in the first domain alpha helix of DR3 and DRw52a beta chains and recognized by both polymorphic antibodies and alloreactive T cells. These results suggest that some alloreactive T cells are insensitive to the presence of peptide occupying the antigen binding site of the DR molecule. Futher mutant DR molecules were produced to fine map the B and T cell epitope. Our analyses may help to define the molecular role of DRw52a as a strong predisposing factor for neonatal alloimmune thrombocytopenia and primary sclerosing cholangitis.

**C 208** THE ROLE OF INVARIANT CHAIN IN ALLO-CLASS II-SPECIFIC T CELL RESPONSES
Jim Miller, Mark Anderson, Michelle Morin, and Mary Peterson
*Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL 60637*
We have recently shown that association of invariant chain (Ii) with Class II MHC molecules influences the conformation of Class II expressed at the cell surface and the ability of Class II to associate with antigenic peptides. To determine whether these effects might alter allorecognition, we compared the ability of Class II-positive EL4 transfectants that either do or do not co-express Ii to stimulate allogeneic Class II-specific T cell responses. Invariant chain negative cells were able to stimulate only a marginal MLR, whereas Ii-positive cells generated a strong response. This effect did not appear to result from a defect in TcR recognition of Class II because Ii was not required for stimulation of allogeneic or antigen specific T cell hybridomas. In addition, a similar requirement for Ii chain expression was observed with Staphylococcal enterotoxin mitogenic responses. To address the possibility that the cell surface-associated, proteoglycan form of Ii may function as an accessory molecule, we transfected the IAd-positive, Ii-negative cells with a mutated form of Ii (Ii-ala201) that no longer encodes the addition site for the chondroitin sulfate side chain. Co-expression of Ii-ala201 imparted all the conformational features to Class II molecules that wild type Ii does, without restoring primary T cell stimulation. These data indicate that in addition to effects of Ii on Class II biosynthesis and antigen presentation, the glycosaminoglycan form of Ii may play a specific role as an accessory molecule that is necessary for efficient activation of primary T cell responses.

**C 209** STRUCTURE AND FUNCTION OF THE CLASS I-LIKE MOLECULE $Q6^d$, Michael I. Nishimura, Keith D. Lewis, Leroy E. Hood, and Iwona Stroynowski, Division of Biology, California Institute of Technology, Pasadena, CA 91125.
Genes located within the major histocompatibility complex (MHC), designated H-2 in the mouse, encode proteins required for immune recognition and response to foreign antigens. Classical class I H-2 antigens are highly polymorphic cell surface glycoproteins that are expressed on virtually all adult tissues. They are involved in the lysis of allografts, virally infected cells, and some tumor cells. More recently, a second group of class I-like molecules, encoded by the Qa and Tla regions of the MHC, was identified. These molecules share considerable structural homology with the H-2 classical antigens but have low polymorphism and a restricted tissue distribution. Function of the Qa-Tla products is not known. We have undertaken a study of one of the nonclassical molecules encoded by the Qa region gene, $Q6^d$. Preliminary DNA sequence analysis indicates that the three external domains of the predicted $Q6^d$ molecule, a1, a2, and a3, encode a polypeptide similar to the H-2 N termini. The putative transmembrane region of $Q6^d$ is truncated and contains hydrophilic residues, suggesting that $Q6^d$ molecules may be secreted. Surprisingly, previous studies have shown that CTL raised against Qa molecules recognize a hybrid antigen ($Q6^d/L^d$) expressing the amino terminal a1 and a2 domains from $Q6^d$ and the a3 and carboxy-terminal regions of $H\text{-}2L^d$. We have now demonstrated that Q7- and Q9-specific CTL do not crossreact with $Q6^d/L^d$ bearing targets. This raises a possibility that secreted $Q6^d$ molecules can induce CTL. Alternatively, the $Q6^d$ gene may encode a novel, membrane-bound antigen. The experiments in progress will distinguish between these possibilities. Function of putative soluble $Q6^d$ proteins in CTL blocking and CTL stimulation will be examined.

**C 210** EMPTY MHC CLASS I MOLECULES RECOGNIZED BY ALLO $H\text{-}2^b$ SPECIFIC CTL CLONES, C. Öhlén*, F. Aosai&, H.G. Ljunggren*, P. Höglund*, R. Glas*, E. Wolpert*, L. Franksson*, H. Stauss& and K. Kärre*, Department of Tumor Biology*, Karolinska Institutet, Box 60400, S-104 01 Stockholm, Sweden and Department of Biology&, University College London, England.

It is not clear whether allo-specific CTL's recognize foreign MHC molecules "as such", or if they recognize peptide + MHC complexes. We have investigated the allo reaction against a mutated lymphoma, RMA-S, that are inable to process and present internally derived antigens but remains sensitive to allospecic lysis. Recent result suggest that the 5-10% cell surface $H\text{-}2^b$ expressed on this mutant (compared with the wild type) does not contain peptide but are a labile dimer complex. These complexes, and hence the cell surface expression, can be induced 4 fold by incubation of the cell line at 26°. This treatment does not render the cell line sensitive to $H\text{-}2^b$ restricted lysis. However, we report here that such treatment will increase the susceptibility of RMA-S for some allo specific clones but not other. We suggest that part of the allo killing is comprised of CTL clones that are specific for "empty" MHC class I molecules, independent of presented peptides, while another part is peptide specific, MHC restricted.

**C 211** IDENTIFICATION OF CRITICAL RESIDUES IN THE PEPTIDE $D^d$ 61-85 REQUIRED FOR RECOGNITION BY dm 1-SPECIFIC CTLS. Clifford A. Olson and Minnie McMillan*. Department of Microbiology and Molecular Genetics, UCLA, 405 Hilgard Ave., Los Angeles, CA 90024-1489. *Department of Microbiology, USC School of Medicine, Los Angeles, CA 90033.
The dm 1 class I molecule is a hybrid of the $D^d$ and $L^d$ class I molecules. This class I molecules is $D^d$ from amino acids 1-114 with the remainder of the molecule being identical to $L^d$. We have shown that the combination of transfected L-cell fibroblasts expressing $L^d$ class I molecules and the peptide $D^d$ 61-85 is recognized by alloreactive dm 1-specific CTLS. This recognition is peptide specific since peptides synthesized from the identical region of other class I molecules are not recognized in this system. The $L^d$ 61-80 peptide contains six different amino acids when compared to the sequence of $D^d$ 61-80. To identify the amino acids found in the $D^d$ 61-80 peptide which are critical for recognition by dm 1-specific CTLS, we synthesized a series of $D^d/L^d$ hybrid peptides. Our results indicate that the peptide $D^d$ 61-69/$L^d$ 70-80 incubated with the $L^d$ expressing L-cell targets is recognized as a dm 1 target structure. The three amino acids that are different between $D^d$ and $L^d$ in the 61-69 sequence (glutamic acid (63) and arginine (65, 66)) were subsequently substituted with the corresponding residues found in the $L^d$ class I molecule (isoleucine (63), glutamine (65) and isoleucine (66)) in all possible combinations. The results using these peptides indicate that the glutamic acid (63) and arginine (65) are the amino acids which are critical for recognition by dm 1-specific CTLS.

**C 212** MAPPING OF THE REGION OF THE T CELL RECEPTOR β-CHAIN THAT INTERACTS WITH THE SELF SUPERANTIGEN MLS-$1^a$,

Ann M. Pullen, Philippa Marrack, John W. Kappler, Howard Hughes Medical Institute, Division of Basic Immunology, Department of Medicine, National Jewish Center for Immunology and Respiratoy Medicine, Denver, CO 80206.

The self superantigen, $Mls\text{-}1^a$, stimulates T cells with high frequency. $Mls\text{-}1^a$/MHC complexes stimulate T cells via the Vβ element of the αβ T cell receptor, with relatively little input from the other variable elements of the receptor.

In general amongst laboratory inbred strains T cells bearing Vβs 6, 8.1 and 9 are $Mls\text{-}1^a$ reactive. However, during a study of the T cell receptors of natural populations of wild mice we identified $V\beta 8.2^+$ T cells which were unexpectedly reactive to $Mls\text{-}1^a$. Using site directed mutagenesis we modified the Vβ gene of a non-Mls-reactive T cell hybridoma, DO-11.10, and identified residues which were important in conferring $Mls\text{-}1^a$ reactivity. These residues are predicted to lie on a β-pleated sheet on the side of the T cell receptor, well away from the predicted complementarity determining regions which are thought to interact with the complex of conventional peptide antigens and MHC. Further mapping of the interaction site for $Mls\text{-}1^a$ on Vβ8.2 will be reported.

**C 213** ON TEST T RECEPTOR REPERTOIRE AGAINST HUMAN ALLOANTIGENS

Bent RUBIN, Eric CHAMPAGNE, Anne HUCHENQ, Joëlle FABRON, Mogens THOMSEN
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Recently, a variant of HLA-DR1 molecule was described in our laboratory : HLA-DR-BON. This molecule has three amino acid changes from the HLA-DR1 molecule, and they are situated in the third hypervariable region (hv3) of the β chain. Preliminary studies indicate that T cell clones reacting with hv3-HLA-DR-BON use rather similar Vα and Vβ gene segments in the construction of their receptors. T cell lines and clones are produced in the combinations HLA-DR1 anti-HLA-DR-BON or HLA-DR-BON anti-HLA-DR1. At given time points after initiation of such lines, specificity analysis, cell cloning and mRNA isolation will be made. Single-stranded cDNA is produced and amplified by PCR. A reverse Dot blot method is used to characterize the T gene segments used. Such studies will elucidate the frequency, avidity (including accessory molecule-dependence) and Tcr repertoire of human T cells against alloantigens of limited difference. Our results will be discussed in the context of T cell receptor repertoire selection, usage and function.

**C 214** MOLECULAR ANALYSIS OF ANTIGEN/MHC RECOGNITION FUNCTION OF THE αβ T-CELL RECEPTOR, Nilabh Shastri, Department of Molecular and Cell Biology, University of California, Berkeley CA 94720

We have established a model system for the antigen/MHC specific recognition function of the αβ T-cell receptor. Pairs of α and β chain genes isolated from T-cells specific for lysozyme peptides and either $A^b$ or $A^{bm-12}$ class II MHC molecules were transfected into recipient T-cells which lack expression of both the endogenous α and β chain genes. Transfectant T-cells expressed the αβ TCR encoded by the introduced genes but recognized the appropriate antigenic peptide/MHC complex only when the CD4 molecule was also expressed by the cells. We present our findings on the structural features of the variable regions of the α and the β TCR chains and those of the CD4 molecule which determine the specificity of recognition of the antigen with the self and non-self MHC complexes.

**C 215 HETERODIMERIC, DISULFIDE-LINKED αβ T CELL RECEPTORS IN SOLUTION,** Alfred E. Slanetz, Young S. Kim, Ajay Kumar, Rong-Hwa Lin and Alfred L.M. Bothwell, Section of Immunobiology, Yale University School of Medicine, New Haven, CT 06510

Structural and functional analysis of T cell receptor-ligand binding would be greatly advanced by the availability of an intact, assembled T cell receptor (TcR) in soluble form. We have produced such a molecule, by splicing the extracellular domains of a T cell receptor to the glycosyl phosphatidylinositol (GPI) membrane anchor sequences of Thy-1. The molecule is expressed in the absence of CD3 on the cell surface, and can be cleaved from the membrane by treatment with phosphatidylinositol specific phospholipase C (PI-PLC). The α and β chains of the soluble molecule are paired in the native conformation as judged by reactivity with the anti-Vβ8 monoclonal antibody F23.1, and with the anti-clonotypic monoclonal antibody 1B2; it is a disulfide linked dimer with an Mr of 95 kDa on SDS-PAGE under nonreducing conditions, and 47 kDa after reduction. We conclude that we have generated an αβ T cell receptor in soluble form.

**C 216 MHC CLASS II Aa/DQb HYBRID MOLECULES ALTER MOUSE T CELL RECEPTOR REPERTOIRE IN HUMAN HLA-DQb TRANSGENIC MICE,** Paul Zhou, Suresh Savarirayan, Gary Anderson, Hidetoshi Inoko, and Chella David, Department of Immunology, Mayo Clinic, Rochester, MN 55905.
A significant alteration of mouse TCR repertoire was observed in our H-2E negative, HLA-DQw6b+ transgenic mice. Specifically, Vb5.1, Vb5.2 and Vb11-bearing T cells are clonally deleted by $A^{T}_{\alpha}$/DQb hybrid molecules in the context of unknown self peptide(s). These hybrid molecules can also present Mls-$1^{a}$ and clonally delete Vb6 and Vb8.1-bearing T cells. Staining thymocytes with these Vb specific MoAbs demonstrates that only mature thymocytes (TCR bright) are deleted, but not immature thymocytes (TCR dull). Since the same Vb-bearing T cells were reported to be clonally deleted by H-2E in the context of an unknown self peptide or Mls-$1^{a}$ and the clonal deletion has not been observed in the DQw6a/DQw6b double transgenic mice, the results presented here indicate that the Aa/DQb hybrid molecules possess the same self-peptide (or super antigen) presenting epitope(s) as the H-2E molecules leading to the clonal deletion of certain Vb-bearing T cells and suggests that the interaction of mouse CD4 and mouse Aa is required for the negative selection of T cells. Thus, the DQb gene in human may be the analog of H-2E genes in mouse for the thymic selection.

## *Class II Expression and Initiation of Autoimmune Disease*

**C 217 THE MAMMALIAN CCAAT-BINDING FACTOR CP1B (NFY-A) CLONED BY FUNCTIONAL COMPLEMENTATION IN YEAST EXHIBITS A COMPLEX PATTERN OF EXPRESSION,** Daniel M. Becker, John D. Fikes and Leonard Guarente, Department of Biology, MIT, Cambridge, MA 02139

We have constructed a HeLa cDNA library of $>10^{7}$ primary clones in a vector that directs high level expression in the yeast *Saccharomyces cerevisiae*. We transformed this library into a yeast strain deleted for one component of the yeast CCAAT-binding heteromer, HAP2/3/4, and have identified by *in vivo* complementation a cDNA clone encoding the HeLa HAP2 homolog, CP1B. This protein is identical to NFY-A, which contributes to cell-type and stage-specific expression of MHC class II. Sequence analysis of the CP1B cDNA identifies a region at the C terminus of near-identity to the HAP2 essential core, which directs DNA binding and subunit association of the yeast complex. The N terminus of the cDNA encodes a glutamine-rich region, unrelated to the yeast protein, that may serve as a transcriptional activation domain. Northern analysis of human cell lines and primate tissues demonstrates constitutive expression of a 4.1 kb transcript in all cell types, and reveals in addition two major, regulated transcripts. The principal transcript in HeLa cells at 1.6 kb corresponds to the cDNA that we have cloned, and is present as only a minor species in other cell types. A 7 kb transcript appears exclusively in spleen and in the class II-positive Raji B lymphoma cell line.

**C 218** ABERRANT CYTOKINE EXPRESSION BY MACROPHAGES (mϕ) PRECEDES DISEASE IN AUTOIMMUNE (AI)-PRONE MICE, David Beller, Jerold Levine, Daqing Wu Hartwell, Gyorgy Frendl and Matthew Fenton, Department of Medicine, University Hospital at Boston Univ. Med. School, Boston, MA 02118
We have found that peritoneal mf from several AI strains of mice (MRL/lpr, MRL/+, NZB, NZB/W F1) show defective expression of IL-1 and IL-6. When cultured in the continuous presence of LPS, mϕ from normal (N) mice express IL-1 and IL-6 at relatively stable levels for at least 4d. Although IL-1 and IL-6 can be readily induced by LPS in AI mϕ immediately after isolation, cytokine levels decline dramatically after the first day in culture, so that by d 3 to 4, these cytokines are virtually undetectable. That this finding does not represent a general anergy in the AI mϕ is indicated by the undiminished capacity to express LFA-1 determinants in response to either IFNγ or LPS. Class I and II MHC determinants are also normally induced. The cytokine defect is demonstrable in NZB or MRL/+ mice at 4 wk of age, months before the appearance of overt AI disease; in MRL/lpr mice it is expressed during the first wk of life as dramatically as in adults. Cytokine down-regulation also occurs in mϕ isolated from the AI bone marrow (BM). Thus, this defect not only is maintained in mϕ induced to proliferate and differentiate in culture in the presence of CSF-1, but is also clearly expressed independently of the immediate AI environment. This aberrant pattern of cytokine expression appears to be at least in part transcriptionally controlled, as determined from a kinetic analysis of IL-1α and β, as well as IL-6 mRNA levels. Aberrant regulation of IL-1 and IL-6 expression may contribute to the development of AI disease, or may reflect a more fundamental dysregulation within the immune system which itself permits or triggers the the lack of control of lymphocyte growth and function characteristic of AI disease.

**C 219** DIFFERENT ROLES OF CLASS II MHC ANTIGENS EXPRESSED ON CORTICAL AND MEDULLARY THYMIC EPITHELIAL CELLS IN MYASTHENIA GRAVIS, Ljiljana Popesković, Slobodan Apostolski. Nevena Arsenović, Jelena Gospavić and Katarina Isaković, Immunology Research Center, Faculty of Pharmacy and Department of Neurology, University Clinical Center, Belgrade, Yugoslavia
The expression of class II MHC antigens on cortical and medullary thymic epithelial (TE) cells, and macrophages in myasthenia gravis (MG) was investigated.MG thymuses (hyperplastic) were obtained from female patients, 18-25 years of age, while control thymuses, of the same age and sex, were obtained after heart surgery. Cortical and medullary TE cells as well as macrophages were identified by appropriate mAbs using immunoperoxidase staining. Expression of Ia molecules by these cells was demonstrated with OKIa mAb by immunofluorescence (double staining). All tests were performed using single cell suspension prepared from confluent monolayer thymus cultures. In comparison to the controls, cortical TE cells from MG thymuses expressed Ia molecules in a significantly lower extent, whereas medullary TE cells showed an increased expression of these molecules. Expression of Ia antigens by macrophages was similar in MG and control thymuses. Decreased expression of Ia antigens by cortical TE cells was in accordance with our hypothesis that these cells are not able to support maturation of T lymphocytes including induction of tolerance to self components. Therefore, nontolerized T helper lymphocytes can initiate autoimmune response. On the other hand, increased expression of Ia antigens by medullary TE cells is involved in the autoimmune process within the medulla. Therefore, the role of class II MHC components expressed on TE cells in MG depends on the subpopulation of TE cells bearing these antigens (Supported by Republic of Serbia Research Fund, Belgrade).

**C 220** AUTOIMMUNE MYOCARDITIS IN RESISTANT B10.A MICE: ASSOCIATION WITH LPS-INDUCED MHC EXPRESSION, James R. Lane, David A. Neumann, Anne Lafond-Walker, Ahvie Herskowitz, and Noel R. Rose. The Johns Hopkins University, School of Hygiene and Public Health, Department of Immunology and Infectious Diseases, Baltimore, MD 21205

Treatment of B10.A mice with Coxsackie virus B3 (CB3) and lipopolysaccharide (LPS) results in autoimmune myocarditis characterized by the presence of IgG autoantibodies to heart antigens and a mononuclear cell infiltrate in the heart. B10.A mice infected only with CB3 are not susceptible to this ongoing autoimmune disease and recover completely from the virus infection. The ability of LPS to induce autoimmune myocarditis in genetically resistant B10.A mice may be due to the actions of cytokines which are increased as a consequence of the LPS stimulation. We have observed increased MHC Class I and II gene product expression on the heart tissue of mice when treated with LPS or with CB3 and LPS (CB3/LPS). Immunocytochemical studies of heart tissue at various times after CB3/LPS treatment indicated that when there was concurrent MHC expression and CB3 infection, myocarditis was observed. In CB3 infected B10.A mice without LPS stimulation, autoreactivity was not observed. Adoptive transfer of spleen cells from CB3/LPS treated mice resulted in myocarditis in recipients pretreated with LPS and exhibiting increased MHC expression. Similar transfers in animals pretreated with saline and exhibiting no increased MHC expression in their hearts resulted in no significant heart inflammation. Because LPS treatment is capable of increasing cytokine levels as well as MHC gene product expression, our results suggest that these factors may contribute to the adoptive localization of heart-specific spleen cells. Supported by NIH grant HL33878.

**C 221** A HIGHLY SENSITIVE CELL-BASED ASSAY SYSTEM FOR ANALYZING INDUCTION OF CLASS II GENES BY GAMMA INTERFERON. H. T. Nguyen, H. K. Lonial, S. K. Narula, and P. J. Zavodny, Department of Biotechnology-Molecular Biology, Schering-Plough Research, Bloomfield, New Jersey, 07003.

Class II genes (DR, DP, DQ) of the human major histocompatibility complex (MHC) encode surface glycoproteins that are expressed in specific cells of the immune system,including B lymphocytes, macrophages, monocytes, and activated T lymphocytes. The class II gene products are essential for antigen presentation and their level of expression influences the magnitude of the eventual immune response. Interferon-gamma (IFN-g) is a pleotropic modulator of the immune system which can exhibit a variety of biological functions, including antiviral and antiproliferative effects as well as macrophage priming activity. In particular, it has been demonstrated that IFN-g plays a modulatory role in antigen presentation by increasing the level of class II gene transcription in class II-positive cells as well as inducing MHC expression in the otherwise class II-negative cells, such as endothelial cells and astrocytes. Indeed, abnormal levels or aberrant expression of class II gene products have been implicated in the onset of several autoimmune diseases, including experimental autoimmune encephalomyelitis (EAE) which involves presentation of myelin basic protein by MHC classII-positive astrocytes. Finally, a neutralizing antibody to gamma interferon has been found to abrogate the effects of autoimmune disease in the (NZBxNZW) F1 mouse model of systemic lupus erythematosus (SLE). Numerous studies have focused on identifying the cis-regulatory sequences and trans-acting acting factors that are involved in the regulation of class II gene transcription. We have developed an assay system for analyzing further the role of human gamma interferon in the regulation of MHC class II gene expression. A chimeric transcription unit was constructed which contains a genomic MHC class II promoter sequence,including the gamma interferon responsive elements, fused to the coding region of a reporter gene. We have obtained stable mammalian cell transformants that express high levels of the reporter protein in response to IFN-g. The response is blocked by neutralizing monoclonal antibodies to human gamma interferon and markedly reduced by the glucocorticoid dexamethasone. This highly sensitive reporter gene-based assay system is suitable for rapid human gamma interferon agonist/antagonist screening strategies.

**C 223** DESTRUCTION OF AUTOLOGOUS HLA CLASS II POSITIVE KERATINOCYTES BY T LYMPHOCYTES IN PATIENTS WITH RECURRENT ERYTHEMA MULTIFORME. Rudolf Wank and Eckhart Kämpgen, Institute of Immunology, University of Munich, Goethestrasse 31, D-8000 Munich 2, Federal Republic of Germany.

Almost all diseases showing a strong HLA association belong to the group of disorders with autoimmune features. In these diseases the pathogenic agents are not or only insufficiently defined. An interesting exception is the recurrent Erythema multiforme (REM), an inflammatory skin disease, in which the herpes simplex virus (HSV) plays a dominant role in the pathogenesis. In an intensive study, in which HLA and complement factors were studied in patients and in their families all the REM patients were found to have the same HLA class II molecule, namely HLA-DQw3. Thus we could confirm for the first time by HLA association analysis a postulated disease entity subtype with a viral etiology. Despite the repetitive character of REM, i.e. that the generalized outbreak of the disease is regularly observed two weeks after the appearance of an HSV lesion of the lip, the antigen eliciting the destruction of the keratinocytes by T-lymphocytes is not known. So far HSV has not been identified in the vicinity of tissue destruction. We have however produced a monoclonal antibody specific for DQw3 and shown that the local keratinocytes express the epitope seen by this reagent. Furthermore we have isolated T-cell lines and clones from patients and their HLA-identical siblings using fragmented HSV for stimulation. Interestingly in the first cloning of such T-cells two gamma-delta TCR positive clones were identified. In comparison, among more than 200 allospecific T-cell clones we did not identify such clones. The fine specificity and function of these clones will be discussed.

## *LY2/CD5 Cells and Gene Expression*

**C 224** PREFERENTIAL UTILIZATION OF A SINGLE VH GENE AND SUBSEQUENT SOMATIC DIVERSIFICATION BY GENE CONVERSION IN RABBIT B CELLS, Robert S. Becker[1] and Katherine L. Knight, Dept. of Microbiology, Loyola University Chicago, Maywood, IL 60153; [1]presently with Connaught Laboratories, Inc., Swiftwater, PA 18370

Previous analysis of VDJ rearrangements within leukemic rabbit B cells indicated that the 3'-most VH gene, VH1, had been preferentially utilized. To determine whether this preferential utilization of VH1 was also observed in normal B cells, VDJ rearrangements were PCR amplified from the bone marrow of a 1 day old rabbit. Three of the three VDJ rearrangements isolated had utilized VH1 and were germline in nature. The preferential utilization of VH1 and the germline nature of the VDJ genes appears analogous to rearrangements observed in early B cells and CD5+ B cells in other animal species. Results of Southern blots of restricted genomic DNA from B cells of mature rabbits indicated that mature B cells also preferentially utilized VH1. To address how rabbit B cells generate antibody diversity, VH1 rearrangements were isolated from B cells of mature rabbits by conventional phage cloning and by PCR amplification. Nucleotide sequence analysis of these 13 rearrangements and comparison with germline VH1 indicated that these VDJ genes had been somatic mutated and were extensively diversified in discrete regions. In some cases, this diversification included the addition or subtraction of exons suggesting that the diversification was not the result of base pair interchanges. Comparison of these diversified regions with the nucleotide sequences of germline VH genes upstream of VH1 identified germline genes with near identical sequences. These results indicate that rabbits preferentially utilize a single VH gene and subsequently diversify their VDJ rearrangements by gene conversion events.

**C 225** LY-1 (CD5) B-CELLS IN THE SPECIFICITY REPERTOIRE TO SELF- AND NON-SELF ANTIGENS
Clara G. Bell & M.D. Bell, Dept Micro & Immunol, Schl Med, UIC, Chicago, Ill.
The extent to which the CD5 B-cell subpopulation represents a distinct lineage in the acquisition & maturation of the specific self- & non-self Ab repertoire is unclear. The responsiveness of the CD5 (CD5+) vs. the non-CD5 (CD5-) subpops was studied in a mouse (BALB/c) model by assessing, in limiting dilution analysis, the frequencies, activation, expansion & maturation of CD5+ & CD5- B-precursors generating Ab binding to non-self antigens - bacterial dextran (dex) B1355, a class II dex, with sequences of linearly (l) alternating 1,3 & 1,6 α D-glucopyranosyls (αDg) & branched (b) 3,6 αDg & non-reducing end groups; bacterial polysaccharides; haptens; & mitogens LPS & LPH - & to self - recurrent idiotypes (Id); thyroglobulin; & albumin. Id patterns were analyzed for the V gene restriction. Primary spleen, bone-marrow, lymph node & Peyer's patch precursor B-cells from new-born, 55, 155 & 390 day old mice, FACS sorted for CD5+ or CD5-, were transferred in limiting dilution to 1,600 $^{137}$Cs-irradiated BALB/c or CB.20 mice, with or without various T-cells. Fragment cultures from the recipient spleens were stimulated with antigens or mitogens. Supernatants, analyzed for binding to the panel of self- & non-self & to various class I dex, (containing the l 1,6 αDg with various b 1,4 & 3,6 αDg) showed peritoneal Cd5+ to differ markedly from the CD5- precursors as to isotype, antigen-binding specificity & affinity binding. The CD5+ adult peritoneal also differed from the new born repertoire, though both expressed multiple reactivities & low affinities. Self reactive repertoires were found in both, indicating a lack of B lineage distinction in the self- & non-self repertoire acquisition. An Id analysis of the self renewal of the two B lineages accorded with with the fine specificity reactivity patterns.

**C 226** THE ONTOGENY AND FUNCTIONAL CHARACTERISTICS OF HUMAN CD5+ B CELLS,
Neelima M. Bhat, Marcia M. Bieber, Alan M. Stall*, Leonore A. Herzenberg**, and Nelson N. H. Teng, Department of Obstetrics and Gynecology and **Department of Genetics, Stanford University School of Medicine, Stanford, California 94305, *Department of Microbiology, College of Physicians and Surgeons of Columbia University, New York, NY 10032

By multi-parameter FACS analysis, we show that CD5 (Leu-1) is expressed on virtually all the B lymphocytes in human fetal spleen and lymph nodes at the gestational age of 18 to 23 weeks. Similarly, all the B lymphocytes in cord blood at term are CD5+. Following birth, however, there is a gradual reduction of this subset in both the spleen and periphery until it stabilizes in young adulthood, and thereafter remains constant. A parallel increase in conventional B cells (CD5-) is seen only in spleen. In the periphery in contrast, maximum levels of CD5- B cells appear early in infancy and remain relatively constant through adult life.

We have also examined the repertoire of antibodies produced by both human fetal and adult CD5+ B lymphocytes. The CD5+ B lymphocytes from fetal spleen produce polyreactive autoantibodies of the IgM isotype. In a normal adult spleen, which contains both B cell subsets (CD5+ and CD5-), such polyreactive IgM autoantibodies are produced only by CD5+ B cells. The significance of the age related reduction of CD5+ B cells and the polyreactive antibodies they secrete is discussed in context of the possible role of this cell type in immune regulation and resistance to infectious agents.

**C 227** ROLE OF CD5+ B CELLS IN SYSTEMIC AUTOIMMUNE DISEASE,
Kathryn H. Brooks, Laurie Iciek and Mark Stuart. Department of Microbiology, Michigan State University, East Lansing, MI 48824
HIV infection, the murine AIDS model, chronic graft-vs-host disease and autoimmune diseases such as systemic lupus erythematosus and rheumatoid arthritis appear to share a similar hyperactivity of B lymphocytes. This polyclonal B cell activation and production of autoantibodies seems to precede the potential development of B cell lymphomas. We have used both the infection of C57Bl/6 mice with the LP-BM5 murine leukemia virus mixture, i.e. the murine AIDS model, and chronic graft-vs-host disease induced by transfer of DBA2 T cells to nonirradiated $BDF_1$ recipients as model systems to evaluate the role of CD5 (Ly1+) or sister lineage B cells in systemic autoimmune disease. In both models, within a few weeks of cell or virus transfer IgM secretion as determined by a reverse plaque assay or spontaneous in vitro IgM release increased 4-6-fold. Concomitantly, the frequency of splenic CD5 (Ly1+) B cells in the spleen declined. We have also assessed additional phenotypic changes in the T cell and B cell populations found in the spleen and the peritoneal cavity. The significance of the results relative to the hypothesis that CD5 is an activation antigen is discussed.

**C 228** MOLECULAR ANALYSIS OF IMMUNOGLOBULIN VARIABLE REGIONS IN HUMAN CHRONIC LYMPHOCYTIC LEUKEMIA: A STUDY OF CASES EXPRESSING MEMBERS OF MULTIGENE $V_H$ GENE FAMILIES, Saskia B. Ebeling, Mieke E.M. Schutte, Karine E. Akkermans, Andries C. Bloem, Frits H. J. Gmelig-Meyling and Ton Logtenberg, Dept. of Clinical Immunology, University Hospital, Utrecht, The Netherlands.

Previous studies have shown that Ig $V_H$ gene expression in the malignant cells of chronic lymphocytic leukemia (CLL) may be biased towards members of the small $V_H5$ and $V_H6$ families and a single member of the large $V_H1$ family. Strikingly, these particular $V_H$ genes also belong to a set of genes preferentially expressed in B cell ontogeny and, in addition, have been frequently found in association with IgM molecules that display autoantibody activity. To further explore possible relationships between $V_H$ genes expressed in CLL and developmentally-restricted and/or autoantibody-associated $V_H$ genes, we analyzed eight cases of CLL in which members of large, multigene $V_H$ families were expressed. Our results demonstrate that different members of these families may be used in CLL, including $V_H$ genes associated with early B cell ontogeny and autoantibody reactivity. Furthermore, we found a highly biased utilization of the $J_H4$ gene in CLL rearrangements and noted that some but not all CLL cases exhibited considerable intraclonal diversity. The distribution and nature of the observed nucleotide diversification was indicative of a random mutational process. These data support the concept that Ig V region expression in CLL is restricted at the level of $V_H$ and $J_H$ genes and that CLL is a heterogeneous malignancy with respect to the occurrence of mutations.

**C 229** IMMUNOGLOBULIN GENE EXPRESSION IN CD5 B CELL MALIGNANCIES

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The malignant cells from most patients with B cell chronic lymphocytic leukemia (CLL) and related small lymphocytic lymphomas (SLL) co-express B cell differentiation antigens and the pan-T lymphocyte surface antigen CD5 (Leu1). As such, CLL and related lymphomas generally may be considered malignancies of the CD5 B cell, a minor B cell subpopulation implicated in the production of autoantibodies. These malignancies are distinctive in that high proportions of patients have neoplastic B cells that express surface immunoglobulin (Ig) bearing one or more cross reactive idiotypes (CRIs) that commonly are present on monoclonal IgM autoantibodies. In contrast, B cell non-Hodgkin's lymphomas (NHL) of follicular center cell origin typically neither express CD5 nor such autoantibody-associated CRIs. Molecular studies indicate that the frequent occurrence of these CRIs in CD5 B cell malignancies is secondary to the non-random use of highly conserved Ig variable region genes (V genes) expressed with little or no somatic hypermutation. In addition, studies with other neoplastic CD5 B cell populations not selected for their expression of autoantibody-associated CRIs reveal that they too may express V genes that have not substantially diversified from those present in the germline DNA. We also have examined unusual cases of CD5-negative CLL or SLL in which the malignant cells either express pathogenic autoantibodies and/or autoantibody-associated CRIs. In contrast to our findings with conventional CLL, we find intraclonal diversity in the Ig V genes expressed by such neoplasms indicative of ongoing somatic hypermutation. As such, these neoplasms more resemble NHL of follicular center cell origin. These studies indicate that expression of CD5 may help delineate B cell neoplasms that differ fundamentally in the ways they process and express their Ig V genes.

**C 230** THE REGULATION OF MURINE B LYMPHOMA CELL GROWTH IN VITRO BY DIRECT INTERACTION WITH ACTIVATED T HELPER CELLS, E. Charles Snow and Elie E. Mansour, Department of Microbiology and Immunology, University of Kentucky Medical Center, Lexington, KY 40536

Human, CD5+ follicular lymphoma cells do not proliferate in culture unless they physically interact with activated, CD4+ T helper (Th) cells (Umetsu et al., J. Immunol. 144:2550, 1990). This observation may provide important clues to the way in which the growth of such tumors in vivo is regulated. In the present study, we have examined the in vitro proliferative capabilities of a series of murine Lyl+ B cell lymphomas to identify murine lymphomas which also require Th cell involvement to expand in culture. We have utilized paraformaldehyde fixed, anti-CD4-stimulated Th clones as the in vitro stimulus. The further analysis of the selected lymphomas will allow us to dissect the regulatory mechanism(s) operative in controlling the growth of these tumors both in vitro and in vivo.

**C 231** COMPLETE NUCLEOTIDE SEQUENCE OF THE EXPRESSED VH AND VL GENES OF A HUMAN $CD5^+$ B CELL CLONE, Roger W.J. van der Heijden, Virginia Pascual*, Albert D.M.E. Osterhaus and Fons G.C.M. UytdeHaag, Laboratory of Immunobiology, National Institute of Public Health and Environmental Protection, P.O. Box 1, 3720 BA Bilthoven, The Netherlands, *Department of Microbiology, University of Texas, Southwestern Medical Center, Dallas, Texas, 75235.

A human EBV transformed **$CD5^+$** B cell (EBV383), producing an IgM,λ anti-idiotypic monoclonal antibody (MAb383), showed **extensive somatic variability** in the expressed $V_H$ gene segment compared to its germline $V_HV$ counterpart (Van der Heijden et al., J.Immunol. 1990). These unique data on CD5 B cell $V_H$ gene expression demanded a detailed characterization of EBV383. We showed the presence of the CD5 precursor RNA as well as the CD5 mRNA transcript, the expression of the CD5 antigen on the cell surface and the remarkable high affinity monoreactive binding pattern of MAb383 to its corresponding idiotype only (Van der Heijden et al., J.Immunol. submitted). In addition, we will present the nucleotide sequence of the expressed $V_\lambda$ gene and suggest a possible role of such $CD5^+$ B cells, as an alternative for persisting antigen, in the maintenance of memory B cells.

## *B Cell Tolerance Mechanisms*

**C 232** IgM AND IgD ANTIGEN RECEPTORS IN THE REGULATION OF B-LYMPHOCYTE DEVELOPMENT. Robert Brink, Jeffrey Crosbie, Antony Basten, Helle Jorgensen, Elizabeth Adams, Suzanne Hartley and Christopher Goodnow*. Centenary Institute of Cancer Medicine and Cell Biology, c/- University of Sydney, NSW 2006, AUSTRALIA. *Present address Howard Hughes Medical Institute and Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, CA 94305.

During differentiation, B-lymphocytes (B-cells) initially express IgM membrane antigen receptors and subsequently acquire IgD so that mature B-cells coexpress both receptor isotypes. Double-transgenic (DTg) mice were obtained by mating mice expressing a hen-egg lysozyme (HEL) transgene with others sequentially expressing anti-HEL IgM and IgD encoded by rearranged heavy and light chain transgenes. Tolerant anti-HEL B-cells persisted in the DTg mice and exhibited selective downregulation of membrane IgM with no change in IgD levels. To examine the respective roles of IgM and IgD in B-cell function, the Ig heavy chain construct was modified so that transgenic B-cells expressed one or other of the receptor isotypes. This approach has yielded interesting results regarding the respective involvement of IgM and IgD not only in the induction of tolerance in self-reactive B-cells, but also in the development of non-tolerant B-cells.

**C 233** **AUTOREACTIVE B-CELLS SYNTHESIZING SECRETED IgM MOLECULES ESCAPE FROM TOLERANCE INDUCTION,** Frank Brombacher, Georges Köhler and Hermann Eibel, Max-Planck-Institut f. Immunbiologie, Stübeweg 51, 7800 Freiburg, FRG.

Two strategies have been used to study B-cell tolerance in transgenic mice (lysozyme/α-lysozyme Ab and α-MHCI Ab tg. mice), finding clonal deletion and/or clonal anergy.

We used a third strategy, where only the α-CD8.2 specific immunoglobulin heavy chain gene (μ), synthesizing either the membrane **and** secreted ($\mu^{m+s}$) form or **only** the secreted form ($\mu^s$), were introduced into the genome of mice. Similiar to normal differentiation, B-cells expressing the transgenic μ have to rearrange their endogenous light chain (L) genes first in order to supply L-chains for retaining the original antibody specificity. In $CD8.1^+$ transgenic mice we find the α-CD8.2 specificity in sera and in transgenic μ expressing LPS stimulated B-cell hybridomas (freq.: 1/420), whereas in the presence of the (auto)antigen ($CD8.2^+$ mice) we are neither able to detect the specificity in sera, nor can we find an α-CD8.2 specific hybridoma (freq.: <1/7000) in $\mu^{m+s}$ transgenic mice. We do find α-CD8.2 specific hybridomas (freq.:1/820) in $\mu^s$ transgenic mice. Our results show, that B-cells expressing high affinity autoantibodies on their surface induces B-cell tolerance, whereas cells synthesizing the same antibodies in their secreted form can escape the immune surveillance and continue to persist in the B lymphocyte repertoire.

C 234 **TOLERANCE INDUCTION IN IG-TRANSGENIC MICE:DEFINITION OF ANTIGEN AND ANTIBODY DEPENDENT VARIABLES.** R. Carsetti, G. Köhler, R. Lamers MPI f. Immunbiologie, D-7800 Freiburg, FRG.

We have exploited transgenic mice to design a flexible model for tolerance induction which could enable us to define the variables depending on the antigen (T-dependent or independent form, number of epitopes, amount,time point and duration of exposure) and on the antibody (isotype, cell-bound and soluble form). In mice carrying μ heavy and κ light chain trangenes specific for TNP, T-dependent (TNP-BSA) and T-independent (TNP-dextran) self-antigens (5-200 μg/mouse i.p. every 3 days beginning on the day of birth) cause deletion of TNP-specific B cells that are brighly stained for μ, TNP and transgenic idiotype in the bone marrow and in the spleen. The extent of the effect is dose-dependent. After 4-5 weeks from the last antigen administration the mice are repopulated with cells of normal phenotype. Transgenic B cells from untreated adult mice appear to be functional when stimulated with thymus-dependent antigen and polyclonal activators. Depending on the dose of "self"-antigen and on the route of administration elimination of μ bright, TNP-specific splenic B lymphocytes can be observed in 8-32 weeks old mice. TNP-dextran injection in double transgenic F1 neonates (μκ x δ) eliminates δ positive B cells bu only those that are also bright for μ.

C 235 EXPRESSION OF ANTI-DNA IMMUNOGLOBULIN TRANSGENES IN NON-AUTOIMMUNE AND MRL/lpr MICE, Jan Erikson*, Marko Z. Radic*, Sally A. Camper†, Richard R. Hardy*, and Martin Weigert*, *Institute for Cancer Research, Fox Chase Cancer Center, 7701 Burholme Avenue, Philadelphia, PA 19111 and †University of Michigan Medical School, Department of Human Genetics, Ann Arbor, MI 48109-0618

To understand how autoantibodies are normally regulated we have generated mice that have transgenes coding for anti-DNA antibodies. These mice were derived by mating a mouse bearing a heavy chain transgene taken from the anti-DNA antibody, 3H9, with a mice bearing either a $V_\kappa 8$ or a $V_{\lambda 2}$ light chain transgene. Both light chains in combination with 3H9 heavy chain form anti-DNA antibodies. In addition, as the 3H9 heavy chain in association with a variety of light chains can bind to DNA, the transgenic mice carrying only the heavy chain were also informative. In both the $V_H3H9/V_\kappa 8$ and the $V_H3H9$ heavy chain-only transgenics the majority of splenic B cells bind DNA. Strikingly, we fail to detect secreted anti-DNA. We suggest that as a consequence of their self-reactivity these B cells are developmentally arrested. Phenotypic changes that coincide with the tolerized B cell state will be discussed. In the $V_H3H9/V_{\lambda 2}$ transgenics fewer splenic B cells co-express both the heavy and light chain transgenes on their surface. Those that do, however, show a dramatic reduction in surface IgM levels. Once again there is no evidence of the secreted transgene product. In vitro, the $V_H3H9/V_\kappa 8$ and the $V_H3H9/V_{\lambda 2}$ antibodies show distinct DNA specificities which may account for the different mechanisms employed to silence them in vivo. The $V_H3H9/V\kappa 8$ transgenes have been backcrossed onto the autoimmune MRL/lpr genetic background. Evidence for the failure to regulate the anti-DNA transgenes in the MRL/lpr mice will be reported.

C 236 IMMUNE TOLERANCE TO BLOOD GROUP ANTIGENS IN MAN, Uri Galili and Aron Thall, Department of Laboratory Medicine, University of California, San Francisco CA 94143

The purpose of this study was to determine whether immune tolerance to self blood group antigens is mediated by active suppression of circulating autoreactive B lymphocytes, or results from the absence of such B lymphocytes from the circulation. B lymphocytes were separated from buffy coats of blood units of normal donors and placed in four plates of 96 wells each (50,000 cells per well). The cells were transformed by Epstein–Barr virus and the supernatants were subsequently assayed for anti-blood group A and anti-blood group B activity by hemmaglutination. Under these conditions, each well contained an average of 10 transformed B cell clones. Four donors of each blood type were studied. In blood group O individuals, 5 wells out of 384 wells contained anti-blood group A, and 5 other wells contained anti-blood group B antibodies. In blood group A individuals, 5 wells contained anti-B antibodies, but no wells contained anti-A antibodies. In blood group B individuals, 5 wells contained anti-A reactivity, and no wells had anti-B antibodies. Our data imply that no B lymphocytes capable of producing antibodies to self blood group antigens can be found when 4000 individually transformed B lymphocytes are screened for antibody production, whereas 0.1% of the circulating B lymphocytes produce antibodies to foreign A or B blood group antigens. Our data imply that immune tolerance to blood group antigens correlates with the absence of autoreactive B lymphocytes in the circulation.

**C 237** CLONAL DELETION OF PHOSPHOCHOLINE (PC)-SPECIFIC B-CELLS IN M167-TRANSGENIC MICE EXPRESSING AN X-LINKED IMMUNODEFICIENCY GENE, XID, James J. Kenny[1], Donna Sieckmann[3], and Dan L. Longo[2], PRI/DynCorp.[1], and the Biological Response Modifiers Program[2], NCI-FCRDC, Frederick, MD 21701, and the Naval Medical Research Institute[3], Bethesda, MD 2081
The combined expression of the M167 $\mu/\kappa$ anti-PC transgenes with the xid gene in (B6.CBA/N x $\mu/\kappa$ 207-4) F1 mice results in an almost total failure to develop B cells in the peripheral lymphoid organs of the immune defective, F1 male progeny, whereas, the phenotypically normal F1 females have large numbers of PC-specific B cells in their spleens. Immune deficient, xid male mice derived from crosses of B6.CBA/N and $\mu$-243-4 M167 H-chain transgenic mice have large numbers of splenic B cells expressing the M167 heavy chain, but none of these B cells are PC-specific. However, the phenotypically normal F1 $TG^+$ female progeny from this cross have 1 to 3% of their B cells which are PC-specific. These B cells appear to express the M167 $\mu$-transgene in association with an endogenous light chain (presumably $V_\kappa$ 24) which gives rise to exclusively M167 $\mu/\kappa$ idiotype positive B cells. T15-idiotype positive B cells are not detectable among these PC-specific B cells. The absence of the T15-idiotype is some what surprising in that this is the dominant idiotype in normal mice. However, transfection experiments show that the M167 H-chain can associate with a $V_\kappa$ 22 gene product to produce a T15-idiotype positive antibody, but this antibody is not PC-specific. These data suggest that: 1) $TG^+$, PC-specific B cells are clonally deleted in an antigen-specific manner in mice expressing the xid gene, and 2) PC-specific B cells expressing the M167 H-chain in association with the appropriate $V_\kappa$ 24 endogenous light chain are specifically selected and expanded in normal M167 H-chain $TG^+$ mice.

**C 238** BCL-2 DISPLAYS A RESTRICTED LYMPHOID TISSUE LOCALIZATION AND EXTENDS B CELL MEMORY. Gabriel Nuñez, David Hockenbery, Timothy J McDonnell and Stanley J Korsmeyer. HHMI at Washington University, Saint Louis, Missouri. The Bcl-2 proto-oncogene encodes a 25 KD inner mitochondrial integral membrane protein that extends the survival of certain hematopoietic cell lines by blocking their programmed cell death following growth factor deprivation. Moreover, we have previously shown that transgenic mice bearing a Bcl-2-Ig minigene demonstrate a polyclonal expansion of resting surface IgM/D B cells which display a prolonged survival in vitro. Immunohistochemical examination with a monoclonal anti-Bcl-2 Ab revealed Bcl-2 protein predominantly in the follicular mantle zone, comprised of long-lived B cells. Within the germinal center Bcl-2 protein was confined to the apical light zone, where a subset of B cells survive. We immunized Bcl-2-Ig and control mice with FITC-KLH to assess their response to T-dependent antigens. Both Bcl-2-Ig mice and control littermates exhibited similar primary and classic 4 wk secondary responses as assessed by serum antibody (ELISA) and splenic plaque-forming cell (PFC) assays. However, Bcl-2-Ig mice exhibited 5-fold and 20-fold greater anti-FITC PFC's/$10^6$ spleen cells than control littermates when assayed at 25 and 75 days respectively after secondary antigen challenge. Transgenics demonstrated 349 ± 95 PFC/$10^6$ spleen cells versus control mice (15 ± 7 PFC/$10^6$ cells) at 75d post $2^o$ antigen challenge. In order to assess Bcl-2's role in B cell memory, donor mice were primed with FITC-KLH and 2-3 months later 6 x $10^6$ resting spleen cells were adoptively transferred to BSA primed but KLH naive recipients. Recipients receiving either control or transgenic B cells responded equally when challenged with FITC-BSA on the day of transfer and assayed 7 days later. In contrast, Bcl-2-Ig mice demonstrated 252 ± 55 PFC/$10^6$ spleen cells and high titer anti-FITC IgG when challenged with antigen 35 days after transfer whereas control littermates failed to mount a response(14 ± 7 PFC). These results indicate an important role for Bcl-2 in an anamestic immune response and antigen-specific B cell memory.

**C 239** INHIBITION OF LPS INDUCED IG SECRETION IN B CELLS OF ANTI TNP-ANTIBODY TRANSGENIC MICE BY TNP-CONJUGATES, A. Schimpl, I. Berberich, C. Pröschel, D. Hallmann, K. Bothe and I. Horak, Institut für Virologie und Immunbiologie, Universität Würzburg, Versbacher Str. 7, D-8700 Würzburg, F.R.G.
Antigen receptor cross-linkage by anti $\mu F(ab')_2$ enhances proliferation, but inhibits IgM secretion in LPS or Il-5 stimulated murine B cells. At the RNA level this is brought about by a selective reduction in $\mu$s-mRNA steady state levels (1), caused by the preference of pre-RNA processing towards $\mu$m rather than $\mu$s and a certain lability of $\mu$s mRNA. In addition, antigen receptor engagement in doubly stimulated cells also prevents J chain induction.
In order to investigate whether massive cross-linkage by antigen will cause the same defects of secretion in proliferating cells we established Ig transgenic mouse lines using a DNA clone coding for the H and L chain of an anti-TNP-antibody (2). B cells from transgenic animals show in vitro that anti-TNP antibody secretion in LPS stimulated cells can be prevented by high doses of TNP-carrier conjugates, while proliferation is unaffected. Studies are on the way to elucidate the role of carrier specific T cells in influencing this phenomenon and on their role in tolerance versus memory induction.
1) U. Chen-Bettecken, E. Wecker & A. Schimpl (1985). Proc. Natl. Acad. Sci. USA 82, 7384
2) Ochi et al. (1983). Proc. Natl. Acad. Sci. USA 80, 6351.

**C 240** RHEUMATOID FACTOR EXPRESSING TRANSGENIC MICE, Dorit Zharhary, Sally A. Camper, Martin G. Weigert, Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, PA 19111

It is clear that in healthy individuals, B cells expressing self reactive specificities are regulated. In order to understand how autoantibodies are regulated, we have generated mice which express a transgene encoding a rheumatoid factor (RF) antibody which arose spontaneously in an MRL/*lpr* mouse. This antibody termed AM14, which expresses an Ig heavy chain product of the J558 family along with a light chain of the $V_\kappa 8$ group, binds to $IgG_{2a}$. This specificity is characteristic of the pathogenic RFs found in MRL/*lpr*. Moreover, the antibody binds to the MRL $IgG_{2a}$ allotype ($IgH\text{-}1^J$) and not to that of C57BL/6 ($IgH\text{-}1^b$). This allotype discrimination allows us to study the expression of this RF in and out of the context of self antigen by introducing the transgene into mice of different IgH allotypic background. The regulation of the transgene expression in mice expressing the $IgH\text{-}1^b$ vs. $IgH\text{-}1^J$ allotype will be discussed.

**C 241** SIGNALLING THROUGH SURFACE IgM IN TOLERANCE-SUSCEPTIBLE IMMATURE MURINE B LYMPHOCYTES, Amy J. Yellen, William Glenn, and John G. Monroe, Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA 19104

During the course of B lymphocyte development, newly emerging surface immunoglobulin-positive B cells pass through a stage when antigen-antigen-receptor interactions lead not to immune responsiveness but to a state of functional tolerance. We have explored the molecular basis of antigenic nonresponsiveness and tolerance susceptibility using tolerance-susceptible surface immunoglobulin-positive splenic B lymphocytes from neonatal mice and anti-μ chain antibodies as a polyclonal ligand. In this population of cells, surface IgM appears to be uncoupled from the PI-hydrolysis pathway at a point proximal to the receptor; anti-μ antibodies did not stimulate soluble inositol phosphate generation despite the fact that PI-hydrolysis was observed following treatment with $AlF_4^-$, implicating the existence of a functional G protein and phospholipase C. Further evidence for a difference early in the signal transduction pathway stems from the finding that stimulation with phorbol diester but not anti-μ induces the expression of two immediate/early PKC-linked genes *Egr-1* and *c-fos*. This appears to be the primary signalling difference between the mature and immature populations, as we were able to cause these cells to undergo a $G_0$-$G_1$ phase transition by bypassing sIgM using phorbol diester and calcium ionophore. Interestingly, despite undetectable levels of PI-hydrolysis, we observed equivalent receptor-mediated changes in intracellular calcium when comparing the immature and mature populations. These results indicate incomplete coupling of sIgM to the signal transduction machinery operative in mature, immunocompetent B cells and suggests a molecular mechanism accounting for the differential processing of sIgM signals into activation versus tolerogenic responses observed in these two stages of B cell development.

**C 242** RHEUMATOID FACTOR EXPRESSING TRANSGENIC MICE

Dorit Zharhary, Sally A. Camper, Martin G. Weigert, Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, PA 19111

It is clear that in healthy individuals, B cells expressing self reactive specificities are regulated. In order to understand how autoantibodies are regulated, we have generated mice which express a transgene encoding a rheumatoid factor (RF) antibody which arose spontaneously in an MRL/*lpr* mouse. This antibody termed AM14, which expresses an Ig heavy chain product of the J558 family along with a light chain of the $V_\kappa 8$ group, binds to $IgG_{2a}$. This specificity is characteristic of the pathogenic RFs found in MRL/*lpr*. Moreover, the antibody binds to the MRL $IgG_{2a}$ allotype ($IgH\text{-}1^J$) and not to that of C57BL/6 ($IgH\text{-}1^b$). This allotype discrimination allows us to study the expression of this RF in and out of the context of self antigen by introducing the transgene into mice of different IgH allotypic background. The regulation of the transgene expression in mice expressing the $IgH\text{-}1^b$ vs. $IgH\text{-}1^J$ allotype will be discussed.

**C 243** **DOWNREGULATION OF EXPRESSION OF A TRANSGENIC IMMUNOGLOBULIN WITH DEGENERATE SPECIFICITY.** Zöller,M., Inst.Radiol.Pathophysiol., German Cancer Research Center, Heidelberg, FRG.

Transgenic mouse models have revealed evidence for clonal deletion as well as clonal anergy of monospecific, high avidity autoreactive B cells. Function and fate of naturally activated B cells, many of them displaying degenerate specificity including autoreactivity are still a matter of debate. The question was pursued in Sp6-transgenic mice. Sp6, a monoclonal IgM antibody, binds strongly to TNP but also reacts with a variety of self antigens.

Early after birth the thymus of transgenic mice contained over 1000 times more TNP-specific B cells than the thymus of non-transgenic mice. The number of intrathymic B cells decreased slightly during postnatal development, but was strongly diminished after antigenic stimulation. The fate of splenic B cells expressing the transgene differed inasmuch as there was no indication for an actual decrease in the number of B cells during postnatal maturation. But B cells did not expand after antigenic stimulation. Two additional observations, which could be interdependent, seem to offer an explanation for the unresponsiveness of transgenic B cells: The frequency of antigen- and idiotype-specific helper T cells was reduced in transgenic mice and, in particular after contact with the nominal antigen, B cells could not present the nominal antigen or their own idiotype.

The data are interpeted in the sense that transgenic B cells apparently become deleted in the thymus, the underlying mechanism being unknown until now. In the periphery, on the other hand, B cells are driven in a stage of anergy after contact with TNP, the nominal antigen which for Sp6 has highest affinity. This in turn hampers activation of antigen- and idiotype-specific help and creates a stable state of "unresponsiveness".

# Self Reactivity and Its Regulation

*Autoimmune Susceptibility Genes*

**C 300** POSSIBLE DELETION OF A DEVELOPMENTALLY REGULATED Vh GENE IN AUTOIMMUNE DISEASE, Pojen P. Chen, Nancy J. Olsen*, Katherine A. Siminovitch+, Tsaiwei Olee, Franklin Kozin, Dennis A. Carson, Pang-Ming Yang, Department of Medicine, University of California, San Diego, La Jolla, CA 92093; *Department of Medicine, Vanderbilt University, Nashville TN 37232; +Department of Medicine, University of Toronto, Toronto, Ontario M5T 2S8, Canada

Several autoantibody-associated variable region (V) genes are preferentially expressed during early ontogenic development, suggesting strongly that they are of developmental and physiological importance. As such, it is possible that polymorphisms in one or more of these genes may alter susceptibility to autoimmune disease. We have searched extensively for a probe related to a developmentally regulated V gene that has the power to differentiate among highly homologous V genes in human populations. Using such a probe (i.e. Humhv3005/P1) related to both anti-DNA and anti-IgG autoantibodies, we studied restriction fragment length polymorphisms in patients with rheumatoid arthritis and systemic lupus erythematosus, and found an apparent Vh deletion that was nearly restricted to the autoimmune patients. These data suggest that deletions of physiologically important Vh genes may increase the risk of autoimmunity through indirect effects on the development and homeostasis of the B cell repertoire.

**C 301** NO RECOMBINATIONS BETWEEN TCRα-V AND TCRα-C GENE SEGMENTS, Lars Gleditsch[1], Ralph Snodgrass[2] and Bjarne Bogen[1]. [1]Department of Immunology and Rheumatology, University of Oslo, Norway [2]Lineberger Cancer Research Center, University of North Carolina, USA

Because *Tcr* genes may possibly function as non-MHC immune response genes or predispose for autoimmune diseases, it is important to know how these genes are inherited. Inheritance of Vα and Cα RFLPs were investigated in 669 offspring from P X $F_1$ backcrosses. We did not find any recombinations in the offspring. In seven B X D and B X H recombinant inbred strains with known recombinations between the *Tcra-C* and *Es-10* loci, all *Tcra-V* RFLPs cosegregated with the *Tcra-C* RFLP. We have also produced a Tcrα congenic mouse by placing the *Tcra-* region of BALB/c on a B10.D2 background (N12, F4). No recombinations between Vα and Cα RFLPs had occurred during the breeding process. From these studies we conclude that *Tcra-V* and *Tcra-C* gene segments are very tightly linked. Such a finding is significant if germline *Tcra* gene segments (in addition to combinatorial and junctional diversity) heavily influence the Tcr repertoire and the magnitude of T-cell responses.

**C 302** IMMUNOGLOBULIN VH CLANS DEFINE EVOLUTIONARILY CONSERVED STRUCTURES.

Perry M. Kirkham and Harry W. Schroeder, Jr., Departments of Microbiology and Medicine, University of Alabama at Birmingham, WTI 263, Birmingham, AL 35294

Immunoglobulin variable heavy chain (VH) gene family identity can be predicted by the extent of sequence homology within residues 6-24 of Framework I and 67-85 of Framework III. These sequences are highly conserved and allow grouping of all known mammalian VH families into one of three clans based upon shared nucleotide and peptide homology. Multisequence analysis of germline VH genes reveals that conserved nucleotide motifs, which are readily apparent upon visual examination, encode clan-specific consensus peptide residues. Of these 12 residues, 7 are found in FWI and 5 in FWIII. Molecular modelling of known immunoglobulin structures demonstrates that these clan-specific peptides are clustered into three groups of interacting residues on a solvent-exposed face of the heavy chain variable region at 90 degrees rotation from the classic antigen binding site. Cluster 1 (residues 23, 73, 75, and 77) is physically adjacent to the CDR and may influence antigen binding. Cluster 2 (residues 6, 9, 71, and 81) is a primary component of the beta pleated sheet supporting the CDR loops. Cluster 3 (residues 12, 13, 16, and 19) participates in the formation of a solvent-exposed beta loop antipodal to the classic antigen binding site. This loop differs in structure and size by up to 3 Angstroms in a clan-specific fashion. Thus, not only are the nucleotide and peptide residues within a clan conserved, but the structure of an immunoglobulin clan-member is preserved as well. The physical location of this latter loop may allow cluster 3 residues to participate in intermolecular interactions. Our analysis suggests that there are a limited set of immunoglobulin structures in the germline. These structures may play an important role in immunoregulation.

**C 303** AUTOIMMUNITY AND T CELL VACCINATION IN A MODEL IDIOTYPIC NETWORK, Alan S. Perelson and Rob J. De Boer, Theoretical Division T-10, Los Alamos National Laboratory, New Mexico 87545.

Empirical evidence on T cell vaccination (TcV) suggests that the resistance to autoimmune disease can be induced by activating an idiotypic network. Here we show that a simple mathematical model of the idiotypic network, involving only one type of T cell, is able to account for TcV. The only difference between the T cell clones is that they lie in the different idiotypic layers of the network. Clones of adjacent layers activate one another according to a bell-shaped activation function. The use of such a function is well accepted for B cell activation but is a matter of debate for T cell activation. A bell-shaped activation function implies that large populations are suppressive, intermediate populations are stimulatory, and small populations have little or no effect. For a model that is based upon these assumptions we show that (1) the network maintains tolerance, (2) antigen may evoke autoimmunity, (3) a large dose of idiotypic T cells may evoke autoimmunity, (4) a small dose of idiotypic T cells protects (or vaccinates) against a subsequent induction of autoimmunity, and (5) peptides or treated cells of the idiotypic population also vaccinate against autoimmunity. In conclusion, a simple network model is able to account for TcV.

**C 304** CELIAC DISEASE (CD) IS PRIMARILY ASSOCIATED TO A CIS OR TRANS ENCODED HLA-DQ$\alpha\beta$ HETERODIMER, Ludvig M. Sollid, Knut E.A. Lundin, Anne Spurkland, Gunnar Markussen, Frode Vartdal and Erik Thorsby, Institute of Transplantation Immunology, The National Hospital, 0027 Oslo 1, Norway.

Most CD patients are either DR3 positive or DR5/7 heterozygous. Interestingly, DR5DQw7/DR7DQw2 heterozygous individuals carry in trans position the same DQA1 and DQB1 alleles (DQA1*0501 and DQB1*0201) as found in cis position on the DR3DQw2 haplotype. DR3 and DR5/7 heterozygous CD patients might thus potentially express identical DQ molecules. Genomic typing of 94 random Norwegian CD children revealed that 93 (i.e. 99%) carried the DQA1*0501 and DQB1*0201 alleles (90 DR3 positive and 3 DR5/7 heterozygous). Typing of the same patients for all known DPA1 and DPB1 alleles showed that the frequencies of the DPA1*0201 and the DPB1*0101 alleles were moderately increased among the patients compared to the controls. However, the increase of these DP alleles seemed to be caused by linkage disequilibrium of these DP alleles with the CD associated DQ alleles. Studies with alloreactive T cell clones further demonstrated that the DQA1*0501 and DQB1*0201 alleles encode a functionally expressed DQ molecule both when positioned in cis and in trans. Thus, our results strongly indicate that the susceptibility to develop CD is primarily conferred by a cis or trans encoded HLA-DQ$\alpha\beta$ heterodimer.

**C 305** CHROMOSOMAL ORGANIZATION OF DEVELOPMENTALLY EXPRESSED HUMAN $V_H$ GENES, Ko Willems van Dijk, Laurie A. Milner, Eric H. Sasso, and Eric C.B. Milner, Virginia Mason Research Center, Seattle, WA 98101

Preferential expression of a limited number of germline $V_H$ elements has been observed during human fetal development. To delineate the molecular basis for this phenomenon, we determined the relative positions of seven distinct, developmentally expressed $V_H$ genes in the human $V_H$ locus. Individual $V_H$ elements were first mapped to individual restriction fragments using $V_H$ family-specific, and gene-specific probes. Haplotype assignments in a single individual were inferred from segregation analysis of the donor family. Subsequently, haplotype-specific maps were constructed from the pattern of deletions detected in a panel of the rearrangements of monoclonal lymphoblastoid cell lines derived from the donor. The developmentally expressed germline genes were mapped with respect to the recombination breakpoints in the deletion lines by hybridization analysis using both conventional and pulse field electrophoresis to size-separate DNA. We have found that the developmentally expressed $V_H$ genes appear to be located within the first 1000 kb of DNA upstream of $J_H$, supporting the hypothesis that chromosomal location proximal to JH contributes to preferential rearrangement. However, this region of the locus contains approximately 30 to 50% of all $V_H$ elements. Since many of these are likely not developmentally expressed, chromosomal location cannot be the sole determinant of developmental expression.

*Autoimmune B Cell Repertoires: Specificity, Idiotype, V Gene Usage*

**C 306** B CELL DIFFERENTIATION IN THE RAT: COMPLEXITY AND ORGANIZATION OF THE $V_H$ GENE LOCUS

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Selection of the B cell repertoire during differentiation of B cells is not very well understood. B cell differentiation in the rat is well characterized. Several subpopulations of B cells can be distinguished on the basis of reactivity with a large panel of monoclonal antibodies which react with differentiation antigens.
The investigation of possible different B cell repertoires in those B cell subpopulations has been hampered by the poor knowledge of the organization of the variable region loci in the rat.
Therefore Southern blotting analysis of rat liver DNA, digested with different restriction enzymes and hybridization to the 12 different mouse $V_h$-gene family probes has been performed. The results show large differences in the complexity of the rat $V_h$ gene locus compared to the mouse locus. Currently the organization of the rat $V_h$ gene locus is being investigated by Southern blotting analysis of rat hybridomas which have deleted the non-functional VDJ-rearrangement.

**C 307** SELECTION OF PRIMARY B CELL REPERTOIRES ACTING DIFFERENTLY IN ADULT AND NEONATAL LIFE, Leif Carlsson, Charlotta Övermo and Dan Holmberg, Unit for Applied Cell- and Molecular Biology, University of Umeå, Umeå, Sweden.

PCR-amplification and nucleotide sequence analysis of a large number of rearranged immunoglobulin heavy chain V-regions, derived from normal nonmanipulated 1 day old and 14 weeks old BALB/c mice were performed. These analyses confirm previous results indicating a limited junctional diversity in newborn compared to the adult repertoires. This is evident both from a lower frequency of N-additions as well as lower exonuclease activity in the former. Furthermore, a unique and restricted utilization of the 7183 $V_H$-gene family was observed in neonatal as well as adult repertoires. These data strongly suggest that selecting mechanisms exist, acting during the development of antibody repertoires.

**C 308** THE ANTI-Sm RESPONSE IN MRL/lpr MICE: GENE USE, CLONALITY, AND RELATIONSHIP TO THE ANTI-DNA RESPONSE. Stephen H. Clarke, Jean-Luc Davignon, Deborah D. Bloom, Philip L. Cohen, and Robert A. Eisenberg. Departments of Microbiology and Immunology and Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC 27514

The response to the ribonuclear protein antigen complex Sm is unique to systemic lupus erythematosus (SLE), but occurs in only 25% of patients. This response occurs at the same frequency in mice of the autoimmune MRL strains, and is attributed to stochastic events. To determine whether this low incidence in MRL/lpr mice is due to severe restrictions in Ig V gene rearrangement or somatic mutation we generated a series of 14 hybridomas from three mice. All were determined to be specific for the D protein of Sm. Ig sequence analysis indicates that the repertoire of $V_H/V_k$ combinations that contribute to the Sm response is large and that the degree of somatic mutation is variable. Thus, the stochastic events that govern B cell specificity do not control the incidence of the Sm response in mice. However, in individual mice the response is clonally restricted, and there is a bias in $V_H$, $J_H$, and $V_k$ use, indicating that it is antigen selected, probably by Sm itself.

Nine of the 11 clones identified express a $V_H$ or $V_k$ gene also used in the anti-DNA response. The probability that this overlap could be the result of chance occurrance is less than 0.05. Moreover, the antibodies from half of these clones bind ssDNA. These data suggest that the specificity for ssDNA and Sm are both selected, and therefore, that there is a relationship between the Sm and DNA responses of MRL/lpr mice.

**C 309** IMMUNOGLOBULIN VH AND VL GENE UTILIZATION AND STRUCTURE IN HUMAN IgG ANTI-DNA AUTOANTIBODIES FROM PATIENTS WITH SLE, Frits H.J. Gmelig-Meyling, Johan van Es, Henk Aanstoot and T. Logtenberg, Dept. of Clinical Immunology, University Hospital, Utrecht, The Netherlands.

Recently, many data have accumulated concerning the immunoglobulin heavy and light chain variable regions ($V_H$ and $V_L$ respectively), expressed in human IgM autoantibodies from healthy individuals and from patients with autoimmune diseases. However, little is known about IgG autoantibodies, which may be more relevant for pathogenesis. We have generated a small panel of IgG anti-DNA secreting, monoclonal EBV-transformed cell lines from patients with SLE. Analysis of the nucleotide sequences of the $V_H$ and $V_L$ regions of these antibodies revealed the presence of somatic mutations (as compared to the patients' own germline sequences) in both $V_H$ and $V_L$ segments. The pattern and extent of somatic mutations were strongly indicative of a process of (auto)antigen selection of these autoreactive B cells. One of the antibodies contained light chains expressing the kv325 gene element frequently associated with paraproteins having autoantibody activity, and with Ig molecules of malignant CD5-positive B cells. Also, this antibody utilized a $D_H$ segment which has been repeatedly found in multireactive, low affinity IgM anti-DNA antibodies. We conclude that pathogenic IgG anti-DNA antibodies in human SLE may arise through antigen-driven selection of somatic mutations in gene elements, some of which may have originally encoded multireactive IgM (auto)antibodies.

**C 310** DIVERSITY OF THE HUMAN FETAL ANTIBODY REPERTOIRE, Jan L. Hillson and Ina R. Oppliger, Department of Medicine, University of Washington, Seattle, WA 98195.

During the second trimester the fetal liver is a site of B-lymphopoesis, containing cells that are predominantly at pre-B and earlier stages of development. Analysis of antibody heavy chain transcripts from this population reveals preferential utilization of certain $V_H$, $D_H$ and $J_H$ gene elements (Schroeder et al, 1987, 1990). To determine the extent to which this restriction is reflected in fully-developed B cells present at the same gestational age, we generated Epstein-Barr Virus-transformed, monoclonal, antibody-producing B cell lines from fetal livers and spleens of 108 and 134 days gestation. Complete heavy chain sequences from 15 liver-derived B-cell clones resemble sequences previously reported for developing cells in several respects. Like developing cells, the clones express germline genes without evidence of somatic mutation; four $V_H$ genes are represented in both populations, including the two most $J_H$-proximal elements $V_H$15p1 and $V_H$5-1R1; and members of the $V_H$3 family predominate in both populations. However, the B cell clones appear to use a more diverse set of $V_H$ elements than is used by developing cells ($P < 0.1$). Further, $D_H$ use strikingly dissimilar ($P < 0.02$): the $J_H$-proximal element $D_H$Q52 implicated in more than 50% of transcripts from developing cells is used by none of the clones. Finally, the predominance of $J_H$4 and $J_H$3 use reported for developing cells is less marked ($P < 0.05$) in the clones. Preliminary analysis of heavy chain sequences from spleen-derived clones suggest the splenic B cell repertoire is still further diversified, in part by somatic mutation. These results indicate that EBV-immortalization samples, or induces, a population of B lymphocytes capable of expressing a more diverse antibody repertoire than that expressed by pre-B cells of the same gestational age. Intriguingly, many of the EBV-transformed B cell clones synthesize antibodies with self-specificities, with 40% binding one or more of 8 soluble and cellular self-antigens tested.

**C 311** ANALYSIS OF SELF-REACTIVE REPERTOIRE AND THE EXPRESSION OF V GENE FAMILIES IN AUTOANTIBODIES FROM TIGHT SKIN MICE, Kuppuswamy N. Kasturi, Tai Muryoi, Yuki Saitosh and Constantin A. Bona, Department of Microbiology, Mount Sinai Medical School, New York, NY 10029.

Tight Skin mouse develops cutaneous hyperplasia and histopathological alterations of skin similar to that observed in diffuse scleroderma patients. Further more, older TSK mice also show increased levels of anti-topoisomerase I autoantibodies as seen in PSS patients. The autoimmune phenomena occurring in connective tissue diseases was investigated using TSK mouse model. A large panel of hybridomas were established from TSK mice and the specificties of autoantibodies secreted by these hybridomas were studied using a panel of 14 autoantigens available in our laboratory. The results are summarized below.

Frequency of hybridomas producing self-reactive autoantibodies

| Origin | No. Studied | binding to | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | topo I | RNA Pol | CENP-B | Coll I | Coll III | Coll IV | DNA | Fc R |
| Non-stimulated lymphocytes | 615 | 4 | 12 | 0 | 3 | 0 | 0 | 71 | 0 |
| LPS stimulated lymphocytes | 444 | 11 | NT | 0 | 0 | 2 | 0 | NT | 3 |

V gene repertoire analysis were done by determining the frequency of $V_h$ and $V_k$ genes expressed in total spleen cell population as well as among hybridomas secreting autoantibodies. Analysis of $V_h$ gene usage in autoantibodies show that majority of them are encoded by J558 gene family. Total V gene repertoire analysis is in progress.

**C 312** DEVELOPMENT OF B CELL SUBSETS AND ANTIBODY REPERTOIRES, John F. Kearney, Department of Microbiology, Division of Developmental and Clinical Immunology, University of Alabama at Birmingham, Birmingham, AL 35294

Autoreactivity of the early B cell repertoire appears to be a hallmark of this stage of development. The early B cell repertoire is also characterized by restricted use of $V_H$ (and $V_L$) genes and a large proportion of early B cells in man and mice belong to the CD5 subset. We have investigated the development of this subset of B cells from various embryonic sources in BALB/c mice and have shown that both 13 day fetal liver and omentum reconstituted $CD5^+$ B cells in the peritoneal cavity. Furthermore, we have extended our analysis of the early B cell repertoire to include the C57/BL strain of mouse. In our previous work with BALB/c mice we demonstrated early development of multireactive and idiotype directed B cell specificities. Similar findings have been made in C57BL/6 with additional isolation of clones of anti-idiotypic cells from perinatal mice towards idiotypes such as $NP^b$ which are characteristically expressed in C57BL but not BALB/c. In addition, we have also detected a high frequency of clones reactive with normal thymocytes and subsets of peripheral T cells. These studies extend our observations and further support our proposal that the development of the early B cell repertoire is dominated by CD5 positive cells whose idiotype and self-reactive antibodies are important for the establishment of the adult B cell repertoire. (Supported by NIH Grants AI30979, AI14782 and CA13148)

**C 313** CROSS-REACTIVITY OF AUTOIMMUNE B CELLS FROM LUPUS-PRONE MICE, Dennis M. Klinman, Division of Virology, CBER, FDA. Bethesda, MD, 20892.

An assay was developed to simultaneously analyze the isotype, antigenic specificity and cross-reactivity of single in vivo activated B cells. Up to 22% of IgM secreting lymphocytes from lupus prone mice were found to spontaneously secrete antigenically cross-reactive antibodies. This was not significantly different from the cross-reactivity of IgM secreting cells derived from normal Balb/C and DBA/2 mice. However IgM anti-DNA (but not IgM anti-TNP) secreting cells from autoimmune NZB/W mice were approximately 50% more cross-reactive than cells of the same specificity of DBA/2 origin.

By comparison, less than 0.1% of IgG anti-TNP producing lymphocytes from TNP-stimulated mice were antigenically cross-reactive. This is consistent with antigen selecting for the expansion of high affinity clones expressing low self/cross-reactivity. In contrast, autoreactive B cells from lupus-prone mice produced IgG antibodies whose cross-reactivity ranged from 0.8 - 6.5%. These findings indicate that i) a significant fraction of physiologically activated IgM secreting B cells in normal and autoimmune mice are cross-reactive, ii) immunization results in the selective proliferation of non-cross-reactive IgG secreting cells and iii) lymphocytes secreting IgG autoantibodies in lupus prone mice are more cross-reactive than would be expected if their activation was entirely autoantigen driven. Calculations suggest that antigen non-specific stimulation (polyclonal activation) may be responsible for up to one-third of the B cell activation characteristic of adult murine lupus.

**C 314** CHARACTERIZATION AND SPECIFICITY OF ANTI-LYMPHOCYTE MONOCLONAL ANTIBODIES FROM PERINATAL MICE, A. Lehuen, R. Vozab and J.F. Kearney, Dept. of Microbiol., Div. Develop. Clin. Immunol., UAB, Birmingham, AL.

Previous studies have shown that the early B cells in mouse and man are different from those of adult with respect to their $V_H$ usage, the specificity of Ig produced, their surface phenotype, and their function in determining the adult B cell repertoire. In this work, we have investigated antibodies of a new specificity, anti-lymphocyte antibodies. For this purpose, we have screened hybridomas obtained by fusions of perinatal liver and splenic B cells from BALB/c and C57BL/6 mice, by immunofluorescent staining of thymocytes. Anti-lymphocyte cell mAbs (all IgM isotype) were detected at a high frequency, 11% (28/262). Three mAbs react differently with thymocytes from mice of different ages: 88A8 binds more thymocytes from adult (82%), than from newborn (38%), and fetal mice (5%), whereas 88C66 and 15-2 bind 80% of fetal thymocytes but only 20% of adult thymocytes. The heterogeneity of these anti-lymphocyte mAbs was also demonstrated by the sensitivity of their specific target to enzymatic treatments. The epitopes recognized by 6 mAbs are sensitive to trypsin and pronase, 2 mAbs appear to recognize carbohydrate moieties since their binding is inhibited by neuraminidase treatment, and 11 mAb recognize molecules sensitive to PI-PLC. Among the 7 mAbs which bind mature T cells, 6 recognize the same subsets (60% of $T4^+$ and 80% of $T8^+$ cells). Inhibition experiments suggest that these 6 mAbs share the same molecular specificity. Furthermore, 3 mAbs immunoprecipitate the same thymocyte surface protein of 100 kD (70 kD under reducing conditions). In conclusion, anti-lymphocyte antibodies are expressed at high frequencies by perinatal B cells, have heterogeneous specificities, and 6 of them appear to recognize the same molecule of 100/70 kD. (NIH Grants AI30879, AI14782 and CA13148)

**C 315** SELF REPERTOIRE OF TIGHT SKIN MOUSE: IMMUNOCHEMICAL AND MOLECULAR CHARACTERIZATION OF ANTI-TOPOISOMERASE I AUTOANTIBODIES, Tai Muryoi, Kuppuswamy N. Kasturi, Osamu Usuba, Yukiko Saitoh, Martin J. Kafina, Jerome S. Perlish¶ Raul Fleischmajor* and Constantin A. Bona, Department of Microbiology and Dermatology¶ Mount Sinai School of Medicine, New York, NY 10029
Tight skin (TSK) mice develope a cutaneous hyperplasia accompanied by histopathological alterations of skin and collagen metabolism similar to those described in human scleroderma. Diffuse scleroderma, the most severe form of progressive systemic sclerosis, is associated with the production of autoantibodies (autoabs) specific for Scl 70 antigen (topoisomerase I). Our studies show that there is a raise in serum anti-topoisomerase I (topo I) autoabs in old TSK mice. The monoclonal abs isolated from TSK mice bind to epitopes interacting with autoabs from scleroderma patients. An important fraction of TSK anti-topo I abs and serum Ig from old TSK mice bear a cross reactive idiotype (Id) recognized by a syngeneic monoclonal anti-Id ab obtained from 2 month-old TSK mice. Analysis of V gene usage of anti-topo I monoclonal abs showed that majority of them are encoded by $V_H$ genes derived from $V_H$ J558 family and are paired with $V_K$ genes from various families in a stochastic manner.

**C 316** MOLECULAR CHARACTERIZATION OF THE HEAVY CHAIN OF HUMAN AUTOANTIBODIES DERIVED FROM PATIENTS WITH AUTOIMMUNE DISEASES. Virginia Pascual and J. Donald Capra. Department of Microbiology. University of Texas Southwestern Medical School. Dallas, TX 75235.
Using oligonucleotides specific for each of the six human $V_H$ families, we have amplified and sequenced the $V_H$ regions expressed in 21 human autoantibodies. They were derived from EBV transformed B cell lines from patients with a diverse array of autoimmune diseases, and belonged to the IgM and IgG isotypes. We have found that a small repertoire of $V_H$ genes from the $V_H$III, IV and V families gives rise to the vast majority of autoimmune heavy chains. While some specificities, like Rheumatoid Factors (RF) and anti-acetyl choline receptor (anti-AChR) antibodies, do not show restriction in their $V_H$ family usage , some others like the cold agglutinins express a single $V_H$ gene segment ($V_H4$-21) which also seems to be the basis for the idiotypic cross-reactivity found in this particular system. Some monospecific autoantibodies of the IgM isotype use $V_H$ genes related to the fetal repertoire in nearly germ line configuration, whereas most of the monospecific IgG autoantibodies seem to be heavily mutated. We did not find restriction in the usage of D or $J_H$ segments among autoantibodies with the same specificity. Our study suggests that a limited number of germ line $V_H$ genes is giving rise to the repertoire of autoantibody heavy chains.

**C 317** INFLUENCE OF SELF-REACTIVITY ON LIGHT CHAIN REPERTOIRE IN ANTI-DNA HEAVY CHAIN TRANSGENIC MICE. Marko Z. Radic, Jan Erikson, Jim Mackle, and Martin Weigert, Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, PA 19111. The expression of $V_H$3H9, a functionally rearranged anti-DNA H chain transgene, in non-autoimmune mice is regulated. One aspect of that regulation is revealed in hybridoma lines obtained after <u>in vivo</u> LPS treatment of $V_H$3H9 mice. Two types of hybrids were recovered, one specific for ssDNA, the other incapable of DNA binding, despite their use of the transgene. Sequence analysis of 24 L chains revealed a limited number of $V_L$ genes that recurred in independent isolates. These results suggest that most germline encoded L chains can not alter the specificity of $V_H$3H9 for dsDNA and therefore do not contribute to our sample. By ways of <u>in vitro</u> mutagenesis we have constructed $V_H$3H9-derived H chains with increased affinity for dsDNA. The extent of their specificity dominance will be tested by transfections. H chains that are found to give rise to dsDNA binding when combined with this initial set of light chains will be used for the construction of additional transgenic lines in hopes of further limiting the L chain repertoire.

**C 318** A NOVEL ASSAY TO DETECT SOMATIC MUTATION IN IMMUNOGLOBULIN VARIABLE GENES, Asad Umar, Peter Schweitzer, and Pat Gearhart, Department of Biochemistry, The Johns Hopkins University School of Hygiene and Public Health, Baltimore, MD 21205

To investigate the role of *cis* sequences in targeting mutation to rearranged immunoglobulin variable genes, we developed a genetic assay to easily detect mutation. The assay features a reporter gene, a bacterial suppressor tRNA gene, that permits visual identification of mutants in procaryotes. Plasmids containing a reporter and kappa variable gene were injected into fertilized eggs to make transgenic mice, and mutation was induced by immunizing the mice. The plasmid shuttle vectors were then rescued and transformed into an indicator strain of bacteria. The integrity of the marker gene was monitored by colony color in the presence of X-Gal and IPTG, and sequenced to confirm mutation. Three constructs were analyzed in three transgenic lines: pVxSC, which had the tRNA gene placed proximal to the variable gene; pVxCS, which had the marker gene located distal to the variable gene, and pP$^-$VSC, which had the reporter gene adjacent to the variable gene but had a deletion of the immunoglobulin promoter region. Transcription of the transgenes was measured by nuclear run-off assays; the pVxSC and pVxCS lines had transcription of the variable gene, but pP$^-$VSC did not. Mutation was detected at a low frequency in DNA from B cells of the pVxSC line, but not in DNA from the tails of these mice. Sequence analysis of the mutated transgenes revealed that mutations were predominantly single nucleotide deletions in the tRNA gene. No mutation was seen in DNA from B cells of the pVxCS or pP-VSC lines. Thus, mutation in the reporter gene (i) is tissue-specific, (ii) is dependent on its position next to the variable gene, and (iii) requires transcription of the variable gene.

**C 319** DIVERSITY OF EXPRESSED ANTIBODIES (Abs) OF THE HUMAN $V_H6$ FAMILY. William S. Varade, Elides Marin, Ann Kittelberger, Richard A. Insel. University of Rochester, Department of Pediatrics, Rochester, NY 14642.

To understand the development and selection of somatic mutations (SM) in germline (GL) immunoglobulin variable region genes encoding self-reactive Abs, we have studied the nucleotide (nt) and deduced amino acid (aa) sequences of IgG transcripts containing the single member $V_H$ gene family, $V_H6$. The $V_H6$ gene, the most $J_H$ proximal human $V_H$ gene, has been found to be preferentially expressed early in ontogeny, encodes in its GL form Ab that binds ssDNA, and has not been shown to encode IgG Ab or myeloma proteins. cDNA prepared from 2 human spleens was amplified by PCR using $V_H6$- and IgG-specific oligo primers. The amplified DNA was cloned into an M13 vector, and clones were selected with an internal $V_H6$-specific probe and sequenced. Of 25 clones sequenced, the nt sequences of 6 were redundant. There were 142 different nt substitutions in the $V_H$ segments of the 20 distinct clones. In 279 nt in the $V_H$ segment, 49 nt positions had SM in multiple clones. Five sequences derived from the same spleen shared nearly identical $V_H$ SM, although derived from 2 independent gene rearrangement events with different D and $J_H$. The SM in CDR1, CDR2, and FR3 were usually associated with aa replacement. Fourteen distinct D sequences were observed. There was bias in use of $J_H$ segments, with 1-$J_H$1, 6-$J_H$3, 6-$J_H$4, and 6-$J_H$6.

Thus, human $V_H6$ was used in the expressed repertoire with frequent SM and aa replacement, parallel evolution of SM in distinct clones was observed, and while SM leading to aa replacement were probably the result of antigen selection, identical silent nt SM in distinct clones may indicate intrinsically unstable "hot spots" of SM.

*Molecular Mimicry of Self and Foreign Antigens*

**C 320** **MOLECULAR MIMICRY, AUTOIMMUNITY AND INFECTION,** Susan M. Antone and Madeleine W. Cunningham, Department of Microbiology and Immunology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190

Antibodies crossreactive with the streptococcal M protein and myosin have been investigated for their ability to cause damage to heart cells in culture and for their association with disease. In addition to myosin, other alpha-helical proteins have been involved in these crossreactions. The heart cell surface protein laminin was associated with antibody-mediated cytotoxicity. These antibodies found in acute rheumatic fever shared an idiotype with similar antibodies in systemic lupus erythematosus, sjogren's syndrome, and post-streptococcal glomerulonephritis. Furthermore, these streptococcal antibodies neutralized certain viruses and may be a first line of defense against infectious agents. However, antibodies which react with cell surface or matrix proteins may be inflammatory in the host and initiate rheumatic or autoimmune disease.

**C 321** MOLECULAR RECOGNITION OF A SELF MIMICKING ANTIGENIC DETERMINANT. Vatsala Bhardwaj, Vipin Kumar*, H. Mario Geysen#, Suzanne J. Horvath*, Leroy Hood* and Eli Sercarz. Department of Microbiology and Molecular Genetics, UCLA, 405 Hilgard Ave., Los Angeles, CA 90024-1489; #Coselco Mimotopes Pty Ltd., Cnr Duerdin and Martin Streets, Clayton, Victoria 3168, Australia. *Division of Biology, Caltech, Pasadena, CA 91125.

Molecular mimicry or immunological crossreactivity has been proposed as an initiating factor for several autoimmune disease. It also plays a role in the evasion of the immune response by microorganisms and parasites and is a major mechanism involved in the positive selection that shapes the T cell repertoire during ontogeny. We have established a prototypic model of "mimicry" at the clonal level in the MBP system, whereby T cell clones and hybridomas utilizing different $V\alpha$ and $V\beta$, specific for the immunodominant encephalitogenic determinant Ac1-9/IA$^u$ could be activated by a set of mouse MBP chimeric peptides [CP's 7-11:35-43, 7-11:35-44 and 7-11:35-47]. As with Ac1-9 this response is A$^u$ restricted. A reciprocal phenomenon was observed when B10.PL mice were immunized with the chimeric peptides, and the response recalled with Ac1-9 in vitro. T cell hybridomas derived from a chimeric peptide-induced, Ac1-9 reactive T cell line, could react with both Ac1-9 and the CP's, and not with 35-47 or 9-11:35-47. Interestingly, these clones also recognize a set of variants of Ac1-9 at residue "4" in a manner identical to the T cells derived from Ac1-9 immunized mice. Preliminary experiments using a replacement net at residues 7-11 in 7-11:35-43 indicate that substitution of N-terminal 7, 8 or 9 has little or no effect on T cell activation, in parallel to the finding with Ac1-9 where the C-terminal residues, 7, 8 and 9, comprise the non-specific "tail" amino acids. Experiments are underway to define the residues that are recognized by the T cells on the mimic peptide. Our results imply that effective T cell mimicry can occur in the absence of obvious sequence homology and cannot be predicted solely on the basis of the primary amino acid sequence. Sponsored in part by grants from the NIH and the Multiple Sclerosis Society to E.S.

**C 322** IDENTIFICATION OF A MURINE DNA RECEPTOR AND ITS ASSOCIATION WITH AUTOIMMUNITY. S.H. Hefeneider, R.M. Bennett, S.L. McCoy, J.I. Morton, A.C. Bakke, K. Cornell. VA Medical Center and The Oregon Health Sciences Univ., Portland, OR 97201

Autoimmunity to a 28-29 kD cell-surface receptor for DNA has previously been described in patients with SLE and related autoimmune diseases. We now report the existence of both a murine DNA receptor and autoantibodies to this receptor in lupus-prone mice. $^3$H-DNA binding to murine spleen and kidney cells was a saturable phenomenon which was inhibited by excess cold DNA and trypsinization. Cell-surface reactivity of DNA was confirmed by flow cytometry. Biotin-labeled DNA bound >60% of spleen cells at two hours, using PE-avidin. To determine whether the murine DNA receptor served as a target antigen for the development of autoantibodies in lupus mice, sera from lupus strains (MRL lpr/lpr, MRL +/+, BXSB), and normal mice (BALB/c, C3H.SW) were tested. It was found that only sera from lupus strains when incubated with DNase treated mouse spleen cells had inhibitory activity, suggesting that the DNA receptor was a target antigen for autoimmunity. Characterization of the serum inhibitory component showed that inhibition was mediated by an IgM autoantibody. Time course studies demonstrated that young female MRL/lpr (four week old) mice lacked detectable anti-receptor activity. At 8-10 weeks maximal anti-receptor activity (25%-35%) was observed; this occurred prior to the development of detectable anti-nuclear antibodies. With the appearance of overt disease and anti-DNA antibodies, anti-receptor activity was undetectable. These data showing the development of anti-receptor activity prior to the appearance of anti-DNA antibodies are consistent with the hypothesis that DNA receptor antibodies have anti-idiotypes displaying anti-receptor activity. This murine model should be useful in defining the role of DNA receptor idiotypic mimicry as an explanation for the phenomenon of anti-DNA antibodies.

**C 323** SUSCEPTIBILITY FACTORS INVOLVED IN THE PATHOGENESIS OF YERSINIA-INDUCED ARTHRITIS, Kristine L. Hogen, Cheryl L. Nickerson-Nutter, Harvinder S. Luthra, and Chella S. David, Departments of Immunology and Rheumatology, Mayo Clinic, Rochester, MN 55905.

Following an intravenous infection with a sublethal dose of Yersinia enterocolitica 0:8 WA, DBA/2 (H-2$^d$) mice developed arthritis, while other strains including C57BL/6 (H-2$^b$), DBA/1 (H-2$^q$) and CBA (H-2$^k$) were negative (Yong et al. 1988). To determine which genetic factors may be involved in the susceptibility to Yersinia induced arthritis (YIA), several strains of mice were inoculated intravenously with $10^7$ to $10^8$ Y. enterocolitica 0:8 WA organisms grown on magnesium oxalate agar plates to remove the virulence plasmid. We found that BALB/c and CBA mice were susceptible to YIA. In addition, B10.D2 (H-2$^d$) mice and several B10 recombinant strains were susceptible to YIA as shown in the table:

| | MHC | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Strain | K | A | $E_\beta$ | $E_\alpha$ | S | DL | IE | TCR Deletion | Athritis | % |
| BALB/c | d | d | d | d | d | dd | + | ± | 18/44 | 41 |
| B10.D2 | d | d | d | d | d | dd | + | ± | 6/23 | 26 |
| B10.RDD | d | d | d | b | b | dd | - | - | 2/13 | 15 |
| B10.RKD6 | k | k | k/q | q | q | dd | - | - | 6/15 | 40 |
| CBA | k | k | k | k | k | kk | + | + | 15/21 | 71 |

These results implicate a role for H-2 genes in addition to other parameters in the susceptibility to YIA. Preliminary studies also suggest a role for T cell receptor $V_\beta$ genes. Analyses of several recombinant strains and transgenic mice is currently underway and will be presented.

**C 324** **IMMUNE RESPONSIVENESS TO A 65kD HEAT SHOCK PROTEIN, GroEL, IS SIGNIFICANTLY AFFECTED BY NON-MAJOR HISTOCOMPATIBILITY COMPLEX GENES,** Christopher J. Krco, Thomas G. Beito, David Yu and Chella S. David, Department of Immunology, Mayo Clinic, Rochester, MN 55905, and Department of Medicine, UCLA, Los Angeles, CA.

Immunity to 65kD heat shock proteins from mycobacteria have been demonstrated in patients with rheumatoid arthritis and inflammatory conditions. In some instances both T cell and B cell autoreactivity has been reported. However, it has been reported that the γδ T cell receptor is associated with restricted reactivity to hsp65 proteins. However, there is little information concerning the characterization of immune response genes that affect immunity to hsp65 as well as the identity of genes that might confer the capacity for autoimmunity to self heat shock proteins. We have tested a number of inbred mouse strains for T cell reactivity to the GroEL form of hsp65. We have found that B10 genetic background is associated with nonresponsiveness to GroEL (<2 U/ml of IL-2) upon challenge irrespective of H-2 haplotype (b,d,k,q,s). However, the same corresponding H-2 haplotypes expressed on a non-B10 genetic background (e.g. DBA/1 or C3H) were associated with strong reactivity to GroEL (>7.5 U/ml IL-2). In an attempt to identify the nature of the non-MHC genes, the responses of B10.D2, BALB/c and BALB.D2-Mls$^a$ were compared. While B10.D2 mice were unresponsive (1.5 U/ml IL-2), BALB/c mice were very responsive (11.2 U/ml IL-2). However, BALB.D2-Mls$^a$ congenic mice were intermediate to poor responders (IL-2 release of 50% of that of BALB/c) and (BALB/c x BALB.D2-Mls$^a$)$F_1$ mice were also unresponsive. BALB/c and BALB.D2.Mls$^a$ differ in Mls-1 locus and the data is consistent with the notion that Mls-1 may affect the repertoire for reactivity to heat shock proteins.

**C 325** AMINO ACID SEQUENCES SHARED BY A DEFINED REGION OF HLA B27 α-CHAIN AND ARTHRITIS TRIGGERING MICROBES ARE NOT RECOGNIZED BY IMMUNE SYSTEM OF PATIENTS WITH HLA B27 ASSOCIATED DISEASES, Riitta Lahesmaa, Mikael Skurnik, Martti Vaara, Marjatta Leirisalo-Repo, Martti Nissilä, Kaisa Granfors and Paavo Toivanen, Departments of Medical Microbiology and Medicine, Turku University, Turku, Dept. of Bacteriology and Immunology, and II Dept. of Medicine, Helsinki University, Helsinki, and Rheumatism Foundation Hospital, Heinola, Finland.

We describe here two new examples of amino acid homology between HLA B27 and microbes triggering HLA B27-associated diseases. The *yadA* gene of the virulence plasmid of *Yersiniae* codes for an outer membrane protein, Yad1, which shares a linear tetrapeptide with HLA B27. Moreover, a kationic outer membrane protein OmpH of *Salmonella typhimurium* shares homology of 5 amino acids with HLA B27. Interestingly, the area of identity in all examples of this molecular mimicry described so far (*Klebsiella pneomoniae* nitrogenase, outer membrane protein of *Salmonella* and plasmid encoded proteins of *Shigella* and *Yersinia*), is located in the same place of HLA B27 molecule; between amino acids 70-78. Based on crystals of other class I molecules, HLA A2 and HLA Aw68, these amino acids are putatively located in variable region of $\alpha_1$ domain α-helix.

To evaluate the significance of this molecular mimicry in the pathogenesis of reactive arthritis or Reiter's disease and ankylosing spondylitis, we studied immune responses against synthetic peptides based on those portions of Yad1 and OmpH, which included the HLA B27 homologous areas. Neither of the two peptides nor their control peptides were able to stimulate bulk lymphocyte cultures or T cell lines of HLA B27+ patients with *Yersinia*- or *Salmonella*-triggered reactive arthritis. Antibodies against the synthetic peptides were observed in one third of the patients, and also in the HLA B27- patients with recent Salmonella or Yersinia infection without postinfectious complications. Studies with control peptides revealed that the HLA B27 homologous portions of the synthetic peptides were not responsible for antibody stimulation.

Our results including the appropriate control patients and panel of control peptides challenge the much-reviewed concept regarding a direct role of cross-reacting antibodies in the pathogenesis of HLA B27 associated diseases.

**C 326** CELLULAR IMMUNE RESPONSE IN MICE WITH POST-VIRAL MYOCARDITIS: RECOGNITION OF VIRAL ANTIGENS AND AUTOANTIGENS, D.A. Neumann, S.M. Wulff, C. Frondoza, A. Herskowitz & N.R. Rose. Department of Immunology & Infectious Diseases, School of Hygiene & Public Health, The Johns Hopkins University, Baltimore, MD 21205

A/J mice develop chronic autoimmune myocarditis following infection with coxsackievirus B3 (CB3). This inflammatory heart disease is characterized by circulating heart-reactive antibodies, principally recognizing cardiac myosin, and by extensive IgG deposition within the myocardium. C57BL/10.A (B10.A) mice do not exhibit this autoimmune sequela to CB3 infection. The co-occurrence of inflammation and tissue-specific autoantibodies suggests that auto-reactive T cells may contribute to myocardial pathology. To test this hypothesis spleen cells collected from infected and control A/J and B10.A mice at various times after treatment were stimulated in vitro with virus, cardiac myosin, and other antigens. Cells from CB3-infected A/J mice responded to stimulation by CB3 (stimulation index [SI] range: 2 to 10) and to myosin (SI: 2 to 5). Cells from control A/J mice did not respond appreciably to any of the antigens tested. Cells from infected B10.A mice responded to CB3 stimulation (SI: 2 to 5), but not to cardiac myosin. These results suggest that the induction of autoimmune myocarditis in A/J, but not B10.A, mice may be related to T cell recognition of myosin. It is possible that T cell responsiveness to cardiac myosin reflects recognition of epitopes shared by CB3 and myosin; the development of T cell clones should help to clarify this issue. (Supported in part by NIH grant number R01 HL33878)

**C 327** MOLECULAR MIMICRY: A MECHANISM FOR THE INDUCTION OF AUTOIMMUNITY. Bellur S. Prabhakar, Kirk W. Beisel* and J. Srinivasappa**, Department of Microbiology. The University of Texas Medical Branch at Galveston, TX 77550, *University of Nebraska, Omaha, NE 68198 and **Oxford Laboratories, MN.
Earlier we screened over six hundred monoclonal antiviral antibodies and showed that approximately 4 percent of those were also capable of reacting with self antigens. To understand the basis for cross reactivity one of the antibodies was studied in detail. A neutralizing anti-cosackievirus $B_4(CB_4)$ antibody (356-1) which reacted specifically with the heart was chosen. Immunohistochemical and fractionation studies revealed that the reactivity was primarily against the murine cardiac heavy myosin. The monoclonal antibody 356-1 was then used to screen a λ gtll mouse cardiac expression library. Fourteen clones that reacted strongly were analyzed and inserts were amplified using the PCR. These inserts hybridized to a 6Kb message in RNA from heart but not liver. Sequence analysis of these clones revealed that they encoded for approximately 1/3 of the amino terminal end of the light meromyosin. Comparison with known sequences of rat alpha and beta cardiac heavy chains showed that the sequences from the alpha chain represented the putative cross-reactive autoantigenic epitope. This putative antigenic site is represented by amino acid residues 1631-1647 of the alpha cardiac myosin. These and other studies imply that molecular mimicry is one mechanism by which autoimmunity could develop.

**C 328** ANTIGEN MIMICRY: HUMAN CYTOTOXIC T CELLS RECOGNIZE AUTOLOGOUS EPSTEIN BARR VIRUS TRANSFORMED LYMPHOBLASTOID CELL LINES AND ALLOGENEIC CLASS I MOLECULES PRESENTING SELF PEPTIDES. Schendel, D.J., Nößner, E., Nelson, P.J., Königes, A., Pullen, L., and Multhoff, G., Institute of Immunology, University of Munich, Goethestrasse 31, D 8000 Munich 2, Federal Republic of Germany
Since the major function of T cells is to recognize foreign peptides presented by autologous MHC molecules the fact that many T cells recognize alloantigens remains enigmatic. Allorecognition may occur because of molecular mimicry if allogeneic MHC molecules display determinants that look like complexes of nominal peptides and autologous MHC molecules. Whether a self-peptide associates with the MHC allotype is a matter of recent speculation. If so, it is easier to envision how one TCR mediates both MHC restricted recognition and allorecognition. We have identified CTL that recognize LCL which are self-restricted by the HLA-C locus. It has been presumed that HLA-C molecules can present peptides, but the importance of HLA-C restriction remains to be determined. For some individuals these CTL are the prevalent effectors against EBV-transformed LCL. The EBV associated component is undefined but active viral replication may be important to express the target antigen. These CTL also recognize some allogeneic class I molecules. Population and family studies have shown that the alloantigen is a complex formed by the interaction of two MHC linked genes. One component is a class I molecule, encoded by the HLA-B locus; the other seems to be a self-peptide. These data support of the peptide model of allorecognition and suggest that a structural equivalence is created by the association of *foreign peptides with self-MHC* and *self-peptides with allo-MHC*. T cell receptor antibodies specific for these CTL show that they are a dominant peripheral population in some donors which may be maintained at high levels to control outgrowth of EBV transformed cells in vivo.

**C 329** A SCLERODERMA MONOCLONAL AUTOANTIBODY CROSSREACTS WITH HERPES SIMPLEX VIRUS RIBONUCLEOTIDE REDUCTASE AND CELL AUTOANTIGENS POTENTIALLY IDENTIFIED AS HEAT SHOCK PROTEINS. P.S. Thomas, Oklahoma Medical Research Foundation, 825 N.E. 13th, Oklahoma City, OK 73104.
A human hybridoma (328) was constructed by fusing peripheral blood lymphocytes of a scleroderma patient with a human-mouse heterohybrid cell line (F6). The 328 line produces an IgG2,k monoclonal antibody (mab) which reacts with a nuclear cell antigen of exponentially growing HEp-2 cells, as well as a cytoplasmic antigen under some conditions. The mab also precipitates a ~140 kDa polypeptide from Herpes Simplex Type 1 (HSV-1) and Type 2 (HSV-2) infected cells. This viral polypeptide is the viral enzyme, ribonucleotide reductase. The reaction of the 328 mab with HSV-1 infected cells is removed by prior treatment with a mouse mab which specifically precipitates this enzyme. The 328 mab strongly precipitates the HSV-1 enzyme, weakly precipitates the HSV-2 enzyme, and does not react with the ribonucleotide reductases of the related herpes viruses, VZV, CMV, and EBV. This mab also does not react with the ribonucleotide reductase enzymes of mammalian cells, vaccinia virus, or *E. coli*. These data identify a potential epitope of the HSV ribonucleotide reductase that is reactive with mab 328. This potential epitope (a pentapeptide sequence) is shared between the heat shock protein 70 (HSP70) and Herpes Simplex Virus (Type 1,2) ribonucleotide reductase.
The patient serum has antibodies to the major glycoproteins of HSV-1 and not HSV-2, suggesting that HSV-1 might have elicited this response. Studies in progress indicate that scleroderma patients have an increased incidence of antibody to HSV-1 (77%) compared to a normal population (41%), with $p < 0.001$ by the Fischer exact test. The possible role of HSV-1 in scleroderma is being investigated further.

**C 330** CYTOTOXIC T LYMPHOCYTE RECOGNITION OF ADENOVIRUS E1A IS LIMITED BY SELF-TOLERANCE, Ralph A. Tripp and Linda R. Gooding, Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, GA 30322

The anti-viral CTL response is characterized by profound selection toward a few dominant epitopes. The mechanism of epitope dominance is currently unknown. In the present study, mapping of E1A epitopes recognized by anti-adenovirus 5 (Ad5) CTL derived from two H-$2^b$ strains, C57BL/6J (B6) and C57BL/10SnJ (B10), revealed that B6 CTL fail to recognize N-terminal E1A epitope(s) which are recognized by B10. To determine whether the response to N-terminal epitope(s) is a function of T cell receptor (TcR) gene availability or a hole in the CTL repertoire induced by tolerance to a self protein of B6 but not B10, (B6xB10)$F_1$ CTL responses were examined. $F_1$ mice fail to recognize N-terminal epitope(s) indicating self-tolerance as the mechanism underlying B6 non-responsiveness. CTL responses of (B6xB10)$F_2$ mice further indicate that tolerance is due to a single gene.

**C 331** ***T CELL CLONES ILLUMINATE THE ROLE OF HSP65 IN EXPERIMENTAL AUTOIMMUNE ARTHRITIS***

***W. van Eden, C.J.P. Boog, E.J. Hogervorst, M.H.M. Wauben, R. van der Zee*, J.D.A. van Embden*, I.R. Cohen*****

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***T and B cells recognizing self antigens can be easily cloned from healthy individuals. The issue is how potentially autoaggressive cells can become activated and how the immune system can achieve their safe containment. Autoimmune disease can be evoked by immunization with autoantigens that feature antigenic relationships with self antigens. In both situations transfer of disease has been shown with cloned T cells. In addition, specific control of disease using the same cloned T cells. Adjuvant arthritis has been illustrative in these respects. By means of T cell cloning, a 65KD heat shock protein was identified as a crucial antigen in the disease. Immunization with this antigen has been found to affect disease. Immunization with this antigen has been found to affect disease, including forms elicited without mycobacterial immunization. Since the same 65KD antigen may be crucial in human chronic arthritis as well, it is possible that extrapolation of the experimental findings to the human situation will help the development of specific means, either T cells of antigens, to control human autoimmune arthritis.***

**C 332** SELF-TOLERANCE CAN CRIPPLE THE IMMUNE SYSTEM, Damir Vidovic[1,2] and Catherina Servis[3], [1] Basel Institute for Immunology, CH-4005 Basel, [2]Central Research Units, F. Hoffmann-La Roche Ltd. CH-4002 Basel, [3] Friedrich Miescher-Institut, CH 4002 Basel, Switzerland.

Two sets of genes control the immune response of H-$2^d$ mice to the synthetic antigen GT. One set involves class II MHC loci encoding an $A^d$ product that serves as a recognition context to GT-reactive helper T cells. The other one is a background gene, the product of which, in association with the same MHC-restricting element, mimics the GT/$A^d$ complex. Mice expressing the GT-mimicking background encoded structure (Im$g^t$), which is preferentially displayed on B lymphoblasts, do not respond to GT as a consequence of self-tolerance. On the other hand, elimination of cells bearing Im$g^t$ renders these mice responsive to GT. Analysis of expression and structure of Im$g^t$ reveals that Im$g^t$ is probably not identical to GT, but resembles it in the way it forms complexes with $A^d$ molecules of MHC.

*T Cell Tolerance Mechanisms II*

**C 333** THE EFFECTS OF CYCLOSPORIN A AND FK506 ON THYMOCYTE NEGATIVE SELECTION IN VITRO. J. Scott Cairns, Jeffrey A. Walker, Dana Banks, and Susan A. McCarthy*. Pittsburgh Cancer Institute and Departments of Pathology and Surgery*, University of Pittsburgh. Pittsburgh, PA 15213

The processes of negative selection of immature thymic T cells and antigenic stimulation of mature T cells appear to share several features. Both processes involve engagement of the T cell antigen receptor (TcR)/CD3 complex, and both are initially accompanied by a rise in intracellular calcium levels. However, these early activation events, which lead to the activation and clonal expansion of mature T cells, result in the negative selection via apoptotic cell death of immature thymocytes. The mechanism by which a similar signal can induce different responses in T cells at different stages of their development is unclear. We are therefore investigating the intracellular signals involved in the process of negative selection of immature thymocytes. Our models of negative selection include the in vitro stimulation of thymocytes with either cross-linked anti-CD3 mAb, or calcium ionophore, which mimics one component of the intracellular cascade initiated by TcR/CD3 engagement. Both of these stimuli result in the death of a subpopulation of $CD4^+8^+$, double positive thymocytes. Our results demonstrate that, in contrast to mature T cell activation, thymocyte apoptosis induced by either of these stimuli is not inhibited by the immunosuppressive drugs cyclosporin A (CsA) and FK506. These results suggest that the responses of immature and mature T cells to TcR engagement diverge before a CsA/FK506-sensitive step. These results further suggest that the previously reported ability of CsA to inhibit negative selection when administered in vivo reflects an effect of CsA on thymic accessory cells. To test this hypothesis, we are currently examining accessory cell dependent in vitro models of negative selection for their sensitivity to CsA/FK506.

**C 334** LONG-TERM ALLOGRAFT ACCEPTANCE IN THE ABSENCE OF CLONAL DELETION/ANERGY, Marilyne Coulombe, Kevin J. Lafferty, Ronald G. Gill, Barbara Davis Center for Childhood Diabetes, University of Colorado Health Sciences Center, 4200 East 9th Ave, Denver, CO, 80262

Long-term acceptance of allografts in immunocompetent adult animals is often equated with the elimination/inactivation of donor-reactive T cells. We have challenged this notion by examining the status of host anti-donor reactivity in animals bearing long-term pancreatic islet allografts. Experimentally-induced diabetic BALB/c ($H\text{-}2^d$) mice were grafted with C57Bl/6 ($H\text{-}2^b$) islets which had been cultured in 95% $O_2$ to eliminate tissue immunogenicity. Such grafts survive and reverse diabetes indefinitely (>100 days). During the immediate post-transplant period, the established islet graft is vulnerable to rejection through immunizing the recipient with donor-type spleen cells. With the passage of time, however, recipient animals become progessively resistant to immunization such that, by 120 days post-grafting, all animals resist graft rejection. Despite resisting the spleen cell challenge, the majority of these animals were capable of rejecting secondary donor-type thyroid grafts without rejecting the primary islet graft. Further, these long-term allograft recipients demonstrated normal anti-donor reactivity in vitro as assessed by CTL generation and lymphokine production (IL-2,IL-3). These results demonstrated that protection of the primary islet graft could occur without gross impairment of donor reactivity either in vivo or in vitro. We conclude that protection of allografts from rejection *can* occur without evidence of clonal elimination/anergy.

**C 335** MOLECULAR MECHANISMS OF T-CELL ANERGY, Cindy Go and Jim Miller, Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL 60637

Th1 cells are activated to produce lymphokines when stimulated by a specific antigen/MHC complex. This complex must be presented by a conventional antigen presenting cell (APC), such as a B cell or a macrophage, in order for the proper activation signals to be given. If the provided costimulatory signal by the APC is absent, TCR occupancy will not result in activation; rather a non-responsive state will be induced. The non-responsive state is characterized by lower levels of IFN-$\gamma$, IL-3, and IL-2 receptor production, and normal induction of the T-cell receptor complex. However, extremely low levels of IL-2 mRNA are observed when normal T cell clones are anergized, indicating that a block exists which prevents expression of the IL-2 gene. The presence of cyclohexamide or cyclosporine A during the anergic stimulus can prevent the non-responsive state from occuring. To investigate the molecular basis for the induction of T-cell anergy, we pretreated Th1 cells by incubating them overnight on tissue culture plates coated with anti-CD3 mAb and subsequently derived nuclear extracts from these cells. By the gel mobility shift technique, preliminary results have shown a different nuclear factor pattern for anergized T-cells compared to the one previously reported for activated or resting T-cells. We are currently analyzing whether these alterations in the expression of certain transcriptional factors are necessary and/or sufficient for the induction of T-cell non-responsiveness.

**C 336** STUDIES OF TOLERANCE IN TRANSGENIC MICE, CARRYING RECOMBINANT H-2K$^b$ GENES. Sandra D. Husbands[1], Karen L. Philpott[1], Peter D. Tomlinson[1], Phillip Chandler[2], Elizabeth Simpson[2] and Andrew L. Mellor[1], [1]Immunology Division, National Institute for Medical Research, Mill Hill, London NW7 1AA, U.K. and [2]Transplantation Biology Section, Clinical Research Centre, Harrow, Middlesex, U.K.

The murine immune system is able to distinguish exquisitely between self and non-self, which is a function of both B and T cells. This phenomenon is known as self tolerance, and for T cells is thought to result from deletion of immature T cell clones with self-specificity, during selection in the thymus. In order to investigate the mechanism(s) involved in inducing tolerance to self antigens expressed outside the thymus, we have generated two lines of transgenic CBA mice (H-2$^k$) carrying recombinant H-2K$^b$ genes, expressed under the control of a tissue-specific milk protein gene promoter. A third line of transgenic CBA mice carries the entire H-2K$^b$ gene, including its own H-2 promoter.

Mice carrying the milk protein gene/H-2K$^b$ recombinants express H-2K$^b$ mRNA in mammary gland and thymus. Immunohistological analysis of tissues to determine expression at the protein level is under way. These mice are tolerant towards skin grafts bearing H-2K$^b$ antigens, and fail to produce H-2K$^b$ specific CTL *in vitro*. However, peripheral T cells from these mice proliferate specifically to H-2K$^b$ in the presence of interleukin 2.

**C 337** THE IMMUNE RESPONSE TO AZOBENZENEARSONATE: MECHANISMS OF FETAL, NEONATAL, AND ADULT TOLERANCE. S. Jerrold-Jones, K. Yui, M. Katsumata, S. Thayu, K-N. Tan, and M. Greene. Dept. of Pathology, University of Pennsylvania, Philadelphia, PA 19104-6082.

The immune response to the azobenzenearsonate (ABA) hapten is dominated by a major idiotype in both the B and T lymphocyte compartments. An idiotypic network appears to be involved in the response to this hapten. ABA reactive T lymphocytes have increased expression of the Vα3 T cell receptor gene and transfer of the Vα3.1 chain from an ABA responsive clone confers ABA responsiveness to a T cell clone lacking this specificity. Using this association of antigen reactivity and receptor expression we are determining the mechanisms of fetal, neonatal and adult tolerance to the ABA hapten through analysis of expression of the Vα3.1 T cell receptor.

C57Bl/6 mice which are transgenic for the Vα3.1 T cell receptor have been developed and are being administered ABA coupled to protein or syngeneic cells during different developmental stages. Using monoclonal antibodies to Vα3.1 that we have developed we are determining whether Vα3.1 expressing cells have been deleted or are present in the tolerant transgenic and non-transgenic mice. We are further distinguishing between anergic and suppressive mechanisms. In addition, we are investigating the possible influence of the immunoglobulin heavy chain locus on the selection and usage of Vα3.1 by ABA specific T cells.

**C 338** LACK OF SUPPRESSOR CELL INVOLVEMENT IN NATURALLY ACQUIRED TOLERANCE TO A MINOR H ANTIGEN, Lawrence L. Johnson, The Trudeau Institute, Saranac Lake NY 12983

The hypothesis that suppressor cells may be responsible for naturally acquired tolerance to the H-24 minor histocompatibility antigen was studied. B6 (H-24$^b$) and HW54 (H-24$^c$) congenic mice that differ only with regard to alleles at the H-24 locus were used in cell transfer experiments. B6 hosts were given HW54 skin grafts along with HW54 lymphoid cells to assess their tolerance of the H-24$^c$-encoded antigen. The hosts were either (i) normal, nonimmune B6 mice, (ii) B6 mice made immunodeficient by thymectomy and irradiation (TXB) and repopulated with H-24-immune B6 lymphocytes, or (iii) TXB B6 hosts repopulated with nonimmune B6 lymphocytes. In each case it was found that additionally infused HW54 lymphoid cells did not suppress the ability of these hosts to reject HW54 skin grafts. Thus HW54 mice appear not to have suppressor cells that might maintain H-24$^c$-specific natural tolerance.

**C 339** SELF-TOLERANCE TO A SECRETED AUTOANTIGEN IS MAINTAINED BY A PERIPHERAL MECHANISM, Judith A Kapp, Nancy J. Poindexter, Carol Landon and Phyllis J. Whiteley, Department of Pathology, Washington University Medical Center, St. Louis, MO 63110

We have examined the mechanism of self-tolerance to secreted autoantigens in mice that are transgenic for human insulin. These mice express mRNA for human proinsulin, the precursor form of insulin, only in pancreatic islets and secrete physiological amounts of human and mouse insulin. These mice are tolerant to human insulin whereas, normal syngeneic mice are not. No differences were found in B cell responses to human insulin suggesting that they are not tolerant. Although lymph node T cells from insulin primed transgenic mice provided no helper activity for antibody responses, they developed significant proliferative responses to human insulin. Human insulin specific T cell hybridomas from transgenic mice displayed similar specificities and avidities as hybridomas from nontransgenic mice. Thus, tolerance is not the result of clonal deletion. Moreover, APC from transgenic mice did not stimulate human insulin-specific hybridomas in the absence of exogenous insulin suggesting that clonal deletion does not occur because thymic APC lack the necessary levels of human insulin-Ia complexes. Thus, tolerance is preserved by a peripheral mechanism.

**C 340** TWO MECHANISMS FOR SELF TOLERANCE: CLONAL DELETION IN BONE MARROW (BM) CHIMERAS AND T-CELL SUPPRESSION IN NORMAL MICE. Benny Leshem, The Lautenberg Center for General and Tumor Immunology, The Hebrew Univ.-Hadassah Medical School, Jerusalem 91010, Israel. We have previously demonstrated that highly potent $CD8^+$ CTL directed against syngeneic target cells are prevalent among splenocytes of normal mice. The anti-syngeneic activity is normally blocked by specific autologous $CD8^+$ T-suppressor cells (Ts), suggesting that suppression of anti-syngeneic effector cells might be the mechanism of self tolerance. On the other hand, transplantation tolerance in BM chimeras has been shown to result from thymic functional deletion of CTL clones directed against the tolerogen. The same mechanism was ascribed to self tolerance. In an attempt to reconcile the apparent discrepancy between the two approaches, we compared the frequency of anti-syngeneic CTL among splenocytes from lethally irradiated mice that were reconstituted with syngeneic BM cells, with that among splenocytes from normal, untransplanted mice. We found that although the anti-allogeneic response in the syngeneic BM recipients was fully restored, the frequency of splenic anti-syngeneic (=anti tolerogen) CTL was 10-fold lower than that found among splenocytes derived from normal mice. Splenic anti-syngeneic CTL frequency was also reduced in neonatally thymectomized syngeneic BM recipients, indicating that thymic selection is not involved in the deletion of splenic anti-syngeneic CTL clones in the transplanted mice. These data suggest that tolerance in BM chimeras and in normal animals might be explained by two different mechanisms: clonal deletion and suppression, respectively. The data also pose the question as to whether the clonal deletion mechanism suggested for transplantation tolerance can be directly extended to explain self tolerance.

**C 341** REGULATION AND MECHANISM OF ACTION OF THYMOCYTE APOPTOSIS, S.A. McCarthy, J.A. Walker, R.N. Cacchione, and J.S. Cairns, U. of Pittsburgh, Pittsburgh PA 15213. Negative selection in the thymus is thought to involve induction of apoptosis, one property of which is DNA fragmentation. Apoptosis can be induced in thymocytes by signalling through the TcR/CD3 complex, or alternatively, by exposure of thymocytes to calcium ionophores or phorbol esters, which may mimic the intracellular second messengers activated by TcR/CD3-mediated signalling. We have used calcium ionophore treatment to directly analyze purified cell populations for functional "apoptotic machinery" capable of effecting DNA fragmentation. We have found that the majority of immature $CD4^+8^+$ double positive cells, including those from newborn mice, respond efficiently to calcium ionophore induction of apoptosis. In contrast, fully mature splenic T cells do not activate apoptosis in response to calcium ionophore, suggesting that they no longer possess functional apoptotic machinery. We will next analyze very immature double negative thymocytes and relatively mature single positive thymocytes to identify precisely the developmental stages at which the apoptototic machinery is intact. In additional studies, we have used anti-CD3 mAb to trigger apoptosis, to identify the cell subsets in which the TcR/CD3 complex is functionally linked to the apoptotic machinery. We have found that only a minority of double positive cells appears to have this functional linkage. We are now analyzing whether additional signals, perhaps mediated through CD4 and/or CD8, regulate the ability of TcR/CD3 engagement and cross-linking to induce apoptosis. Finally, we are investigating whether components of the lytic machinery of CTL (which induce apoptosis in target cells) such as proteases/granzymes are also involved in thymocyte apoptosis.

**C 342** IN VIVO ANTIGEN-INDUCED DELETION OF $CD4^+8^+$ $TCR^{lo}$ THYMOCYTES PROCEEDS VIA INTRATHYMIC APOPTOSIS, Kenneth M. Murphy*, Amy B. Heimberger, and Dennis Y. Loh, Howard Hughes Medical Institute, Departments of Medicine, Pathology*, Genetics and Molecular Microbiology, Washington University School of Medicine, St. Louis, MO 63110.

To examine the cellular mechanisms by which clonal deletion of autoreactive T cells takes place, we have investigated the ability of a specific peptide antigen to induce deletion of antigen-reactive thymocytes in vivo. In transgenic mice expressing a unique T cell receptor (TCR) reactive to a known peptide from chicken ovalbumin, thymocytes develop through an immature CD4+8+ $TCR^{lo}$ phenotype and, in the $H\text{-}2^d$ haplotype, progress to a $CD4^+8^-$ $TCR^{hi}$ stage and are exported to the periphery as mature ovalbumin-reactive $CD4^+$ T cells. Intraperitoneal administration of the reactive ovalbumin peptide to transgenic mice results in a rapid deletion of the immature $CD4^+8^+$ $TCR^{lo}$ thymocytes. Apoptosis of most cortical thymocytes is seen within 20 hours of treatment, evident by nearly complete degradation of thymocyte DNA into oligonucleosomal fragments and by the appearance of highly condensed chromatin and pyknosis of thymocyte nuclei. These results provide direct evidence for an in vivo role of apoptosis as a mechanism for clonal deletion in antigen-induced tolerance. K.M.M is a recipient of a Juvenile Diabetes Foundation Career Development Award. D.Y.L. is an investigator of the Howard Hughes Medical Institute.

**C 343** NEGATIVE SELECTION OF IMMATURE $CD4^+CD8^+$ THYMOCYTES BY ANTIGEN-INDUCED T CELL RECEPTOR SIGNALS, Toshinori Nakayama, Lawrence E. Samelson, Carl H. June, Terry I. Munitz, Michael Sheard and Alfred Singer, Experimental immunology Branch, National Cancer Institute, Cell Biology and Metabolism Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892, Immunobiology and Transplantation Department, Naval Medical Reserch Insttute, Bethesda, MD 20814

Thymic selection of the developing T cell repertoire occurs in immature $CD4^+8^+$ thymocytes, with the fate of individual thymocytes determined by the T cell antigen receptors (TCR) they express. However, most $CD4^+8^+$ thymocytes express few TCR molecules and are incapable of transducing intracellular signals in response to antigen. Recently we demonstrated that TCR expression and function in immature $CD4^+CD8^+$ thymocytes was actively inhibited by CD4-mediated signals, and that release from CD4-mediated inhibition resulted in significant increases in both TCR expression and TCR signaling function. In the present study, we have examined the reactivity of immature $CD4^+CD8^+$ thymocytes that have been released from CD4-mediated inhibition. We specifically examined the responses of competent $CD4^+CD8^+$ thymocytes expressing TCR-V$\beta$6,8, and 17a to the antigen Staphylococcal Enteroxin B and to the B cell alloantigen $I\text{-}E^d$. We found that antigen engagement of TCR on competent $CD4^+CD8^+$ thymocytes stimulates TCR-mediated signals that result in internalization of surface TCR, re-phosphorylation of the TCR-zeta chain, and induction of programmed cell death (apoptosis).

**C 344** ANERGY AND PROGRAMMED CELL DEATH IN POST THYMIC TOLERANCE. Atsuo Ochi, Kouichi Yuh and Kiyoshi Migita and Yojiro Kawabe. Department of Immunology and Medical Genetics University of Toronto. Division of Neurobiology and Molecular Immunology Samuel S. Lunenfeld Research Institute, Mount Sinai Hospital, 600 University Ave. Toronto, Ontario, Canada, M5G 1X5.

Both clonal deletion and functional inactivation of self-reactive cells have been invoked as mechanisms underlying the intrathymic development of T cell tolerance. The relative role of these mechanisms in the tolerizing of more mature, peripheral T cells either to self or to exogenous antigen is unclear, although the predominant paradigm ascribes the development of T cell tolerance in the periphery to clonal anergy. We have investigated the cellular basis of T cell tolerance to the bacterial superantigen, staphylococcus enterotoxin B (SEB) which stimulates the V$\beta$3, 7, $8^+$ T cells in mice. In the SEB-tolerant mice, $CD4^+$, V$\beta8^+$ cells become anergized but the $CD8^+$, V$\beta8^+$ T cells do not, in spite of the fact that both subsets can respond to SEB. Our data also showed that both anergy and deletion of peripheral V$\beta8^+$ T cells occured in SEB tolerant mice. The mechanism of post thymic tolerance against SEB and the relative roles of anergy and programmed cell death will be discussed.

References

Selective anergy of V$\beta8^+$, $CD4^+$ T cells in Staphlococcus enterotoxin B-primed mice. Kawabe, Y and A. Ochi. J. Exp. Med. October in press.

Programmed cell death and extrathymic reduction of V$\beta8^+$, $CD4^+$ T cells in Staphylococcus enterotoxin B-specific tolerance. Kawabe, Y and A. Ochi. submitted.

C 345 TOLERANCE AND INDUCTION OF DIABETES IN ANTIGEN/T CELL RECEPTOR DOUBLE TRANSGENIC MICE, Pamela Ohashi, Stephan Oehen, Kurt Buerki*, Hanspeter Pircher, Cara Ohashi, Bernhard Odermatt, Bernard Malissen+, Rolf M. Zinkernagel and Hans Hengartner. Institute of Pathology, University Hospital, 8091 Zurich Switzerland. * Sandoz Pharma Ltd. 4002 Basel Switzerland. + Centre d'Immunologie INSERM-CNRS, 13288 Marseilles Cedex 9 France.

To address the mechanisms of tolerance to extrathmymic proteins, we have generated transgenic mice expressing the lymphocytic choriomeningitis viral (LCMV) glycoprotein (GP) in the ß islet cells of the pancreas. The fate of T cells specific for LCMV-GP was examined by breeding the GP transgenic mice with T cell receptor transgenic mice bearing T cells specific for the LCMV-GP presented in association with H-2D$^b$. The presence of T cells bearing the transgenic T cell receptor (TCR) in the thymus and periphery, and the normal functional response to the GP antigen suggests that neither clonal deletion nor clonal anergy are responsible for maintaining tolerance to the GP transgenic antigen expresed in the pancreas. Results show that tolerance is readily broken by infecting either single or double transgenic mice with LCMV. Induction of "transgenic self" reactive cytotoxic T cells in vivo results in a CD8+ T cell mediated diabetes. These findings suggest that similar mechanisms may operate in several so called "T cell mediated autoimmune diseases."

C 346 SELF-TOLERANCE IN MICE TRANSGENIC FOR CD8 AND A SELF-REACTIVE, CLASS I-RESTRICTED T CELL RECEPTOR, Jane R. Parnes, Rho H. Seong, Paul von Hoegen, Gregory D. Frank, Sherman M. Weissman and John W. Chamberlain, Department of Medicine, Stanford University Medical Center, Stanford CA 94305 and Department of Human Genetics, Yale University School of Medicine, New Haven, CT 06510

Negative selection of thymocytes expressing self-reactive T cell receptors (TCRs) has been shown to play a major role in T cell tolerance to self-antigens. We have examined the role of CD8 in this process by crossing mice expressing high levels of transgenic CD8 driven by constitutively active human $\beta$-actin control sequences to mice transgenic for a TCR specific for the male antigen HY in association with the class I major histocompatibility complex protein D$^b$ (kindly provided by Drs. H.S. Teh and H. von Boehmer). We have succeeded in breeding mice that are transgenic for the $\beta$-actin/CD8 and the TCR $\alpha$ and $\beta$ genes. Male H-2D$^b$ mice expressing these transgenes have a high proportion of peripheral T cells expressing all three transgenic proteins despite predictions that such cells should be deleted by negative selection. The *in vitro* reactivity of these T cells will be discussed.

C 347 Clonal deletion versus T cell activation

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Transgenic mice were generated with T cell receptor (TCR) genes originally isolated from a lymphocytic choriomeningitis virus (LCMV) specific T cell clone P14. LCMV variants wich contain alterations in the transgenic T cell epitope were isolated from infected transgenic mice. Functional assays revealed that transgenic TCR expressing mature T cells exhibited no/or only weak reactivity towards the LCMV variants. TCR transgenic mice were neonatally infected with wildtype and variant LCMV to study T cell tolerance in virus carrier mice. These studies adress the sensitivity difference of clonal deletion versus T cell activation.

**C 348** ONTOGENY OF THE TOLERANT PHENOTYPE IN MICE RENDERED NEONATALLY TOLERANT OF MHC CLASS II. T J. Powell, I. E. Joo, S. Socarras, and J. W. Streilein. University of Miami Sch of Med, Dept of Microbiology and Immunology, Miami FL 33101.

Induction of neonatal tolerance to MHC Class II alloantigens in mice of the A strain background results in an immunologic paradox: while maintaining a healthy skin graft bearing the tolerogen(s), tolerant mice also retain tolerogen-reactive T cells detectable in the MLR. However, while the normal anti-tolerogen MLR is dominated by TH1-like cells, the tolerant anti-tolerogen MLR is dominated by TH2-like cells. We have investigated the ontogeny of the TH2-like cells in tolerant mice, by examining the MLR of thymocytes from tolerant mice following tolerance induction on day 0. Normal neonatal (day 0) thymocytes proliferated strongly and secreted IL-2 when stimulated with Class II tolerogen or with 3rd party alloantigens. Thymocytes from normal 9 day old mice maintained this TH1-like phenotype, while age-matched tolerant thymocytes failed to proliferate or secrete IL-2 in response to the tolerogen but not to 3rd party. Thymocytes from 23 day old tolerant mice had regained proliferative reactivity to tolerogen, but did not secrete IL-2 or IL-4. Day 36 tolerant thymocytes had developed the typical adult tolerant phenotype characterized by proliferation and IL-4 secretion. The transient loss of specific reactivity early following tolerance induction may represent deletion and/or anergy of the TH1-like cells, followed by the emergence of the tolerogen-specific TH2-like cells. Thus, it appears that Class II tolerance in this system may involve suppression (previously demonstrated), perhaps maintained by the TH2-like cells, as well as clonal deletion/anergy of the normal TH1-like cells. This hypothesis suggests that all three mechanisms may be required for induction of tolerance to alloantigens. If the same is true for the induction of tolerance to autoantigens, then a breakdown or failure in any one of these mechanisms may be sufficient for the development of pathogenic autoimmune responses.

**C 349** FUNCTIONAL EFFECTS OF ANTI-CD4 ANTIBODY TREATMENT ON SELF-REACTIVE CELLS RESCUED FROM INTRATHYMIC DELETION, Joseph L. Roberts, Michael Sheard, Susan O. Sharrow and Alfred Singer, Experimental Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

Self-tolerance of the T cell repertoire can be generated during intrathymic development by clonal deletion or clonal inactivation of potentially autoreactive thymocytes following encounter with self antigen. Anti-CD4 mAb treatment is known to rescue $CD8^+$ thymocytes bearing self-reactive TCR from deletion, presumably by blocking TCR engagement of self ligands by these cells at an earlier $CD4^+CD8^+$ precursor stage. It has recently been demonstrated that anti-CD4 mAb administration also releases immature $CD4^+CD8^+$ thymocytes from CD4-mediated inhibitory signals with resultant increases in both TCR expression and TCR signalling by $CD4^+CD8^+$ cells. In the present study we have examined the relationship between induction of TCR expression on $CD4^+CD8^+$ thymocytes by anti-CD4 mAb and rescue from clonal deletion. We found that the ability of anti-CD4 mAb to rescue self-reactive cells from deletion is a consequence of its interference with CD4-mediated inhibitory signals on double positive thymocytes.

**C 350** NEGATIVE SELECTION OF AUTOSPECIFIC THYMOCYTES: SIGNALLING BY CD4 AND CD8 CORECEPTORS REQUIRES $p56^{lck}$, Hung-Sia Teh*, Alex M. Garvin$^{\Delta}$, Nicolai van Oers*, Douglas A. Carlow* and Roger M. Perlmutter$^{\Delta}$, *Department of Microbiology, University of British Columbia, Vancouver, Canada V6T 1W5 and the $^{\Delta}$Departments of Immunology, Biochemistry and Medicine (Medical Genetics), University of Washington, Seattle, WA 98195

Deletion of immature $CD4^+8^+$ thymocytes expressing autospecific αβ T cell receptors (TCR) requires the participation of CD4 or CD8 coreceptors. This involvement of CD4 or CD8 coreceptors probably reflects both putative functions of these molecules: enhancement of TCR binding to peptide-MHC complexes and signal transduction. The signalling function of CD4 and CD8 may result from interaction of the cytoplasmic tails of these molecules with the lymphocyte-specific protein tyrosine kinase, $p56^{lck}$. Data demonstrating that overexpression of the intact CD4 coreceptor, but not a truncated form of the CD4 coreceptor lacking the cytoplasmic tail, inhibited the intrathymic deletion of $CD4^+8^+$ thymocytes in male transgenic mice expressing a TCR specific for the male (H-Y) antigen plus $H\text{-}2D^b$ class I MHC molecule will be presented. These findings argue that CD4 and CD8 coreceptor compete for interaction with $p56^{lck}$ and directly implicate a role for $p56^{lck}$ in the deletion process.

*Strategies in Overcoming Autoimmune Disease: MAB to TcR and Other Surface Molecules; Agretypic Peptides; TcR Peptides; Tolerance; Immunotoxins*

**C 400 DEFINITION OF POTENTIAL IMMUNOMODULATORY PEPTIDES BY FINE STRUCTURE ANALYSIS OF AN ARTHRITIS ASSOCIATED T CELL EPITOPE**

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**Dept. Immunol, Veterinary Faculty, Univ. of Utrecht. *Dept. of Bact., Nat. Instit. Public Health & Envir. Hygiene, Bilthoven. **Central Vet. Instit., Lelystad, The Netherlands.**

**Immune intervention in experimental autoimmune diseases by the use of synthetic peptides is the subject of intensive research. In this respect information on MHC and TcR contact residues within relevant T cell epitopes is necessary for a guided structural manipulation of such epitopes. We now show that the PEPSCAN method modified for T cells can be used efficiently for such analysis. We analysed an arthritis associated T cell epitope on the mycobacterial 65 kD heatshock protein at the level of single amino acids. Rat T cell clones developed in the adjuvant arthritis model with specificity for this epitope were used to define in vitro stimulatory and inhibitory substituted peptides for the classification of MHC and/or TcR contact residues. Through this detailed molecular characterization of this epitope, we could design non-stimulatory peptide analogues that can compete efficiently for antigen presentation not only with the original T cell epitope but also with the mycobacterial 65 kD protein and whole Mycobacterium tuberculosis. The feasibility of immune intervention with these peptide analogues in experimental autoimmune diseases is currently under investigation.**

**C 401** IMMUNOREGULATION OF AUTOIMMUNE DISEASE BY VACCINATION WITH T CELL RECEPTOR (TCR) PEPTIDES, Steven W. Brostoff and Mark D. Howell, The Immune Response Corporation, San Diego, CA 92121

Restricted TCR gene usage in animal models of autoimmune disease has led to strategies for control of these diseases by targeting the idiotypic determinants within the TCR sequence. Rats can be rendered resistant to EAE by immunization with synthetic peptides representing sequences contained within the V$\beta$, J$\alpha$ and VDJ$\beta$ regions of the TCR that are conserved among encephalitogenic T cells. We propose that the mechanism of immunoregulation thus produced results from the stimulation of an anti-clonotypic response directed at endogenously synthesized TCR peptides presented by Class I MHC on the surface of the autoreactive T cell, and that this mechanism may be part of the natural immunoregulation of T cell responses. The experimental data demonstrate the utility of this therapeutic approach and its potential for treatment of any pathogenic condition mediated by specific, oligoclonal T cell populations.

**C 402** ANTIGEN-SPECIFIC SUPPRESSION OF AN ONGOING AUTOANTIBODY RESPONSE BY MULTIVALENTLY-ARRAYED PEPTIDE, Claire Coeshott, John Cheronis, Jim Blodgett, Chris Ohnemus, Lisa Allen, Ellen Roper and Brian Kotzin, Cortech, Inc., Denver, CO 80221 and Division of Basic Sciences, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO 80206.

In the NZB/NZW mouse model of SLE, a linear peptide consisting of residues 3-12 from the N terminus of histone H2B has been identified as a major autoimmune epitope. We have conjugated a modified peptide containing this sequence to size fractionated dextran and injected this construct into mice having high IgG antibody titers to this epitope. This conjugate was found to suppress specifically the antibody response to this defined epitope with suppression persisting for several weeks even after administration of the conjugate was halted. In addition, the numbers of splenic cells secreting anti-peptide antibodies were specifically reduced in the treated mice when compared to anti-DNA antibody secreting cells from the same mouse. Thus the construct was not exerting its effect by simply adsorbing circulating antibodies.

While mortality was not affected by the suppression of anti-histone antibody production, these data suggest that it will be possible to suppress other deleterious ongoing antibody responses to defined epitopes in autoimmune states by the administration of these epitopes in multivalent arrays which are themselves non-immunogenic.

**C 403** TCR - PEPTIDE THERAPY : EFFECT ON T CELL RESPONSE

Amitabh Gaur and C. Garrison Fathman, Dept of Medicine, Div. of Immunology, Stanford University School of Medicine, Stanford, CA 94305.

Autoimmune disorders like EAE involving T cells bearing TCRs of limited heterogeneity have been shown to be treated by the use of a TCR peptide corresponding to the CDR II region of the beta chain. Direct T cell-T cell interactions have been invoked to explain this phenomenon. We have attempted to use the same approach towards understanding these interactions and their regulatory effects. The response to sperm whale myoglobin epitope, 110-121 in the DBA/2 mice is essentially contained in the compartment of T cells bearing 8.2 Vbeta element . This was found in the various clones derived from immunized animals and has now been confirmed in vivo. Treatment of DBA/2 mice with a TCR peptide corresponding to the CDR II region of Vbeta 8.2 elicits a response to the peptide but in our case fails to down regulate the 8.2 driven response to the SpWMb epitope . The role of TCR peptide immunization in generating /augmenting a regulatory cellular interaction will be discussed.

**C 404** IN COLLAGEN-INDUCED ARTHRITIS T CELLS ARE IMPORTANT NOT ONLY FOR THE INDUCTION OF THE ANTI-COLLAGEN ANTIBODY RESPONSE BUT ALSO FOR THE PROGRESSION AND MAINTAINANCE OF THE ARTHRITIS AS DEMONSTRATED BY ANTI-TCR TREATMENT IN VIVO, Tom J. Goldschmidt*# and Rikard Holmdahl*. *Dept. Medical and Physiological Chemistry, University of Uppsala, Uppsala, Sweden. #Dept. Inflammation Research, Pharmacia Therapeutics, Uppsala, Sweden.

In the type II collagen (CII) induced arthritis (CIA) model for rheumatoid arthritis it is unclear whether T cells are important only in the inductive phase of the anti-CII immune response to trigger B cells for production of pathogenic autoantibodies or if they also are required for the progression and maintainance of arthritis. In the present study we have addressed this question using a monoclonal anti-TCRab antibody (R73) for treatment of rats induced to develop CIA after immunization with autologous CII.

It was found that the anti-TCR treatment arrested the developing arthritis both when administered repeatedly from immunization or just before onset. The anti-TCR antibody also had a therapeutic effect in already arthritic rats. The given doses of R73 was demonstrated to cause reduction of the lymph node cells but a significant part of the cells remained with R73 bound to their surface. No reduction of the level of anti-CII antibodies was seen exept in the rats treated from immunization. However a strong anti-R73 response was recorded during the antibody treatment concomitantly with the loss of the inhibitory effects which lasted for a period of 10 days. The arthritis then progressed fast to the same level as in the untreated group suggesting that the arthritis was supressed as long as free anti-TCR antibody was available to block involved T cells. We concluded that R73 influenced the arthritis development by blocking the activities of T cells and suggest that T cells are important not only for the induction of the anti-collagen antibody response but also for the progression and maintainance of arthritis.

**C 405** INDUCTION OF DONOR SPECIFIC ALLOGRAFT TOLERANCE IN THE ADULT ANIMAL

Liming Hao, Filippo Calcinaro, Ronald Gill, & Kevin Lafferty. Barbara Davis Center for Childhood Diabetes, University of Colorado Health Sciences Center, Denver, CO 80262

The development of clinical transplantation requires procedures for tolerance induction in the adult animal. This technology is now a reality in the case of cellular replacement therapy such as pancreatic islet transplantation. RS-61443, a prodrug of micophenolic acid, prolongs islet allograft survival and induces donor specific tolerance in adult mice (B6 --> BALB/c; BALB/c --> CBA). Recipient animals were treated with the drug (80 mgm/Kgm/day) orally for 30 days. 50-70% of recipients retain their graft for >100 days and accept a donor strain thyroid graft, but reject a third party thyroid. Tolerant animals do not show any in vitro evidence of clonal deletion or clonal anergy. The specific tolerance can be transferred to SCID mice. This model will be used to determine whether tolerance achieved under these conditions is dominant or recessive immune function.

**C 406** INDUCTION OF UNRESPONSIVE T CELLS BY NEONATAL CD3 INJECTION IN NOD MICE, Anthony R. Hayward, Michelle Shriber, Ralph Kubo & Marcia McDuffie. Departments of Pediatrics & Microbiology, University of Colorado Health Sciences Center & National Jewish Center, Denver, CO.

NOD mice injected with CD3 antibody as newborns have a reduced incidence of diabetes, raising the possibility that the neonatal injection caused a long lasting change in circulating T cells. The present study shows that NOD mice injected with soluble CD3 antibody in the first two days of life sustained an 80-95% reduction in the number of circulating T cells for 2-3 weeks, with T cells returning after 4 weeks, and reaching control values after 6 weeks. They had a similar distribution into CD4 and CD8 subsets as uninjected controls, and a similar usage and cell surface expression of 4 T cell receptor Vß families. Labelled CD3 antibody was detected in the serum for up to 2 weeks after injection into neonates and was enriched in the thymus. Adoptively transferred T cells continued to be cleared from the circulation for 4 weeks following injection. The properties of T cells which had been exposed to CD3 neonatally were investigated in animals who were first injected with CD3 antibody and then thymectomized. These animals had reduced numbers of T cells at 12 weeks of age but the percentage distribution of 4 different Vß families amongst these cells was the same as in the uninjected controls. A diminished fluorescence staining intensity for $V_{\beta}8$ among the reduced number of cells utilizing this family in the CD3-injected, thymectomized, mice suggested that their density of expression of the T cell receptor was reduced. The surviving T cells showed a $Ca^{++}$ flux when stimulated but their proliferation in response to Con A was reduced, even in the presence of supernatant co-stimulator factors. The data indicate that the limited number of T cells which survive the neonatal CD3 injection have functionally deficient proliferative responses to mitogen. An intact thymus is required for full T cell repopulation following neonatal CD3 injection into NOD mice.

**C 407** PREDAMINISTRATION OF A 65KDa HEAT SHOCK PROTEIN, GroEL, INHIBITS COLLAGEN INDUCED ARTHRITIS IN MICE, Jun Ito, Christopher J. Krco, David Yu, Harvinder S. Luthra and Chella S. David, Departments of Immunology and Rheumatology, Mayo Clinic, Rochester, MN 55905, and Department of Medicine, UCLA, Los Angeles, CA 90024-167022.

T cell and B cell reactivities to a heat shock protein, hsp65, have been reported in association with several rheumatoid and inflammatory diseases. Prechallenge with mycobacterial hsp-65KD suppresses adjuvant arthritis and type II collagen induced arthritis (CIA) in rats. In this study, we investigated the effect of a hsp65 protein, GroEL, on collagen induced arthritis in mice. Three experimental groups were made of B10.RIII mice; Group 1 (G1) was not pretreated. Group 2 (G2) was pretreated with emulsion of PBS and incomplete Freunds adjuvant (IFA) intraperitoneally. Group 3 (G3) was pretreated with emulsion of IFA and 20 µg of GroEL. Bovine type II collagen (BII) was emulsified with IFA and injected intradermally at one week after pretreatment. Arthritic score, incidence of arthritis and anti-GroEL antibody in sera was measured. Twelve weeks after injection with BII, mean arthritis scores were 2.62 in G1, 2.4 in G2, 1.0 in G3. Incidence were 48% (14/29 mice) in G1, 53% (8/15) in G2, and 28% (11/39) in G3. Anti-GroEL antibody (O.D. 414 nm) in ELISA were 0.135 in G1, 0.124 in G2, 0.866 in G3. These results indicate that GroEL has a suppressive effect in autoimmune CIA.

**C 408** REGULATION OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS WITH SYNTHETIC T CELL RECEPTOR PEPTIDES, Richard E. Jones, Arthur A. Vandenbark, George A. Hashim and Halina Offner, Neuroimmunology Laboratory, V.A. Med. Ctr., Portland, OR. 97201.

Encephalitogenic T cells induced by immunization of rats with myelin basic protein express a limited repertoire of T cell receptor (TcR) V region genes, namely the Va2:Vb8 combination. To test the idea that these common TcR sequences could serve as targets of immunoregulation, we identified a region of the Vb8 TcR which was predicted to be antigenic and which was modelled to contribute contact residues (CDR2) interactive with the BP/MHC and we synthesized a peptide analogue of this region, Vb8-39-59. As a control, we synthesized the corresponding peptide from the Vb14 TcR sequence. Immunization of Lewis rats with each of these peptides induced both T cells and antibodies specific for the respective immunogen. Vb8-39-59 but not Vb14-39-59 immunized rats were protected from clinical signs of EAE induced by challenge with an encephalitogenic dose of GP-BP or either encephalitogenic peptide for lewis rats (S69-84 or S87-99). Both T cell lines and antibodies specific for Vb8-39-59 transferred protection to naive rats challenged with GPBP/CFA, implicating both cellular and humoral mechanisms of disease resistance. The Vb8-39-59 peptide was also highly effective as <u>therapy</u> for EAE. Depending on the dose and route, the Vb8-39-59 peptide halted disease progression and/or shortened the disease course. Rats undergoing EAE had increased T cell responses to the Vb8-39-59 peptide without prior exposure to the peptide suggesting that the rapid therapeutic effect observed after treatment with the TcR peptide may have resulted from the activation of a pre-existing regulatory pathway initiated during the development of EAE.

**C 409** T LYMPHOCYTE RECOGNITION OF HLA MOLECULES ASSOCIATED WITH SUSCEPTIBILITY TO INSULIN DEPENDENT DIABETES MELLITUS (IDDM) AND CELIAC DISEASE (CD), Knut E.A. Lundin, Ludvig M. Sollid and Erik Thorsby, Institute of Transplantation Immunology, The National Hospital, 0027 Oslo 1, Norway.
HLA restricted T lymphocyte responses are probably of importance for the pathogenesis of HLA associated diseases. DQβ chains having a non-Asp amino acid (aa) at residue 57 appear to be involved in IDDM susceptibility. To study the importance of this residue for antigen presentation, we generated alloreactive DQw8 specific or herpes simplex virus specific HLA-DQw8 restricted T lymphocyte clones (TLC). We could demonstrate that in both cases the DQβ residue 57 was of critical importance for T cell recognition and antigen presentation. We have also found that CD is strongly associated with the combination of the DQA1*0501 and DQB1*0201 genes in cis or in trans position. Using DQ specific alloreactive TLC we could demonstrate that in both cases the same DQαβ heterodimer is encoded. The results demonstrate that disease-associated HLA molecules may be precisely defined by TLC. Removal of T cells recognizing antigens together with disease-associated HLA molecules may be a method for immune intervention.

**C 410** SUPPRESSION OF AUTOIMMUNITY BY TARGETING OF THE AUTOANTIGEN: EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS (EAE) IN RATS IS SUPPRESSED BY TARGETING THE ENCEPHALITOGENIC PEPTIDE OF MBP TO B CELLS VIA PEPTIDE-ANTI IgD CONJUGATES, Don Mason, Michael Day, Ferenc Antoni, Iain MacPhee and Albert Tse, MRC Cellular Immunology Unit, Sir William Dunn School of Pathology, University of Oxford, Oxford OX1 3RE.
EAE in Lewis strain rats can be induced by the injection of guinea-pig myelin basic protein in complete Freund's adjuvant (MBP/CFA). Pre-immunization of rats intravenously with a conjugate between the encephalitogenic peptide of MBP and a monoclonal anti-rat IgD monoclonal antibody virtually prevents the development of EAE on challenge with MBP/FCA. Targeting the encephalitogenic peptide to B cells in this way may be expected, on current understanding of T cell induction, to lead to a Th2 type response which antagonises the subsequent development of auto aggressive Th1 type T cells.

**C 411** SPECIFIC IMMUNOREGULATION OF THE INDUCTIVE AND EFFECTOR STAGES OF RELAPSING EAE VIA NEUROANTIGEN-SPECIFIC TOLERANCE INDUCTION, Stephen D. Miller, Lit-Jen Tan, Mary K. Kennedy, and Mauro C. Dal Canto, Departments of Microbiology-Immunology and Pathology, Northwestern University Medical School, Chicago, IL 60611
The effects of tolerance, specifically induced to mouse spinal cord homogenate (MSCH), myelin basic protein (MBP), and proteolipid protein (PLP), on the inductive and effector stages of relapsing experimental autoimmune encephalomyelitis (R-EAE) were examined in SJL/J mice. The incidence of clinical and histologic signs of actively-induced R-EAE, and accompanying neuroantigen-specific DTH responses were dramatically reduced in SJL/J mice tolerized 4-14 days prior to priming with MSCH in CFA by the i.v. injection of injection of syngeneic splenocytes coupled via carbodiimide with MSCH, PLP, and encephalitogenic PLP peptides (but not with MBP or encephalitogenic MBP peptides). Intravenous injection of neuroantigen-coupled splenocytes is also an effective means for treating established R-EAE: a) clinical and histologic expression of R-EAE is reduced in mice treated after immunization with MSCH/CFA, but before onset of clinical signs; b) disease expression of adoptive R-EAE is inhibited in an MBP encephalitogenic peptide-specific, MHC-restricted manner by tolerization of recipient SJL/J mice or Lewis rats up to six days post adoptive transfer of MBP-primed T cells; and, c) treatment of SJL/J mice after the first incidence of clinical disease effectively prevents future paralytic relapses. Specific tolerance induction is thus useful for identification of potential autoimmune determinants, and has potential for regulating ongoing (auto)immune responses without the necessity for eliminating entire T cell subsets, or prior identification of immunodominant epitopes or of a limited repertoire of (auto) antigen-specific T cells.

**C 412 A SIGNIFICANT REDUCTION IN THE INCIDENCE OF COLLAGEN INDUCED ARTHRITIS IN MICE TREATED WITH ANTI-TCR $V_\beta$ ANTIBODIES,** Kevin G. Moder, Gary D. Anderson, Harvinder S. Luthra and Chella S. David, Departments of Immunology and Rheumatology, Mayo Clinic, Rochester, MN 55905.
Collagen induced arthritis (CIA) is an animal model of inflammatory polyarthritis. Previous studies have shown that T cells bearing specific receptors (TCR) are necessary for the development of CIA. The incidence of CIA in B10.RIII mice injected with porcine type II collagen was significantly reduced in those treated with a single injection of the monoclonal antibody F23.1 which deleted $V_\beta 8$ bearing T cells (33% vs. 74% in controls, n=63, $p<.05$). Arthritis score and antibody level to type II collagen were also reduced in the treatment group. No deleterious effects were noted in the treated animals. Use of the monoclonal antibody 466B5 to delete $V_\beta 6$ in combination with F23.1 was no more effective than F23.1 alone and the incidence of CIA in animals treated with 466B5 alone was not significantly different from controls. While F23.1 is a mouse antibody, 466B5 is a rat IgM antibody and is neutralized rapidly in mice in vivo. Thus, $V_\beta 8$ family of TCR's may be expressed on self reactive T cells in CIA. Deletion of only the specific subset of T cells involved in a disease is a promising therapy for autoreactive disorders because it will prevent the development of disease yet leave the host immunocompetent.

**C 413** PEPTIDE-MEDIATED IMMUNOTHERAPY IN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS. Dawn E. Smilek, Sunita Dwivedy, David C. Wraith, Dennis J. Mitchell, Lawrence Steinman and Hugh McDevitt. Department of Microbiology and Immunology, Stanford University, Stanford, CA 94305

Peptide binding and lymph node T cell activation studies have been used to characterize T cell recognition of an encephalitogenic T cell autoantigen from myelin basic protein (MBP) in (PL/J x SJL)F1 mice. Amino acids that determine interactions with either the restriction element of the major histocompatibility complex or the encephalitogenic T cell receptor have been defined. This information has enabled the design of MBP peptide analogs which bind to the MHC, and which therefore have the potential to inhibit experimental autoimmune encephalomyelitis (EAE) by competing for binding of the autoantigen to the MHC. EAE is mediated by T cells which recognize the N-terminal acetylated 1-11 peptide of MBP (Ac1-11) in association with $A\alpha^u A\beta^u$, and several peptide analogs of Ac1-11 have been identified which also bind to $A\alpha^u A\beta^u$. Some of these analogs inhibit EAE, while others do not. Inhibition of EAE by one of the analogs (Ac1-11[4A]) is especially surprising, since the analog actually stimulates encephalitogenic T cells *in vitro*. The mechanism of inhibition of EAE by this and other MBP peptide analogs is under investigation.

*Immunogenicity of Self-Peptides*

**C 414 MHC CLASS II DERIVED PEPTIDES STIMULATE PROLIFERATION OF T CELLS FROM SYNGENEIC MICE,** B. Agrawal, M. Manickasundari, E. Fraga and B. Singh, Department of Immunology, University of Alberta, Edmonton, Alberta, Canada, T6G 2H7

T cell reactivity towards self MHC class II molecules has been recognized in syngeneic MLR's in a number of studies, where the T cells are believed to recognize the combination of self/nonself peptide and self MHC molecule. We have used the synthetic peptides of sequences corresponding to the most polymorphic areas on the amino terminal domains of $\alpha$ and $\beta$ chains of $I\text{-}A^d$ molecules to analyze the immunogenicity/tolerance of I-A molecules in syngeneic mice. T cells responded to a number of peptides of $\alpha_1$ and $\beta_1$ domains of self $I\text{-}A^d$ molecules. The response was dependent upon the presentation of $I\text{-}A^d$ peptides by syngeneic APCs and was blocked by anti class II MHC MAbs. We found that some of these $I\text{-}A^d$ peptides inhibit the stimulation of antigen specific $I\text{-}A^d$ restricted T cell hybridomas. This is probably due to the presentation of I-A peptides by self MHC rather than a direct binding of free peptides to the TCR. These results further support the affinity/interaction of self I-A peptides with intact self MHC class II molecules. One of the peptides of $I\text{-}A\beta^d$ showed high affinity towards intact self MHC II molecule and yet was non stimulatory for syngeneic T cells. It therefore represents an MHC determinant that may have induced self tolerance. It is however highly immunogeneic in allogeneic mice. Thus our results show that strong T cell proliferative responses can be generated against the peptides derived from self MHC II molecules which have a high affinity for intact MHC II molecules.

**C 415** SELF PEPTIDES DERIVED FROM MHC CLASS II MOLECULES BIND TO CLASS II AND ARE IMMUNOGENIC, John H. Freed and Edward F. Rosloniec, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO 80206

Two peptides, representing the sequences found in the first and third polymorphic regions (PMR) of the α chain ($\alpha^k$-1 and $\alpha^k$-3) of the I-$A^k$ class II molecule, are capable of inhibiting the presentation of three different HEL-derived peptide antigens to their appropriate T cells. In addition, the $\alpha^k$-1 peptide inhibits the presentation of the OVA(323-339) immunodominant peptide to I-$A^d$-restricted T-cell hybridomas specific for it. Pre-pulsing experiments showed that the PMR peptides are interacting with the APC and not with the T-cell hybridomas. Demonstration that the $\alpha^k$-1 and $\alpha^k$-3 peptides block the direct binding of HEL(46-61) to purified I-$A^k$ and that the $\alpha^k$-1 peptide blocks the binding of OVA(323-339) to I-$A^d$ provides the mechanism by which these self peptides inhibit presentation of foreign antigens. B10.A(4R) mice (I-$A^k$, I-$E^-$) are capable of mounting an immune response to the $\alpha^k$-1 peptide as assessed by lymph node T cell proliferation and by the preparation of $\alpha^k$-1 peptide-specific, I-$A^k$ -restricted T cell hybridomas from immunized B10.A(4R) mice. These combined data, demonstrating that class II-derived peptides can bind to MHC class II molecules, including the autologous molecule from which they are derived, and suggesting that such complexes can be immunogenic in the autologous host, have important implications for the molecular basis of autoreactivity and alloreactivity. Further, they suggest a possible mechanism by which selecting elements, involving only MHC molecules, may be generated in the thymus.

**C 416** EPITOPE DOMINANCE IN MOUSE EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS, William K. Funkhouser, Dennis M. Zaller, Michael I. Nishimura, Leroy E. Hood, Division of Biology, California Institute of Technology, Pasadena, CA 91125

Mouse experimental allergic encephalomyelitis (EAE) is a T lymphocyte-mediated autoimmune disease which is due to immunization with auto- or xeno-geneic myelin basic protein (MBP). $CD4^+$ T cells are necessary, and possibly sufficient, for pathogenesis. These T cells activate in response to at least four different epitopes within the MBP molecule. Interestingly, secondary response to only one of these epitopes, mMBP (Ac1-20), dominates the response to intact mMBP immunization. The basis for this dominance phenomenon is unknown. We have shown that B10.PL mice can get EAE after immunization with the subdominant epitope (31-50), although the incidence and severity of disease are lower than after mMBP immunization. We have made $T_H$ hybridomas that are (31-50)/H-$2^u$ specific, and have shown that they can also recognize processed and presented mMBP, implying that H-$2^u$ APCs do present a peptide closely mimicked by (31-50). We have also shown that the dominant and subdominant epitopes are presented by different class II molecules. The basis for the differences in response to the dominant and subdominant (31-50) epitopes is under investigation.

**C 417** A MONOCLONAL ANTIBODY AGAINST A LINEAR EPITOPE YCRHNYGV ON DRβ CHAINS, Gustav Gaudernack, Institute of Transplantation Immunology, The National Hospital, 0027 Oslo 1, Norway.

In an approach to make monoclonal antibodies (MAb) against polymorphic HLA Class II determinants, we have immunized mice with Class II molecules purified from lymphoblastoid cell lysates using Dynabeads conjugated with a pan-Class II reactive MAb (HKB1, IgM). Of several MAbs produced after such immunizations one has been extensively characterized using the 11th IHWS homozygous cell panel and Class II transfectants. MAb ITI-1E4 reacted with all cell lines except those homozygous for DR7, DR9 and DR1(Dw20). These data indicate that the epitope recognized is not expressed on the gene products of DRB1*0102, 0701, 0702, 0901 and DRB4*0101. The MAb was also unreactive with several DP transfectants and a DQ transfectant. DR specificity was confirmed by blocking experiments with standard Class II MAbs against DR(L243), DQ(PL8) and DP(B7/21). Examination of available sequence data indicate that the epitope recognized by ITI-1E4 may contain the amino acid sequence YCRHNYGV in positions 78-85 of the DRβ chain, and that substitutions in position 78, 81 and 85 may abrogate binding of the MAb.

**C 418 LOW DOSES OF ADJUVANT-FREE SYNGENEIC IgM MONOCLONAL Ab ELICIT CD4+ T-CELL DEPENDENT ANTI-IDIOTYPIC Ab,** **Kristian Hannestad, Kjetil Andreassen and Gunn Kristoffersen, Department of Immunology, Institute of Medical Biology, University of Tromsø School of Medicine, 9001 Tromsø, Norway.**

It is unclear whether only a few unique or a major fraction of native adjuvant-free syngeneic clonotypic Ab are immunogenic. This has implications for the regulation of Ig production by B cells, immune surveillance of B cell neoplasias, serotherapy with monoclonal Ab and the role of idiotypes (Id) in auto-immune disease. We have assessed the immunogenicity of 22 syngeneic IgM anti-DNP Ab with λ2 or λ3 L-chains. Hybridoma cells killed by 10,000 rad ($5x10^6$ cells, followed by $10x10^6$ s.c.) elicited Ab activity against all 22; a single injection of $0.5x10^6$ cells elicited Ab against two of them. Following a single s.c. injection of 10 μg soluble purified IgM, responses were detected against 12 of the 22 mAb, and the activity against five of them persisted for at least 126 days. One μg soluble IgM was not detectably immunogenic. The bulk of the IgG1 Ab of antisera and all hybridoma anti-Id mAb were hapten-inhibitable. Pretreatment of animals with 400 μg anti-CD4 mAb GK1.5 abolished the IgG1 anti-Id responses. This provides evidence that a large fraction of the syngeneic IgM Ab repertoire can act as potent T-dependent antigens in the absence of artificial enhancement of their immunogenicity, i.e. under near physiological conditions.

**C 419** HUMAN AUTOANTIGENS: EPITOPE MAPPING AND T CELL CLONES. Leonard C. Harrison, David Cram, Shirley Chu and Senga Whittingham, Burnet Clinical Research Unit, Walter and Eliza Hall Institute of Medical Research, Parkville, 3050, Victoria, Australia.

Definition of autoepitopes is a prerequisite for understanding the mechanisms of autoimmune diseases and the development of specific immunotherapies. High titre IgG disease-specific autoantibodies were used to clone autoantigens from λgt11 cDNA libraries, which were then expressed as fusion protein fragments from pGEX. B cell epitopes were identified by Western blotting and T cell epitopes by stimulation of thymidine uptake and cytokine secretion by peripheral blood mononuclear cells.

Multiple B cell epitopes were present in p70 U1RNP (mixed connective tissue disease), topoisomerase I (diffuse scleroderma) and La (Sjögren's syndrome), in contrast to a restricted number of T cell epitopes identified in p70 U1RNP and topoisomerase I. B cell epitopes in both p70 U1RNP and topoisomerase I have significant amino acid sequence homology with the p30gag core protein of type C mammalian retroviruses. Patterns of B cell epitope reactivity may define disease subtypes. T cell responses were also demonstrated to non-cloned pancreatic islet antigens in subjects with pre-clinical and recent-onset insulin-dependent diabetes. CD4 positive T cell clones were generated to epitopes in p70 U1RNP, topoisomerase I, and to islet antigens.

Blood T cell response to autoantigen has been used in pre-clinical diagnosis and for monitoring immunotherapy with cyclosporine or photopheresis. T cell lines/clones expanded to disease-specific autoantigens/peptides will enable us to test human T cell vaccines.

**C 420** DETERMINANT SELECTION IN EXPERIMENTAL AUTOIMMUNE MYASTHENIA GRAVIS (EAMG). Anthony J. Infante, Patricia A. Thompson, Keith A. Krolick and Katherine A. Wall, Department of Pediatrics, Microbiology and Biochemistry, University of Texas Health Science Center, San Antonio, TX 78284

The T cell response to immunization with Torpedo acetylcholine receptor (TAChR) was studied in C57BL/6 (B6) and MHC congenic B6.C-H-$2^{bm12}$ (bm12) mice. Although both strains generate a T cell proliferative response to TAChR, B6 produces specific antibody and develops EAMG while bm12 does not. B6 lymph node T cells and T cell clones respond predominantly to a peptide representing residues 146-162 of the TAChR α subunit. This response is crossreactive at the clonal level to peptide 111-126. Mapping of the B6 T cell epitope using truncated synthetic peptides localizes the epitope to residues 148-151 (IWTY). This sequence shares homology with residues 116-119 (IMWT). bm12 T cells respond mainly to peptide 111-126; however, they do not crossreact with peptide 146-162. Although peptide 146-162 can bind to I-$A^{bm12}$ and be recognized by a B6 T cell clone, immunization of bm12 with 146-162 fails to elicit an anti-peptide response. These results identify an immunodominant and potentially disease-related T cell epitope of TAChR in B6 mice which is non-immunogenic in bm12 mice. Determinant selection of T cell specificity for an individual epitope, rather than T cell responsiveness to the whole antigen, appears to control EAMG susceptibility in the B6/bm12 strain combination.

**C 421** THE DIFFERENTIAL ANAMNESTIC T CELL RESPONSE TO "SELF" AND "FOREIGN" ANTIGENIC DETERMINANTS ON MYELIN BASIC PROTEIN, Paul V. Lehmann, Thomas Forsthuber, Alexander Miller & Eli E. Sercarz Department of Microbiology and Molecular Genetics, University of California at Los Angeles, CA 90024, U.S.A.

When susceptible mouse strains are immunized with the self-constituent myelin basic protein (MBP), primed autoreactive T cells can be readily detected in draining lymph nodes and in the spleen, demonstrating that self tolerance has been broken. However, the immunization does not lead to experimental allergic encephalomyelitis (EAE), unless the animals receive intravenous injections of pertussis toxin in addition. What is the fate of primed autoreactive T cells in animals which do not develop disease? Do they persist as dormant memory cells with the potential of inducing EAE if they later gain access to the brain?

We immunized (B10.PLxSJL)$F_1$ mice with guinea pig MBP (gpMBP) with and without additional pertussis treatment. When the mice were tested 2 months later, a strong proliferative response was recalled by gpMBP in splenocyte cultures of both groups. However, the reactivity to mouse MBP and to peptide 1-9 of MBP (the sequence of which is identical in the mouse and guinea pig) was detected only in animals treated with pertussis. Thus, the anamnestic response to foreign antigenic determinants of gpMBP persisted in mice which did not develop disease, but the reactivity to self determinants disappeared. Currently we are investigating the mechanisms involved in the selective loss of the autoreactive T cell memory.

**C 422** RECOGNITION OF REPTIDES THAT ARE IMMUNOPATHOGENIC BUT CRYPTIC, W.J. Lipham, T.M. Redmond, H. Takahashi, J.A. Berzofsky, B. Wiggert, G.J. Chader, and I.Gery, NEI and NCI, NIH, and Howard Hughes Med. Inst., Bethesda, MD. 20892

Recent studies have identified several determinants of tissue-specific antigens which are immunopathogenic but "cryptic", i.e., are not recognized by lymphocytes sensitized against the whole antigen. In addition, lymphocytes sensitized against these cryptic peptides do not recognize the whole protein in vitro and yet, they must do so in vivo, when initiating the pathogenic process. We have examined this contradiction using peptides derived from the retinal interphotoreceptor retinoid-binding protein (IRBP). Main findings: (1) The response to a cryptic peptide ("R4") was competitively inhibited by an immunodominant determinant ("W10") only when W10 was added at concentrations >100 fold higher than those of R4. This rules out the possibility that whole IRBP is not recognized by R4-specific lymphocytes because of competition by immunodominant determinants. (2) R4-sensitized lymphocytes, which cannot recognize native IRBP in culture, proliferated strongly against preparations of IRBP which were previously digested by endoproteinases, Asp-N,V-8, or Glu-C. This finding thus suggests that recognition of IRBP in the eye is made possible by prior cleavage of the protein by retinal proteinases. (3) Analysis of the stimulation of lymphocytes sensitized against cryptic peptides, by IRBP digested by five defined endoproteinases showed that cleavage fragments of ≤38 residues in length are stimulatory, while fragments of ≥75 residues are inactive. These data thus provide new information concerning the processing and presentation of cryptic determinants.

**C 423** TOLERANCE/NON-TOLERANCE TO TWO T CELL DETERMINANTS ON THE SAME MOLECULE: IMPORTANCE OF PEPTIDE-MHC INTERACTION, David Milich*, Alan McLachlan+, Richard Houghten*, Ben Thornton†, Joyce Jones* and Janice Hughes*, Departments of Molecular Biology* and Molecular and Experimental Medicine+, Scripps Clinic and Research Foundation, La Jolla, CA 92037, and R.W. Johnson Pharmaceutical Research Institute†, San Diego, CA 92121

We utilized a neonatal tolerance model and synthetic peptides representing residues 120-140 of the hepatitis B e antigen (HBeAg) to compare the requirements for T cell tolerance induction as opposed to T cell immunization. Peptide 120-140 contains the dominant T cell sites on HBcAg recognized by the B10.S (p120-131) and B10 (p129-140) strains. Neonatal injection of p120-140 into B10.S mice resulted in complete T cell tolerance to p120-131 and to the entire native HBcAg. In contrast, p120-140 was immunogenic but not tolerogenic in B10 mice. Similarly, injection of p120-140 into (B10.S X B10) $F_1$ mice resulted in tolerization of p120-131-specific, I-$A^S$-restricted T cells, but not of p129-140-specific, I-$A^b$-restricted T cells. Furthermore, the p120-131 immunogenic/tolerogenic T cell site could be converted into a immunogenic/non-tolerogenic T cell site by a single amino acid substitution in either residue 127 or 129. These amino acids represent agretopic residues which interact with I-$A^S$. These results demonstrate that the avidity of a peptide-MHC interaction can influence T cell tolerance induction and suggest a higher threshold of peptide-MHC avidity may be required to induce T cell tolerance as compared to that required to immunize T cells. Additionally, B10.S and $F_1$ transgenic mice have been produced which express HBeAg. Studies of T cell tolerance and autoantibody production in Tg mice confirm and extend the results obtained in the neonatal tolerance system, and will be discussed as a model of autoimmunity.

**C 424** CLASS II-RESTRICTED PRESENTATION OF SELF AND FOREIGN ANTIGENS, Paola Panina-Bordignon, Giampietro Corradin* and Antonio Lanzavecchia, Basel Institute for Immunology, Basel, Switzerland and *Institute of Biochemistry, University of Lausanne, Epalinges, Switzerland.

It is well established that the function of class II molecules is to sample from the endosomal compartment and present to T cells peptides which are generated from the processing of exogenous antigens. We have focussed our studies on three aspects: i) the influence of processing on the T cell repertoire; ii) the effect of Class II polymorphism on peptide binding and T cell recognition; iii) the processing and presentation of self molecules and their recognition by T cells.

We find that antigen presenting cells (APC) from donors with identical class II molecules may differ in their capacity to generate a given epitope, indicating that the immune response is controlled at the level of antigen processing. We also find that some T cell epitopes of tetanus toxin are universally immunogenic, since they can bind to a large number of DR alleles. In this case the T cells may or may not distinguish between the different complexes of the same peptde bound to different DR molecules. These results reveal an unexpected degeneracy both at the level of peptide-class II binding and at the level of T cell recognition.

Finally we find that monomorphic serum proteins are constitutively processed and presented by APCs and are recognized by a high frequency of alloreactive T cells. Thus, the processing and presentation of a large number of self epitopes explains the high frequency of alloreactive T cells.

We will discuss these three aspects in the context of the mechanisms that maintain self tolerance and allow response to foreign antigens.

**C 425** INDUCTION OF MURINE AUTOIMMUNE OOPHORITIS (MAO) BY IMMUNIZATION WITH A PEPTIDE FROM MURINE ZONA PELLUCIDA, ZP3, Kenneth S.K. Tung, Sung Hee Rhim, An-Ming Luo, Terecita Yule, Paul Allen, Frank Robey, Sarah Millar and Jurrien Dean, Department of Pathology, Washington University School of Medicine, St. Louis, MO 63110; Lab of cellular and Developmental Biol. and Lab of Cellular Development and Oncology, NIH, Bethesda, MD 20892.

A 15-mer peptide from murine ZP3 can elicit: 1) antibody response to the zona pellucida, 2) T cell proliferative response and 3) MAO, in B6AF1, A/J and BALB/cBy mice. Thus the peptide possesses both B and T cell epitopes of ZP3. Study of truncated ZP3 peptides showed that the B cell epitope resides in a 7-mer peptide and oophoritogenicity resides in a 10-mer peptide. Partial deletion of the B cell epitope eliminated the peptide's ability to induce antibody response but not T cell response or MAO induction. Moreover, MAO was adoptively transferred to normal recipients by CD4+, peptide-specific T cell lines. Thus for MAO induction, T cells are sufficient whereas antibody is not required. Since antibody to the B cell epitope of the peptide is known to cause infertility, it may be possible to design a contraceptive vaccine that encompasses the B cell epitope of ZP3 without the T cell or the oophoritogenic epitope. On the other hand, T cell response to ZP3 may be important for autoimmune oophoritis encountered in patients with premature ovarian failure.

**C 426** TOLERANCE AND IMMUNITY TO HUMAN C-REACTIVE PROTEIN (hCRP) IN TRANSGENIC MICE: A MODEL SYSTEM FOR AN INDUCEABLE SELF-PROTEIN.

Paul Waterhouse, Rainer Döffinger, Mark.B. Pepys#, Ulrich Rüther* and Bruno Kyewski, Institute for Immunology and Genetics, German Cancer Research Center, and *European Molecular Biology Laboratory, D-6900 Heidelberg, F.R.G., #Immunological Medicine Unit, Dep. of Medicine, Royal Postgraduate Medical School, London W12OHS, U.K.

We are studing the immune response against hCRP in normal C57BL/6 mice and in mice transgenic for hCRP. The hCRP-transgene is under the control of its own promoter which tightly regulates its liver specific expression and is induced by acute phase inducers, e.g. interleukin 6. Transgene expression can be induced from undetectable levels (both mRNA and protein) to a serum protein level of greater than 100 µg/ml. This tight regulation of a "neo self-antigen" offers the possibility to study tolerance and immunity as a function of time- and dose-dependent antigen expression. We will report on the peptide recognition and T cell receptor usage of CRP-specific I-$A^b$ restricted T cell clones; dose-dependent self-antigen presentation in central and peripheral lymphoid organs *in vivo*, and on the correlation between experimentally controlled expression of hCRP and T and B cell tolerance in this model.

## *TcR V Gene Expression in Autoimmunity*

**C 427** T CELL RECEPTOR (TCR)-GAMMA/DELTA EXPRESSION BY PERIPHERAL BLOOD, LYMPH NODE AND TUMOUR INFILTRATING LYMPHOCYTES IN HUMAN BREAST CARCINOMA, Syed M. Alam, P. Whitford, W.D. George and A.M. Campbell, Dept. of Biochemistry, Univ. of Glasgow, Glasgow G12 8QQ, U.K.

The quantitative distribution and phenotype of gamma/delta T-lymphocytes in the peripheral blood (PBL), lymph node (LNL) and tumour infiltrating lymphocytes (TILs) were determined in 11 breast carcinoma patients. Using a monoclonal antibody that detects human TCR-gamma/delta on intact, viable cells and reacts with both the disulfide and the non-disulfide forms of the receptor, the immunophenotypic characteristics of the TCR-gamma/delta positive lymphocyte population was delineated by one and two colour flow cytometric analysis. The gamma/delta + lymphocytes express the T cell lineage antigen CD3 and the number of these cells were variable, but generally small in lymphocytes from all the three sources. The percentage of CD3+ T cells that express TCR gamma/delta are: PBL 1.5 to 14% (median 4.0), TIL 0 to 28% (median 8.5), LNL 3.5 to 33% (median 10.5). Phenotypic analysis of these minority T cells revealed that 1) gamma/delta positive lymphocytes in peripheral blood lack both the CD8 and the CD4 marker; 2) In TILs they were predominantly $CD8^+$ ($>$50% of total CD3 + cells) in 8 cases, while in 3 other cases $CD8^+$ cells were present but in significantly lower numbers ($<$5%). $CD3^+$ gamma/delta lymphocytes in tumour infiltrate were negative for the CD4 marker; 3) lymphocytes from the lymph node were more variable in expressing these markers. In the majority of cases they were double negative (CD8-4-) but populations of $CD8^+4^+$&$CD8^+4^-$ were also detected. It is possible that gamma/delta T cells in the tumour infiltrate may play a role in suppressor/cytotoxic phenomena.

**C 428** OVERLAPPING $V_\alpha$ AND $V_\delta$ USAGE BY TCR $\gamma/\delta$ EXPRESSING THYMOCYTES, Bernhard Arden, Sabine Wehr, H.Robson MacDonald* and Guido Miescher*
Max-Planck-Institute for Immunobiology, Freiburg, Germany,
*Ludwig Institute for Cancer Research, Epalinges, Switzerland.

A subset of adult $CD4^-CD8^-$ thymocytes lacking the B2A2 antigen expresses $\alpha/\beta$ TCRs utilizing predominantly $V_\beta$8.2. 10% of the $B2A2^-$ double negative $CD3^+$ thymocytes express $\gamma/\delta$ TCRs. These preferentially use $V_\alpha$7.1. We are trying to amplify by one-sided PCR RNA from the $B2A2^-$, $\alpha/\beta$ $TCR^+$ subset, to determine whether a particular $V_\alpha$ is found along with the dominant $V_\beta$8.2. In $\alpha$-chain message $V_\alpha$7.1 and 7.2 were always rearranged out of frame to $J_\alpha$, suggesting that at a post-transcriptional level they are selected to be expressed exclusively as $\delta$-chains. In neonatal thymocytes we found predominantly $\delta$-chain transcripts from functionally rearranged $V_\alpha$7.1 that was shown by M.P. Happ et al. to be required in most of the autoreactive and mycobacterial PPD-reactive $\gamma/\delta$ hybridomas from newborn thymocytes, but equally frequent we found $V_\alpha$7.2 in frame. Both were absent in day 16 fetal and adult thymocytes, suggesting a wave of $V_\alpha$7.1 and 7.2 expression around birth. In contrast, all neonatal in frame $\delta$-chain transcripts containing $V_\alpha$7.3 were inactivated through a splice event deleting the major portion of the $V_\alpha$7.3 gene segment. In adult thymus $V_\alpha$7.3 is frequently utilized in functional $\delta$-chain message. We have now begun to study the mechanisms that influence the programmed read out of these V gene segments during ontogeny.

**C 429** LIMITED T CELL RECEPTOR V BETA CHAIN USAGE IN THE BB RAT, Donald Bellgrau and Daniel P. Gold, Barbara Davis Center for Childhood Diabetes, Denver, CO. 80262 and La Jolla Institute for Experimental Medicine, La Jolla, CA 92037

BB rats develop insulin dependent diabetes. Disease is associated with several T cell abnormalities including T cell lymphopenia and a reduced proliferative response to alloantigen. On a cell for cell basis BB T cells continue to proliferate poorly to alloantigen suggesting the proliferative defect may not simply be due to a reduced number of T cells caused by the lymphopenia. Because BB T cells proliferate normally when stimulated with the T cell mitogen concanavalin A we hypothesized that the poor proliferative response is also not due to an innate proliferative defect but rather could simply reflect a reduction in the heterogeneity of the response to alloantigen due to an underrepresentation of certain T cell receptor families. To determine the T cell receptor V beta repertoire, PCR analysis was performed using oligonucleotide primers specific for 17 V beta chains representing 13 families. Marked differences were found when comparing the expressed repertoire of diabetes resistant and BB rats. Several V beta families seemed to be underrepresented in the BB compared to diabetes resistant MHC matched controls. It appears that the BB rat T cell repertoire is limited in its capacity to mount a heterogeneous immune response. The limited T cell repertoire may contribute to the autoimmune phenotype of diabetes prone BB rats.

**C 430** $A^k$ AND $A^u$-RESTRICTED RECOGNITION OF THE AUTOANTIGEN MBP: $A_\beta$ RESIDUES CONTROLLING PEPTIDE BINDING AND T CELL RECOGNITION,
Amelia Black, Animesh Sinha, Christopher Lock, Hugh McDevitt, and Patricia Jones, Departments of Biological Sciences and Microbiology and Immunology, Stanford University, Stanford, CA 94305-5020
We are investigating the roles of the polymorphic residues in class II molecules of the *k* and *u* haplotypes in the presentation of the N-terminal peptide of myelin basic protein (MBP) to $A^u$ and/or $A^k$-restricted T cell clones. Mutant $A_\beta^k$-$A_\beta^u$ genes have been engineered which contain various combinations of the 12 amino acids that differ between *k* and *u*-derived $A_\beta 1$ domains. L cell transfectants expressing the mutant class II molecules have been evaluated for their ability to stimulate MBP-specific T cell clones and for their ability to bind MBP peptides in direct binding assays. In addition, the sequences of the T cell receptors expressed by the *k*-restricted MBP-specific T cell clones are being determined. The *k*-restricted clones use a variety of $V_\alpha$ and $V_\beta$ segments, in contrast to the *u*-restricted T cell clones which exhibit limited TCR heterogeneity.

**C 431** STRAIN DEPENDENT NON-SYMMETRIC DECREASES IN T CELL RESPONSES TO V BETA SPECIFIC SUPERANTIGENS DURING AGING, L. Butler, Lilly Research Laboratories, Indianapolis, Indiana 46285
Recent data has shown that certain bacterial toxins are mitogenic for subsets of T lymphocytes. These toxins appear to stimulate the T lymphocytes in a specific manner, restricted by the genotype of the T cell receptor $V_{beta}$ chain. We have used these toxins as tools to examine the decreased T lymphocyte responses characteristic of lymphocytes from aged mice. We have examined the responses of spleen cells to these toxins from three different strains of mice and compared the responses of spleen cells from aged mice to those of young mice of the same strain. Spleen cells from CBA/CaNNIA and Balb/cNNIA mice showed a 30-60% decrease in responses to SEA, SEB, SEE and TSST. However, spleen cells from CBA/CaNNIA aged mice displayed normal responses to SEC1, SEC 2 and SED while spleen cells from Balb/cNNIA aged mice displayed normal responses to SEC2 and SEC3. In contrast to these strains, spleen cells from C57Bl/6NNIA aged mice showed decreases of 75% or more to all the toxins. While the C57Bl/6NNIA strain displays symmetric loss of responsiveness, the other two strains do not. These results suggest that the loss of T cell receptor $V_{beta}$ chains is not always symmetrical during the aging process and is influenced by the genetic makeup of the strain.

**C 432** UNUSUAL RECOMBINASE ACTIVITY IN AN AUTOIMMUNE MOUSE STRAIN, James J. Campbell and Yasuhiro Hashimoto, Department of Pathology, University of Pennsylvania School of Medicine, Philadelphia, PA, 19104
A novel PCR technique has been developed to assay recombinase activity in T cell subsets from normal and autoimmune mice. Although it is widely assumed that recombination of TCR genes must be halted in mature T cells to maintain the selected repertoire, we have found that lymph node T cells from the C3H-*gld/gld* autoimmune mouse display high levels of recombinase activity. The same population of cells from normal mice has no detectable recombinase activity. Transcription of the Recombinase Activating Genes (RAG-1 and RAG-2) agrees with the observed levels of recombinase activity of these cells. Continuing recombinase activity in mature T cells may have a role in the autoimmune nature of the *gld* syndrome, since new TCR molecules arising in peripheral cells after negative selection would be likely to contain self reactive specificities. In addition, analysis of the DNA joints resulting from recombination events in C3H-*gld/gld* mice yielded unusual sequences.

**C 433** HUMAN T CELL RECEPTOR (TCR) V-BETA REPERTOIRE, Yongwon Choi, Joyce Lafferety, Brian Kotzin, Philippa Marrack and John Kappler. Howard Hughes Medical Institute, National Jewish Center, Denver, CO 80206.
The TCR V-beta usages in human peripheral T cells and the interaction of TCR and bacterial toxin superantigens have been studied using quantitative polymerase chain reaction (QPCR) and mouse T hybridoma cells expressing human-mouse chimeric TCR. By this, we have shown that bacterial toxins stimulate T cells bearing appropriate V-beta elements through the side of TCR away from V-alpha and joining regions. By QPCR, we have noticed that the usage of certain V-betas varies a lot among different individuals. To facilitate the study of change in V-beta usage, we have generated two monoclonal antibodies against human V-beta 13 families. The usage of these V-betas is currently studied.

**C 434** ANALYSIS OF T-CELL RECEPTOR $\gamma\delta^+$ CELLS AND THEIR REACTIVITY TO HSP65 USING TRANSGENIC MICE WHICH SPECIFICALLY BLOCK THE MAIN T-CELL DIFFERENTIATION PATHWAY. Hermann Eibel*, Frank Brombacher*, Petra Fiedler*, Georges Köhler*, and Stefan H.E.Kaufmann#, *Max-Planck-Institute for Immunology, Freiburg, and #Institute for Microbiology, Dept. of Med. Microbiology and Immunology, University of Ulm, FRG.
A subset of $\gamma\delta$ TCR$^+$ T-cells shows a high spontanous specificity for the bacterial heat shock protein ***hsp65***. Recently it was demonstrated, that $\alpha\beta$ TCR$^+$ ***hsp65*** specific T-cells can cross-react with autologous heat shock proteins and may cause autoimmune disease. We therefore are interested whether ***hsp65*** specific $\gamma\delta$ TCR$^+$ T-cells exhibit a similar cross-reactivity. In order to adress this question we use transgenic mice expressing an α-CD8 Ig μ heavy chain gene in all populations of T-cells. The expression of the transgene blocks specifically *in vivo* the main maturation pathway of $\alpha\beta$ TCR$^+$ cells at the stage of CD4$^+$8$^+$ thymocytes. This differentiation block causes a 10-50 fold reduction of the immature CD4$^+$8$^+$ and of the mature CD4$^+$8$^-$ and CD4$^-$8$^+$ populations. It does not affect the maturation of $\gamma\delta$ TCR$^+$ cells. Their population develops normally and is highly enriched in the α-CD8 μ transgenic mice. Unselected T-cell hybridomas were generated from transgenic and control mice and analyzed for the expression of $\alpha\beta$ and $\gamma\delta$ TCR chains. As expected, we found a high increase for $\gamma\delta$ TCR$^+$ hybridomas with T-cells from transgenic mice (30% in transgenic, <1% in control fusions). Currently they are tested for their specificity for bacterial ***hsp65*** and their crossreactivity to autologous heat shock proteins.

**C 435 Role of JB in the Positive Selection of VB11+ CD8+ T Cells**
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We have previously shown that non-MHC encoded genes affect the fraction of murine T cells which bear Vβ11 encoded T cell receptors. Thus, in DBA/2 (H-2d) mice, 3.2% of the CD8+ cells bear Vβ11 T cell receptors while in B6.D2 (also H-2d) the corresponding number is only 1.5%. This difference is more pronounced in the recombinant inbred strain BxD28 (also H-2d) which expresses Vβ11 on 4.5% of its CD8+ T cells. This effect is likely due to more efficient postive selection in DBA/2 and BxD28 as evidenced by its dominance in a (BxD28 x B10.D2)F1 mouse. We are currently investigating the role of Jβ gene segments in mediating the increased positive selection of CD8+ Vβ11+ T cells in BxD28 and DBA/2 mice.

**C 436** LACK OF Vβ17 EXPRESSION BY AUTOREACTIVE T CELLS OF (MRL/*lpr* x SJL/*lpr*)F1 MICE, Rachel C. Ettinger*, Ekram El-Laban*, Deborah Ahern†, Man-Sun Sy‡, and Ann Marshak-Rothstein†, Department of †Microbiology and *Pathology, Boston University School of Medicine, Boston, MA 02118, ‡Department of Pathology, Massachusetts General Hospital and Harvard Medical School, Charlestown, MA 02129.

Mice expressing the recessive mutation *lpr* develop a systemic autoimmune disease characterized by a variety of T and B cell immunoregulatory defects. Work from this and other laboratories has demonstrated that class II-restricted autoreactive T cells (ART) readily develop from high density cultures of young MRL/*lpr* or (MRL/*lpr* x SJL/*lpr*)F1 lymph node cells (LNC). These ART do not grow out of LNC from the congenic MRL/+ strain and may play a significant role in the etiology of autoimmune disease. It has been shown that $V\beta17^+$ T cell receptors recognize I-E class II molecules, and in mice that express I-E molecules, self reactive Vβ17-bearing T cells are clonally deleted in the thymus. To test the hypothesis that the prevalence of *lpr*-derived ART is due to T cells that prematurely leave the thymus and thereby escape negative selection, T cell receptor expression was examined by flow cytometry using the Vβ17 specific monoclonal antibody KJ23a. It was found that the (MRL/*lpr* x SJL/*lpr*)F1 ART did not express Vβ17, indicating that the high frequency of ART present in the lymph nodes of young *lpr* mice cannot simply be ascribed to a defective thymic education process.

**C 437 A SINGLE AMINO ACID SUBSTITUTION IN THE PUTATIVE CDR1 OF THE TCR Vβ3 PROTEIN PROFOUNDLY ALTERS TCR USAGE IN AN ANTIGEN-SPECIFIC MHC-RESTRICTED IMMUNE RESPONSE** Sara-Jo Gahm[1,2], B.J. Fowlkes[3], Osami Kanagawa[4], Stephen C. Jameson[5], Nicholas R.J. Gascoigne[5], Louis A. Matis[2]; [1]HHMI-NIH Research Scholar; [2]DCB, CBER, FDA, Bethesda, MD 20892;[3]LCMI, NIAID, NIH, Bethesda, MD 20892; [4]Washington University School of Medicine, St. Louis, MO 63110; [5]Dept. of Immunology, Research Inst. of Scripps Clinic, La Jolla, CA 92037. The αβ T cell receptor (TCR) that mediates MHC-restricted antigen recognition is a heterodimer encoded by V gene segments rearranged to (D), J, and C regions. Previous work has shown that most $E^k$-restricted, pigeon cytochrome c (p. cyt.)-specific T cells from B10.BR (H-$2^k$, Mls-$2^b$) and B10.A (H-$2^a$, Mls-$2^b$) mice express a $V\alpha11/V\beta3$ heterodimer. We have shown that p. cyt.-specific $E^k$-restricted T cells from H-$2^k$, Mls-$2^b$ C57BR mice may use either Vα11 or Vβ3, but that, in contrast to B10.BR and B10.A, the two chains rarely appear as a heterodimer in the C57BR response. This does not result from an inability of Vα11 and Vβ3 to pair in the C57BR strain, since $V\alpha11/V\beta3$ heterodimers appear with equal frequency among unselected $CD4^+$ peripheral T cells in B10.BR and C57BR mice. Pigeon cytochrome c-specific lines from (C57BR x B10.BR) $F_1$ mice express $V\alpha11/V\beta3$, also ruling out negative selection of p. cyt.-specific $V\alpha11/V\beta3$ receptors in C57BR mice. The Vα11 families of C57BR and B10.BR mice are similar, both by RFLP and serological analyses. However, the Vβ3 alleles of these strains encode proteins that differ by a single amino acid residue (31, V->F) in a region corresponding to the putative CDR1 of the TCRβ chain. To explore the influence of this TCRβ polymorphism on receptor expression, we examined TCR usage in $E^k$-restricted p. cyt.-specific T cell lines from B10.A TβL mice, which have the TCRβ locus of C57L mice ($V\beta^a$) bred onto the B10.A strain ($V\beta^b$). The B10.A TβL lines, like C57BR, fail to express $V\alpha11/V\beta3$ heterodimers. The profound influence of an amino acid residue on receptor usage in an antigen-specific, MHC-restricted immune response supports models that propose an Fab-like conformation of the TCR.

**C 438** DIRECT BINDING OF SOLUBLE T CELL RECEPTOR β-CHAIN TO SUPERANTIGEN-MHC CLASS II COMPLEX, Nicholas R.J. Gascoigne, Michael J. Irwin and Kristina T. Ames, Department of Immunology, Research Institute of Scripps Clinic, La Jolla, CA 92037

Both α and β-chains of the T cell receptor (TCR) are required for the recognition of peptide antigen plus MHC. The "superantigens" are a group of molecules that differ from standard peptide antigens in that they appear to act specifically through recognition by the TCR β-chain. We have used a soluble form of the TCR β-chain to investigate the involvement of the β-chain in recognition of a superantigen, staphylococcal enterotoxin A (SEA), complexed to cell-surface MHC class II. We have shown that this interaction is specific for the enterotoxin and that it requires class II expression by the cell. The reaction can be inhibited by antibodies against each of the components of the reaction; class II, SEA and Vβ. This demonstrates that the TCR β-chain is sufficient for interaction with a superantigen-MHC class II complex and that the α-chain and CD4 or other molecules are not required. We will use this interaction to estimate the TCR affinities required to cause deletion of thymocytes in tolerance induction.

**C 439** RESTRICTED V GENE USAGE IN PERIPHERAL T CELLS FROM MYASTHENIA GRAVIS PATIENTS. Johan Grunewald[1], Richard Åhlberg[2], Ann-Kari Lefvert[2], Hans Wigzell[1] and Carl Harald Janson[1]. Department of Immunology, Karolinska Institute[1], Box 60400, S-104 01 Stockholm, Sweden. Karolinska Hospital[2], Stockholm, Sweden.

To determine the extent of clonal heterogeneity of the T cells in patients suffering from Myasthenia Gravis (MG), we used 8 recently available MAb, directed against different α and β V gene products of the variable part of the TCR and covering approximately 25 % of the α/β T cells in normal PBL. Using a two-colour immunofluorescence method, we could calculate the expression of α/β V gene segments within the two major T-cell subsets, the MHC class I restricted $CD8^+$ and the MHC class II restricted $CD4^+$ cells. By comparing these reactivities between 15 MG patients and 24 healthy blood donors, 27% (4/15) of the MG patients showed distinct signs of clonal T cell expansions. Furthermore, among these T cells, a highly restricted V gene usage could be seen, with a preferential usage of the β V 12 gene segment product. Our data thus strongly suggest that the majority of MG patients will have abnormally expanded T cell clones.

**C 440** **DEVELOPMENT OF A SOLUBLE T CELL ANTIGEN RECEPTOR.** **Colin R.A. Hewitt and Mike Owen. ICRF London. UK.**

Exploitation of synthetic peptide antigens (Ag) and the functional polymorphism of MHC, has led to development of structural models for the interaction of MHC with Ag. Models predicting the structure of TcRs, and their interactions with MHC-Ag complexes, have proved more difficult to define. To address this problem we have developed a bacterially expressed soluble TcR from a human T cell clone HA1.7 which recognises influenza haemagglutinin peptide 319-324 in the context of HLA-DR1. HA1.7 TcR α and β chain cDNAs were cloned by anchored PCR. The α and β V(D)J regions were then fused 3' of leader sequences from the bacterial pectate lyase gene (pelB) to facilitate the translocation of the TcR proteins from the bacterial cytosol to the periplasmic space where folding and hetero-association of protein subunits occurs. Dicistronic α–β, and monocistronic α and β constructs were assembled in pUC19 and expressed by induction of the lacz promoter. TcR proteins were detected by immunoblotting with an antibody recognising a "myc-tag" introduced 3' of the β VDJ regions. Results demonstrating correct folding, heterodimerisation and function of the soluble TcR will be presented.

**C 441** CLONAL INFILTRATES OF ACTIVATED Vβ17+ T CELLS IN SYNOVIAL TISSUES OF RHEUMATOID ARTHRITIS PATIENTS. Mark D. Howell, Jocelyn P. Diveley, Abby Esty, Katherine A. Lundeen, Steven T. Winters, Dennis J. Carlo and Steven W. Brostoff, The Immune Response Corporation, San Diego, California, USA 92121.

Rheumatoid arthritis (RA) is thought to be mediated by T cells reactive with synovial antigen(s) and major histocompatibility molecules. The identification and characterization of T cells responsible for the pathogenesis of RA, however, has remained elusive. We have analyzed T cells infiltrating synovial tissue specimens obtained, during joint replacement therapy, from proven rheumatoid arthritis patients. We find clonal infiltrates of activated, Vβ17+ T cells in each of five synovial specimens analyzed. Moreover, we have isolated, from one of these patients, CD4+, Vβ17+ T cell clones that display <u>in vitro</u> cytotoxicity for autologous synovial adherent cells. The presence of these T cells in the diseased tissue of multiple patients, their clonality, and their cytotoxicity for synovial adherent cells, argue a central role for Vβ17-bearing T cells in the pathogenesis of RA.

**C 442** SELF REACTIVE T CELLS FROM NZB/NZW MICE. Joan L. Klotz, Dale Ando and Mitchell Kronenberg. Department of Microbiology & Immunology, University of California at Los Angeles, Los Angeles, CA, 90024-1747.

T lymphocytes appear to play an important role in the development of the systemic autoimmunity which occurs spontaneously in NZB/NZW ($H\text{-}2^d/H\text{-}2^z$) mice. We have isolated ten autoreactive T cell clones from three NZB/NZW females, and have been investigating the phenotypic and functional characteristics of these cells. Our data suggest a restricted useage of Vb gene segments. Eight of the ten T cell clones express the Vb8.3 gene segment in association with Jb1. Studies are in progress to determine the MHC restriction element recognized, and the cell types capable of activating the T cell clones to proliferation. The T cells analyzed to date are restricted to $IA^d$. They are capable of stimulating $H\text{-}2^d$ B cells to secrete immunoglobulin of several isotypes particularly IgM and IgG1. Smaller resting B cells as well as larger cells can be stimulated to Ig secretion by these autoreactive T cell clones. Some of the clones are activated by mitomycin c-treated A20, a BALB/c $IA^d$-positive B cell lymphoma. A second BALB/c B cell lymphoma line which also expresses $IA^d$, 2PK3, lacks the ability to activate these T cells. Thus, these T cells recognize a self antigen expressed in association with $IA^d$ on some, but not all, B cells. These cells may be relevant to pathogenesis in NZB/NZW mice by causing a polyclonal stimulation of B cells and hypersecretion of Ig.

**C 443** **The Role of Cell Mediated Immunity in the Pathogenesis of Poststreptococcal Autoimmune Disease**
Malak Kotb, Mark A. Tomai, Gipsy Majumdar, and Edwin H. Beachey.
*The University of Tennessee, Memphis and The VA Medical Center, 38104.*

*Streptococcus pyogenes* can, in certain individuals initiate autoimmune diseases such as acute rheumatic fever and acute glomerulonephritis. The surface M Protein of group A streptococci are believed to play an important role in the pathogenesis of these poststreptococcal diseases. Clinical and experimental evidence indicate that cell mediated immune responses to M protein may be responsible for the pathogenesis of poststreptococcal auotoimmune diseases. We studied the T cell response to pepsin extracted type 5 M protein (pep M5) because it is the most frequently isolated serotype from outbreaks of rheumatic fever. Our studies revealed that M protein stimulates T cells via a nonpolymorphic receptor that is shared by 5-10% of these cells. The stimulation of T cells by pep M5 was mediated via the TCR because: one, CD3 was modulated from the surface of T cells stimulated by pep M5; two, the proliferative response was blocked by anti-CD3 antibodies; three, a $CD3^-$ mutant of a T cell line failed to respond to pep M5 whereas its $CD3^+$ counterpart responded; and four, the response of T cells to pep M5 was inhibited by cyclosporin A. Although pep M5 stimulated both CD4 and CD8 T cells, subsets of T cells bearing Vß2 and Vß8 sequences were preferentially expanded. T cell activation was dependent on the presence of antigen presenting cells (APC) which express HLA class II antigens. M protein binds to HLA class II molecules on human APC as well as mouse fibroblasts transfected with human HLA class II molecules. The presentation of pep M5 to T cells is not MHC restricted and the requirement for APC can be bypassed by costimulatory signals provided by IL-1, IL-6 and the phorbol ester, PMA. In the absence of these costimulatory molecules, pep M5 failed to induced $Ca^{++}$ mobilization in purified T cells; whereas, in their presence, pep M5 bound to 5% of resting T cells and induced a significant rise in intracellular $Ca^{++}$. Under these conditions pep M5 stimulated the same T cell subsets with preferential expansion of the same Vßs stimulated in the presence of APC, namely those bearing Vß 2 and 8. This indicates that pep M5 interacts directly with the TCR and that it utilizes specific Vßs. Our data show that M protein stimulates human T cells in a superantigenic manner. The superantigenecity of M protein provides an alternative explanation for its role in poststreptococcal diseases. Self reactive T cells that may have escaped tolerance, and which bear the same Vß elements that interacts with the M protein may be expanded beyond a threshold leading to autoimmunity. Supported by research funds from the U.S.Veteran's Administration and funds from NIH GM-3580, MK.

**C 444** TCR USAGE OF AUTOREACTIVE T CELL SPECIFIC FOR HUMAN THYROID AUTOANTIGENS.

M. Londei, S. Quaratino, C. Dayan, M. Feldmann. The Charing Cross Sunley Research Centre, Lurgan Avenue London W6 8LW London, UK.

The definition of the T cell receptor (TCR) repertoire of autoantigen specific T cells clones is of extreme importance to possible therapeutic approaches in autoimmunity. Recently we have established a panel of autoreactive T cell clones obtained from the thyroid of a patient with Graves' disease. We could detect T cell clones specific to Thyroid peroxidase (TPO) which is the classical thyroid microsomal antigen. For some of the TPO specific clones it has been possible to determine the specific peptide recognized. T cells recognizing the first epitope, NP7, use Vβ1 in their TCR, cells recognizing the B6 epitope use Vβ2 in their TCR. We are analyzing the TCR usage of the other T cell clones both TPO specific, and thyroglobulin specific, in order to establish if a restricted usage of Vβ genes is observed in T cell clones recognizing the these autoantigens.

**C 445** **COLLAGEN INDUCED ARTHRITIS (CIA): ROLE OF Mls REACTIVE T-CELLS**, Harvinder S. Luthra, Gary D. Anderson and Chella S. David, Departments of Internal Medicine (Rheumatology) and Immunology, Mayo Medical School, Rochester, MN 55905.

Previously we have shown that inbred strains of mice of the H-$2^q$ or H-$2^r$ haplotype are susceptible to CIA, when immunized with native type II collagen. The prototypic animals used in these studies were B10.Q and B10.RIII animals respectively. In addition it was shown that animals of the same haplotype but with T-cell receptor gene deletions were resistant (SWR and RIII S/J respectively). The role of TCR $V_\beta$ genes was demonstrated by gene complementation analysis. Along with genomic deletion, clonal deletion can also restrict the T-cell repertoire of an animal. Clonal deletion occurs when the T-cell receptor interacts with self gene products like Mls, presented by self MHC.

In order to understand the role of Mls in CIA we studied two Mls congenic strains, BALB/c and BALB.D2.$Mls^a$. These two strains differ at the Mls-$1^a$ locus. Crosses of these with B10.Q were immunized in the traditional fashion with native chick type II collagen in Freund's complete adjuvant. The results are shown in the Table.

| | Cross | Mls | Incidence | Day of Onset | Antibody |
|---|---|---|---|---|---|
| EXP 1 | BALB/c x B10.Q | b | 12/13 (92%) | 31.2 ± 3.2 | 44.7 ± 8.4 |
| | BALB.D2.Mls x B10.Q | a | 7/13 (54%) | 43.6 ± 2.6 | 18.5 ± 6.4 |
| EXP 2 | BALB/c x B10.Q | a | 10/11 (91%) | 37.2 ± 3.6 | 34.5 ± 8.9 |
| | BALB.D2.Mls x B10.Q | b | 5/12 (42%) | 49.2 ± 7.2 | 14.4 ± 4.1 |

In addition, using FACS analysis we were able to show deletion of $V_\beta$ subsets including $V_\beta$8.1, 6, 7 and 9 in BALB.D2.Mls x B10.Q. We conclude that Mls reactive T cells play a role in CIA and that clonal deletion of these cells leads to decreased incidence and delays onset of disease.

**C 446** NON-OBESE DIABETIC (NOD) MICE DELETE EFFICIENTLY BOTH Vß3$^+$ AND Vß17$^+$ T CELLS IN THE ABSENCE OF AN MHC CLASS II I-E MOLECULE, Marcia McDuffie and Barbara Reitz, Barbara Davis Center for Childhood Diabetes, University of Colorado Health Sciences Center, Denver, CO 80262

The non-obese diabetic mouse (NOD) is a model for human Type I diabetes. As is postulated for human insulin-dependent diabetes, diabetes in the NOD mouse is an autoimmune disease caused by T-cell-dependent destruction of pancreatic insulin-producing cells. In order to investigate the role of the T cell repertoire on the development of diabetes in this model, the controls operating on thymic selection in the NOD mouse have been examined in NOD/"Bdc" mice as well as in (C57L/J X NOD)F1, intercross, and backcross mice. Analysis of the use of 13 Vß segments in these mice indicated the expression of powerful non-MHC deleting elements analogous to the $Mls^c$ factors previously described for other mouse strains. $Mls^c$ factors have been confirmed in the NOD background by mixed lymphocyte culture using cells from (H-$2^d$ X NOD)F1 animals. In addition, cells from these animals have been shown to stimulate $Mls^c$-reactive T cell hybridomas (gift of A. Pullen, Denver, CO). The presence of these $Mls^c$ factors results in severe deletion of both Vß3$^+$ and Vß17$^+$ T cells in NOD and (C57L/J X NOD) backcross mice, respectively. In non-diabetes-prone mouse strains, severe deletion of T cells bearing these Vß segments has only been seen in the presence of an I-E molecule. The relevance of this deletion to diabetes among backcross animals is being explored.

**C 447** T-CELL V BETA REPERTOIRE IN PATIENTS WITH RHEUMATOID ARTHRITIS, Xavier Paliard, Brian Kotzin, Yongwon Choi, Sterling G. West, John Kappler and Philippa Marrack. Howard Hughes Medical Institute, National Jewish center, Denver, CO80206.

Rheumatoid arthritis (RA) is an autoimmune disease associated with HLA-DR4 alleles. However, since this association is incomplete, T-cells bearing certain V betas have been thought to be implicated as playing a critical role in the pathogenis of RA.

Using a quantitative PCR technique utilizing V beta specific oligonucleotide primers, we compared the V beta repertoire in the peripheral blood and the synovial fluid from patients with RA. As the synovium from healthy donors contains almost no T-cells, the V beta repertoire in the blood and synovial fluid of patients with Reiter's syndrome was also investigated as a control.

It has been observed that RA synovial fluids contain T-cell bearing almost all known V beta genes, but that this V beta repertoire is skewed in favor of a particular V beta gene. This indicates that some T-cells bearing a certain V beta seem to be specifically recruited to the synovium and to be playing a critical role in RA. In control synovial fluids from patients with Reiter's syndrome, similar V beta repertoire alterations were not observed.

These data demonstrate an apparent oligoclonal expansion in rheumatoid synovial fluids and may lead to a better understanding of the immunological mechanisms involved in the development of this disease.

**C 448 Double Negative(CD4$^-$CD8$^-$)$\alpha\beta^+$ T Cells of Murine Lungs,** Ramanujam Rajasekar, Department of Medicine, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO 80206.

The double negative ( CD4$^-$CD8$^-$ ) $\alpha\beta^+$ T cells constitute about 20% of all $\alpha\beta^+$ T cells in murine lungs. We find that in BALB/c mice, 60% of the double negative $\alpha\beta^+$ pulmonary T cells express receptors of the V$\beta$8 family whereas only 33% of single positive (CD4$^+$/CD8$^+$) pulmonary T cells express V$\beta$8. This difference is mainly due to a higher frequency of T cells expressing V$\beta$8.2$^+$ receptors. However, in C57BL/6 mice, equal frequencies (25%) of double negative and single positive $\alpha\beta^+$ pulmonary T cells express V$\beta$8. Further studies indicate that the high frequency of double negative V$\beta$8$^+$ pulmonary T cells, particularly of V$\beta$8.2$^+$ T cells, is dominantly inherited and is not due to positive selection events involving classical MHC region gene products. Upon exposure to mycobacterial antigens, double negative $\alpha\beta^+$ pulmonary T cells are coenriched *in vitro* in parallel with $\gamma\delta^+$ T cells.

**C 449** PREFERENTIAL UTILIZATION OF T-CELL RECEPTOR $\beta$ CHAIN V, J, AND C GENE SEGMENTS BY HUMAN PERIPHERAL T LYMPHOCYTES. MA Robinson, Laboratory of Immunogenetics, NIAID, NIH, Bethesda, MD 20892

Certain T cell receptor (TCR) $\beta$ chain constant (C), joining (J), and variable (V) gene segments are preferentially utilized by peripheral T lymphocytes in the assembly of functional TCR genes. Genes in each category (V, J and C) were found to be expressed by PHA stimulated peripheral blood lymphocytes from a normal healthy adult individual at frequencies significantly greater than expected on the basis of the number of gene copies. A TCR $\beta$ cDNA library was constructed from RNA using the polymerase chain reaction with anchored primers and primers specific for the TCR $\beta$ C region. Sequences were determined for 88 distinct clones and the frequency of V, J, and C genes was ascertained. C$\beta$2 predominated; 78% of the clones were positive for C$\beta$2. The frequency of utilized J$\beta$ genes was likewise skewed; of the C$\beta$2 positive clones, 39% utilized J$\beta$2.1, 29% utilized J$\beta$2.7 and none used J$\beta$2.4. Of the C$\beta$1 positive clones, 32% utilized J$\beta$1.2 and 32% utilized J$\beta$1.5. No precedent for selective use of J$\beta$ genes has been reported. Seventeen of the 20 reported V$\beta$ genes were found in this sample. In addition, clones representing 4 new V$\beta$ gene families were found. Five V$\beta$ families (V$\beta$1, V$\beta$2, V$\beta$3, V$\beta$4, and V$\beta$7) were utilized at frequencies more than 2.5 times that expected on the basis of their frequencies in the gene complex. There was no apparent reason for the observed preferential TCR$\beta$ gene usage; the blood donor was healthy and not likely to be responding to bacterial superantigen stimulation. It will be of interest to determine what genetic and somatic factors influence the preferential use of TCR $\beta$ genes by peripheral T lymphocytes and to assess the impact that selective expression of TCR$\beta$ genes may have on health and susceptibility to disease.

**C 450** THE T CELL RECEPTOR REPERTOIRE INFLUENCES THE SPERM WHALE MYOGLOBIN T CELL REPONSE, Giovina Ruberti, Amitabh Gaur, Alexandra Livingstone and C. Garrison Fathman, Dept. of Medicine, Div. of Immunology, Stanford University School of Medicine, Stanford, CA 94305.

T cell clones recognizing the Sperm Whale Myoglobin (SpWMb) 110-121 peptide (Ag) in association with IE$^d$ MHC class II molecules display a very limited heterogeneity of T cell receptor (TCR) V$\beta$ elements in DBA/2 mice. All the clones use the same V$\beta$ 8. 2 gene segment and a very restricted junctional regions. To investigate the significance in vivo of this observation we have isolated V$\beta$8$^+$ and V$\beta$8$^-$ helper T cell populations from mice immunized with the Ag. Under these conditions, contrary to long-term T cell culture, very little selection occurs for T cell clones that can adapt to tissue culture. Only the V$\beta$8$^+$ T cells showed a significant response (either IA$^d$ or IE$^d$ restricted) to the Ag. Thus we showed that the T cell clones derived in vitro accurately represent the immune response in vivo. Mice, which as a consequence of antibody (Ab) depletion or genomic deletion (DBA/2 V$\beta^a$) do not express V$\beta$8 gene segment were instrumental in analyzing the impact of the TCR repertoire on the immune response. The DBA/2 V$\beta^a$ mice have a very restricted TCR V$\beta$ repertoire nevertheless they seem to respond better to the Ag when compared with DBA/2 mice. V$\beta$8 Ab depleted mice do respond to the same epitope but only at higher antigen concentrations. The influence of the TCR repertoire in DBA/2, DBA/2V$\beta^a$ and V$\beta$8 Ab depleted DBA/2 mice on the T cell response will be discussed.

**C 451** POSITIVE SELECTION OF HOMOGENEOUS SELF REACTIVE TCR α-CHAIN IN MICE

M. Taniguchi and H. Koseki, Division of Molecular Immunology, School of Medicine, Chiba University, Chiba, Japan.

TCR α-chain isolated from KLH-Ts hybridoma has been characterized by PCR and RNase protection assays. Most (12/13) of KLH-Ts used the same TCR α-chain composed of V14J281 with one-base N-region. As the N-region is consisted of the third base of the codon "glycine", any nucleotide addition in the N-region becomes glycine. Surprizingly, this α-chain is found to dominate at hight level (1.5% of total α-chians) in all laboratory unprimed strains, independent of H-2, Qa-2, Tla, Qa-1, Q10 and Hmt, and also in same wild mice, such as *M.m.domesticus* and *M.m.castaneus*, but not in *M.m.musculus* and *M.m.molossinus*. The frequency of the α-chain expression is $10^5$ times higher than was expected, because the diversity of TCR α-chains is calculated to be $10^8$. The predominant expression of this α-chain seems to be due to positive selection, because 1) The V14J281 expression increases in single positive mature T cells in periphery (>90% of $V14^+$α-chains are this unique α-chain) than in double positive immature thymocytes. 2) The α-chain expression is hardly seen in neonatal stage, but increases and reaches maximum at around 5-8 wks old. 3) Bone marrow chimera (*M.m.molossinus* →C57BL/6) showed that stem cells from *M.m.molossinus* negative for V14J281 α-chain expression do generate this α-chain in C57BL/6 environment. The above results also suggest that the ligand involved in the positive selection of this α-chain is an unknown self molecule with monomorphic in nature, thus indicating that the KLH specificity observed in original Ts seems to be due to the cross-reaction with self molecule. Additional observations, such as the expression of the homogenous V14J281 α-chain in athymic mice, suggest that the positive selection of $V14^+$ T cells occurs extrathymically.

## *T-T, T-B, and B-B Regulatory Interactions*

**C 452** VETO-LIKE DOWN-REGULATION OF T-HELPER CELL REACTIVITY IN VIVO BY INJECTION OF SEMIALLOGENEIC LYMPH NODE CELLS.

Mogens H. Claesson and Thomas Tscherning. Laboratory of Experimental Immunology. Institute of Anatomy A, University of Copenhagen. Copenhagen. Denmark

The aim of the present work was to study the effect on T-helper cell functions in mice injected with semiallogeneic cells. This procedure is known to down-regulate or veto recipient cytotoxic T-cell precursors (CTLp) with specificity for the semiallogeneic donor MHC class I antigens, but its effect on recipient T-helper cell functions remains unclear. (BALB/c x C57BL/6)F1 lymph node cells ($10^7$) were injected iv. into C57BL/6 mice and two days later lymph node and spleen cells from recipient mice were used as responder cells in a MLC against irradiated BALB/c and C3H cells. Cytotoxicity was tested at day 4 and IL-2, IL-3 and IL-4 production was assayed at day 1 to 4 of culture. As expected, the cytotoxicity against BALB/c target cells was decreased by more than 75% as compared to control mice injected with syngeneic cells whereas no inhibiton was observed against third party target cells. Addition of growth factors (ConA-SN) to the MLC did increase the level of cytotoxicity against BALB/c cells although this reactivity remained significantly reduced. Moreover, we found that the MLC responder cells secreted far lower amounts of lymphokines than did responder cells of control mice, whereas no effect on lymphokine secretion was observed when recipient cells were stimulated with third party C3H cells.

**C 453** CYTOKINE-MEDIATED DOWN REGULATION OF CELL-MEDIATED IMMUNE RESPONSES IN VITRO, Bruce H. Devens, Chigusa Terajima, Andrea W. Koontz, Meridith Kelly, and David R. Webb, Institute of Immunology and Biological Sciences, Syntex Research, Palo Alto, CA 94304.

Cytokines exert a variety of biological effects in the generation of immune responses including inhibitory effects. We have investigated the role of interleukin-2, as well as other cytokines, in the non-specific down regulation of in vitro CTL immune responses. Stimulation of murine splenocytes with IL-2, interferons-α or γ, but not IL-1,3,7,10 or TNF-α or TGF-β results in the generation of cells that can be added to mixed lymphocyte cultures and will block the induction of cytolytic T cell activity. The generation of non-specific down regulation is blocked by antibody to the IL-2 receptor (7D4). Antibody to soluble immune response suppressor (SIRS) inhibits the cytokine-induced down regulation. Generation of the non-specific suppression requires CD4+ and CD8+ T cells as well as antigen presenting cells. Once suppression has been generated, suppressive function is seen only with CD8+ T cells. These data clearly show that cytokines, in particular IL-2, previously thought to be involved in growth stimulation also have the capacity to induce the converse response.

**C 454** T CELL RECEPTOR GENES IN A T CELL HYBRIDOMA PRODUCING A DNP/H-2 $K^d$-SPECIFIC IMMUNOREGULATORY MOLECULE, Robert L. Fairchild, Ed Palmer, and John W. Moorhead, Research Inst., Cleveland Clinic Fdn, Cleveland, OH 44195, Natl. Jewish Ctr. for Immunol and Resp. Med., Denver, CO 80206, and Univ. Colorado Sch. Med., Denver, CO 80262

DNP/class I MHC-specific molecules produced by Lyt $2^+$ T cells from dinitrobenzene sulfonate-primed mice inhibit the ability of immune T cells to transfer DNP-specific contact sensitivity. Structural and serological studies of these secreted effector molecules have indicated that they are disulfide-linked dimers bearing T cell receptor (TcR) $\alpha$ and $\beta$ chain determinants. A T cell hybridoma, MTs 79.1, constitutively produces DNP/$K^d$-specific suppressor molecules that are bound by anti-V$\beta$8 and by anti-C$\alpha$ antibodies. Northern and Southern blot analyses have indicated that MTs 79.1 uses the V$\alpha$4 and V$\beta$8 genes to encode the surface TcR. Deletion of either the V$\alpha$4 or the V$\beta$8 gene from MTs 79.1 results in loss of surface receptor expression and ability to produce the soluble suppressor molecule. To further assess the role of the T cell receptor $\beta$ chain in the composition of these immunoregulatory molecules, we have cloned the MTs 79.1 $\beta$ chain gene. A cDNA encoding a membrane form of the $\beta$ chain was inserted into a Moloney virus-driven expression vector and used to transfect $\beta$ chain-deletion variants of the MTs 79.1 parent hybridoma. Although surface TcR expression was absent and mRNA levels of the transfected gene were undetectable by Northern blot analysis, integration and transcription of the $\beta$ chain clone was demonstrable by Southern blot analysis and PCR amplification of cDNA from the transfectants. Preliminary results indicate that the transfectants are reconstituted in the ability to produce the MTs 79.1 suppressor molecule. Supported by UPHS grants AI-17873 and AI-12993.

**C 455** AUTOLOGOUS ANTI-T CELL RECEPTOR RESPONSES, Nina Genthe and David Eckels, The Blood Center of Southeastern Wisconsin and the Medical College of Wisconsin, Milwaukee, WI 53233.

Alloreactive anti-DR1 $CD4^+$ T lymphocyte clones (TLCs) have been established and used to stimulate autologous peripheral blood lymphocytes (PBLs). Attempts to clone the responding T cells were unsuccessful. Primed T lymphocyte lines (PLTs) were generated, however, they did not proliferate beyond 2-3 weeks in culture. Addition of antigen presenting cells (APCs) had no effect. Thus, consecutive primary cultures were set up and responding PLTs were further tested in secondary assays. The PLTs responded to the priming-TLC but not other autologous TLCs expressing the same MHC molecules but different T cell receptors (TCRs). Cells responding to a difference in TCR molecules are said to be "anti-idiotypic" in nature. The anti-idiotypic response was blocked by anti-HLA-DR antibody bound to the priming-TLC. Reciprocal stimulation of the TLC by the PLT did not occur. These results support the hypothesis that a T cell recognizes TCR idiopeptides presented by autologous DR molecules on the surface of another T cell. In promotion of this hypothesis, TCRs from the priming-TLC and other TLCs were immunoprecipitated and purified. These TCR proteins will be used to determine if anti-idiotypic T cell lines respond to TCR protein of the priming-TLC and not other TLCs, when presented by autologous APCs. Further analysis will address the necessity of processing and presentation of TCR protein by APCs. In phenotypic and functional studies, the PLT displayed a relative increase in $CD8^+$ cells when compared to PBLs, although they were not cytotoxic to the priming-TLC. The T cell lines were $CD45^-/CD29^+$ and in co-culture assays, they augmented the response of the priming-TLC to alloantigen. Further immunoregulatory studies are underway. In summary, there is a strong anti-idiotypic T cell response in a human immune response to alloantigen. Responding T cells are short-lived and as a whole population, display an equivocal function.

**C 456** MOLECULAR ANALYSIS OF ANTIGEN RECOGNITION BY Ts CELLS, Ellen Kraig, Ann M. Minter, Kimberly A. Zborowski, Renee Brown, Carl W. Pierce, Judy A. Kapp, and David R. Webb, Department of Cellular and Structural Biology, Univ. Texas Health Science Center, San Antonio, TX, 78284-7762.

We have shown that some Ts hybrids do not use TCR genes to encode their antigen receptors. Thus, we have pursued a serological approach to identify genes that encode antigen-binding suppressive molecules secreted by these cells. A Ts cDNA library was screened with a panel of monoclonal antibodies, including anti-I-J, anti-Tsu, and anti-TsF reagents. Twenty five serologically-reactive cDNA clones were selected and used as probes on RNA and DNA blots; most detected RNAs of very low abundance as expected for Ts antigen receptor. Interestingly, one of these clones, designated pS35, did hybridize to a gene that rearranged in several Ts hybridomas. We have determined the sequence of the cDNA and it would appear not to be one of the known Ig or TCR genes. Thus, we have preliminary evidence for a novel rearranging gene that may encode an antigen receptor on Ts cells. Currently, we are: (1) isolating genomic clones for germline and rearranged genes homologous to pS35; (2) using panels of somatic cell hybrids to map the murine and human chromosomes on which pS35 resides; (3) isolating overlapping cDNA clones; and (4) using the pS35 clone to purify sufficient quantities of the Ts fusion protein to generate a specific polyclonal antibody.

Supported by Welch AQ985 and NIH AI22181.

C 457 ID-SPECIFIC, MHC-RESTRICTED T CELLS ARE OF BOTH TH1 AND TH2 TYPE. Grete F. Lauritzsen#, Sigfried Weiss*, and Bjarne Bogen#. #Department of Immunology and Rheumatology, University of Oslo, Norway. *GBF, Braunschweig, BRD.

The lymphokine secretion pattern by seven independent 91-101.$\lambda 2^{315}$/I-$E^d$ specific CD4+ T cell clones has been investigated. Six of the clones are of the Th1 type in that they secrete IL 2 and IFN-γ, but not IL 4. Some of these clones produce TNFα/β and some produce IL 5 and IL 6. One clone is of the Th2 type in that it produces IL 4, IL 5, and IL 6, but not IL 2, IFN-γ or TNFα/β. Both types of clones produce IL 3. All the T cell clones are cytotoxic *in vitro* and *in vivo* against $\lambda 2^{315}$-transfected B lymphoma cells. The Th1/Th2 classification is independent of fine specificity and Vα/Vβ gene segment utilization. The finding of both Th1- and Th2-like idiotype specific T cell clones is probably significant for the immunoregulation of Id+B cell clones and the elimination of Id+B cell lymphomas.

C 458 **Class II MHC IS EXPRESSED ON MURINE THYMOCYTES AND CAN PRESENT ANTIGEN TO A T CELL HYBRIDOMA**, R. D. Mayforth and J. Quintans. Department of Pathology, The Univ. of Chicago, Chicago IL., 60637. (Support: NIH grants PO1 CA 19266 and T32 GM-07281.)

We found significant levels of fluorescent staining for MHC Class II, or Ia, on the surface of murine thymocytes. Thymuses were removed from prenatal (CBA/caJ X B10BR F1 (H-$2^k$)), neonatal (C3H (H-$2^k$)), and adult (CBA/caJ (H-$2^k$), DBA/2 (H-$2^d$), and B10.BR (H-$2^k$)) mice. Dual fluorescent analysis of single cell suspensions of thymocytes using isotype matched or MHC-disparate controls of α-CD4 vs. α-Ia, α-CD3 vs. α-Ia, or α-Thy-1.2 vs. α-Ia demonstrated that the thymocytes were Ia positive. (α-Ia: biotinylated Mm-Ia-17-2 (α-I-$A^k$), biotinylated Mm-Ia-d (α-I-$A^d$), with PE-conjugated egg-white avidin or 10-2.16 (α-I-$A^k$), MKD6 (α-I-$A^d$) and MOPC 141 with goat-α-mouse Ig FITC; α-CD3: 145-2C11; α-Thy-1.2: AT83A; α-CD4: PE-conjugated α-L3T4 or GK1.5.) In addition, the level of staining for I-$A^k$ appeared to increase gradually from day 15 through day 18 of prenatal development. In order to determine if thymocyte Ia can present antigen, the following studies were performed. Adult DBA/2 thymocytes depleted of adherent cells (T(Adh$^-$), $10^6$ or 3 x $10^5$ cells/well) and an OVA, I-$A^d$-restricted T cell hybridoma (3DO 54.8, $10^5$ cells/well) were cultured with an OVA tryptic digest (200, 20, and 2μg/ml). α-Thy-1.2 plus complement-treated T(Adh$^-$) cells and an FGG tryptic digest were used as controls. 24h supernatants were collected and assayed on IL-2 dependent CTLL cells (3 x $10^4$ cells/well). The CTLL cells were pulsed with $^3$H-thymidine at 20h and harvested at 24h. The OVA-restricted T cell hybridoma secreted IL-2 in response to pulsed T(Adh$^-$) but not to the complement treated thymocytes. Whether the class II MHC is passively acquired or endogenously synthesized by the thymocytes is yet to be determined. It is clear, however, that thymocytes do express Class II MHC on their cell surfaces that can present peptide fragments to other T cells.

C 459 SUPPRESSION OF CONTACT SENSITIVITY RESPONSES BY T CELL RECEPTOR MOLECULES, John W. Moorhead, Robert L. Fairchild and Ralph T. Kubo, Univ. Colorado Health Science Center, Denver, CO 80262, Research Inst., Cleveland Clinic Fdn., Cleveland, OH 44195 and Natl. Jewish Ctr. for Immunol and Resp. Med., Denver, CO 80206

Contact sensitivity (CS) to DNFB is regulated by DNP/class I MHC-specific suppressor molecules produced by $CD8^+$ T cells. Our studies have shown that these soluble suppressor molecules are structurally and serologically similar to membrane T cell receptor (mTcR) proteins in that they are disulfide-linked dimers which bear TcR α and β chain determinants. Preliminary studies have also indicated that the suppressor molecule is larger than the mTcR by approximately 10,000 kd per chain. One explanation to account for these findings is that a structural change has altered the carboxy end of the mTcR, changing it from a hydrophobic to a hydrophilic molecule, allowing for its release from the cell. Such a change could account for the increased size and also be associated with the molecules suppressor effect. To explore this question we have affinity purified mTcR from several Ts hybridomas and compared these TcR molecules with the soluble suppressor molecules produced by the cells for their ability to suppress the transfer of CS to DNFB. Surprisingly, the isolated mTcR molecules and the soluble suppressor molecules suppressed the CS response equally well. Additional experiments have shown that treatment of the Ts hybridoma cells with the enzyme phospholipase-C releases suppressor molecules into the supernatant. Preliminary results indicate that these suppressor molecules bear TcR determinants. These results suggest that there may be two forms of the TcR present on the Ts hybridomas. The conventional mTcR as well as a glycosyl-phosphatidylinositol-linked TcR which may be a membrane anchored form of the soluble suppressor molecule. Supported by UPHS grants AI12993 and AI18785.

**C 460** OX-22$^{high}$ CD4$^+$ T CELLS INDUCE WASTING DISEASE WITH MULTI-ORGAN PATHOLOGY: PREVENTION BY THE OX-22$^{low}$ SUBSET. Fiona Powrie and Don Mason. MRC Cellular Immunolgy Unit, Sir William Dunn School of Pathology, University of Oxford, UK.

CD4$^+$ T cells in the rat can be subdivided into two populations based on the level of expression of the OX-22 (CD45RB) antigen. These two subpopulations mediate distinct functions *in vitro*, OX-22 $^{high}$ cells are the most potent producers of IL-2 and γ-IFN whereas the OX-22$^{low}$ population expresses higher levels of IL-4 mRNA. Data described herein demonstrate profound differences in the functional activity of these populations *in vivo*.

Congenitally athymic rats injected with OX-22$^{high}$ CD4$^+$ T cells from congenic euthymic donors developed a severe wasting disease with inflammatory infiltrates in liver, lung, stomach, thyroid and pancreas. In contrast, recipients of the OX-22$^{low}$ CD4$^+$ T cells remained well and continued to gain weight. Animals given unfractionated CD4$^+$ T cells ( containing 70% OX-22$^{high}$ and 30% OX-22$^{low}$ cells ) were protected from the wasting disease and the incidence of organ specific inflammation was much reduced compared with that found in recipients of OX-22$^{high}$ cells alone. The data suggest that this latter subset of CD4$^+$ T cells has autoaggressive potential which is inhibited in normal animals by cells of the OX-22$^{low}$ phenotype. The pathological changes in the liver, stomach, thyroid and pancreas reported here are not dissimilar to those associated with organ specific inflammatory diseases in man and it may be that some of these diseases develop due to a malfunction in the regulatory interactions between CD45RB$^{high}$ and CD45RB$^{low}$ CD4$^+$ T cells. It is notable in this context that we have recently reported that the CD4$^+$ memory T cell population in man can be subdivided on the level of expression of the CD45RB epitope.

**C 461 CO-STIMULATION, SELF-SUPERANTIGENS AND T CELL MEDIATED IMMUNE REGULATION.** Jose Quintans, Ruth D. Mayforth and Alexander Gottschalk. Univ. of Chicago, Ill.

The immune system is not tolerant of self components expressed transiently by activated cells. Some of the activation markers exhibit co-stimulatory properties and behave as immunogenic self "superantigens" capable of initiating immunoregulatory circuits. The results of our experiments show that upon activation, TH2 cells acquire the ability to stimulate T cells polyclonally, particularly CD8$^+$ cells reactive with activated T and B cells. We speculate that the physiological purpose of this anti-self response is to couple the activation events characteristic of all immune responses with immunoregulatory circuits operating through help and suppression: the engagement of additional CD4$^+$ cells creates amplificatory circuits while the activation of CD8$^+$ cells brings about suppression and/or killing of activated cells. Neither one of these two processes is antigen-specific, although both are initiated by antigen-specific events, i.e. the activation of the "inducer" TH2 clones in vitro or their counterparts in vivo. Bacterial superantigens probably evolved to take advantage of this regulatory loop: the bacteria trip the physiologic immunoregulatory loop and ultimately promote suppression of immune responses, allowing bacterial growth. The link between autoimmunity, infection and immune regulation is also apparent. In our hypothesis, physiologic immune regulation is a controlled autoimmune reaction driven by activation markers present in certain T cell subsets. The elimination and/or suppression of activated clones restores the steady state. Abnormal expression or persistence of self-superantigens induced by infections in cells and tissues automatically perpetuates an autoimmune response characterized by chronic anti - self reactivity and oscillatory patterns in the levels of "non-specific " CD4$^+$ and CD8$^+$ immunoregulatory cells. (Supp. by NIH grants PO1 CA 19266 and T32 GM - 07281).

**C 462** ISLET SPECIFIC CYTOTOXIC T CELLS FROM DIABETES PRONE AND DIABETES RESISTANT RATS AND IDENTIFICATION OF POTENTIAL REGULATORY CELLS IN DIABETES RESISTANT RATS, Joan M. Redd, Karen S. Sellins, Anne-Catherine Lagarde, and Donald Bellgrau. The Barbara Davis Center for Childhood Diabetes, University of Colorado Health Sciences Center, Denver, CO 80262

Biobreeding (BB) rats spontaneously develop insulin dependent diabetes. Paradoxically, while the disease is T cell dependent, it is also associated with poor T proliferative responses to alloantigen. BB proliferative responses to both the T cell mitogen Concanavalin A and an antibody (R73) to the rat T cell receptor are normal which suggests that the poor response to alloantigen is not due to an intrinsic defect in proliferation. R73 stimulation of the T cell receptor induces not only proliferation but also the generation of islet specific cytotoxicity. Interestingly, islet specific cytotoxicity is readily generated in T cells from not only diabetes prone but also diabetes resistant rats; thus the potential for autoreactivity may exist in both diabetes prone and diabetes resistant animals. The diabetes prone phenotype in the BB rat might be due to the absence of a subset of T cells that controls autoreactive T cells. Normally, mature peripheral rat T cells lack Thy 1. Approximately 10% of diabetes resistant peripheral T cells express the Thy 1 antigen; in contrast, as many as 50% of BB peripheral T cells are Thy 1+. The higher percentage of Thy 1+ T cells in the BB rat could actually reflect reduced numbers of Thy 1- cells since the relative numbers of Thy 1+ cells are comparable in BB and diabetes resistant animals. Thy 1- mature T cells in diabetes resistant rats might contain a regulatory subset that somehow inhibits the autoreactive Thy 1+ T cells. Thus, the absence of Thy 1- T cells rather than the presence of Thy 1+ T cells in the BB rat may determine the diabetes prone T cell phenotype. This lack of Thy 1- T cells could be due to a severe reduction in numbers of T cells (BB rats are known to be lymphopenic) or to selective deletion of certain T cell subsets (i.e. skewing of the T cell repertoire). The latter hypothesis would explain why BB T cells can proliferate normally to nonspecific stimuli but poorly in an antigen specific fashion. Further experiments will distinguish between these two hypotheses.

**C 463** CHARACTERIZATION OF A CELL SURFACE MARKER ON REGULATORY T CELLS, David R. Webb, Gloria Semenuk, and Bruce H. Devens, Institute of Immunology and Biological Sciences, Syntex Research, Palo Alto, CA, 94304

Radioresistant regulatory T-cells are detectable during the development of a mixed lymphocyte reaction. These thy $1^+$ $CD8^+$ cells suppress the development of an in vitro allospecific cytolytic T-cell response in an antigen specific fashion. The monoclonal antibodies 984D4.6 and 2441B3.6 remove these cells in the presence of complement. Cell mixing studies demonstrate that the cell surface antigens recognized by these antibodies are on separate cell populations. These antibodies are useful in discriminating regulatory T cells from CTL. Therefore, we have begun to characterize the cellular distribution and biochemical nature of the 984 antigen. Since this marker is present in very low amounts in mixed lymphocyte cultures, we have surveyed a number of cell lines for the presence of this marker. The marker is found on the T suppressor hybridomas 372B3.5, 372D6.5, and the macrophage like line WEHI-3. It is not detectable on B cell lines, T helper cell hybridomas, or other macrophage lines. Studies are in progress to characterize this antigen at the biochemical level.

*Neural and Muscular Autoimmune Disease*

**C 500** ADOPTIVE TRANSFER OF VACCINATION-INDUCED RESISTANCE IN THE RAT MODEL OF EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS. H. G. Archie Bouwer, Greg N. Dietsch, Bruce L. Gibbins and David J. Hinrichs, Chiles Research Institute and VAMC, Portland, OR
In the rat model of EAE, encephalitogen specific T cells lines can adoptively transfer clinical disease following antigen specific activation in vitro. With certain cell lines, recipients not only develop disease but following recovery, acquire resistance to actively induced disease. This phenomenon has been called vaccination. Vaccination has only been achieved with T cell lines. Primary cell cultures derived from lymph node or spleen, following stimulation with encephalitogen, can adoptively transfer clinical disease. Recipients of these cells, following recovery, are fully susceptible to actively induced disease. The mechanism leading to resistance in cell line vaccinated animals in unclear. We have attempted to adoptively transfer the EAE-resistant state by transferring spleen cells or thymus cells from vaccinated donors into naive recipients. When challenged with encephalitogen these donors developed active disease. Thus direct cell transfer does not transfer the resistant state. We were however able to adoptively transfer resistance if the donor cells, obtained from vaccinated rats, were stimulated in culture with encephalitogen prior to transfer. Recipients of such cells did not develop EAE following immunization with encephalitogen. We have also found that the lymphocyte populations present following the culture activation step which can adoptively transfer resistance to actively induced EAE do not influence the development of adoptively transferred EAE and do not alter the development of effector cells in vitro. Supported by NIH Grant NS24130.

**C 501** ENCEPHALITOGENIC DA STRAIN T-CELLS INDUCE RESISTANCE TO EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS IN DA BUT NOT IN (LEW X DA)F1 HYBRIDS, David J. Hinrichs, Ron Barry, Dejan Dordevich and H. G. Archie Bouwer, Chiles Research Institute and VAMC, Portland, OR 97213
In the Lewis rat model of EAE, long-term T-cell lines have been developed from BP-immune lymphoid populations by repeated cycles of antigen stimulation and expansion in lymphokine containing medium. Following recovery from encephalitogenic T-cell line mediated adoptive disease, recipients do not develop EAE when subsequently challenged with BP-CFA. We have developed encephalitogenic T-cell lines from non-Lewis strain rats in order to evaluate general features of cell line induced resistance to EAE. We developed T-cell lines from DA strain rats ($RT1^a$) and found that following adoptive transfer of syngeneic encephalitogenic T-cells, recipient DA rats are resistant to the induction of active EAE, an observation which indicates that this phenomenon extends to other rat strains. We have also evaluated T-cell line mediated resistance to EAE in (P1 x DA)F1 hybrids following transfer of encephalitogenic T-cell lines of DA derivation. Following adoptive transfer of DA encephalitogenic T-cells, (Lew x DA)F1 hybrids develop and recover from adoptive EAE similar to parental controls. However, unlike DA parental recipients of DA encephalitogenic T-cells which do not develop EAE when subsequently challenged with BP-CFA, Lew and (Lew x DA)F1 recipients of DA encephalitogenic T-cells remain susceptible to induction of active EAE following a subsequent challenge with BP-CFA.
Supported by NIH Grant NS24130.

**C 502** CEREBRAL VASCULAR ENDOTHELIAL CELLS ARE EFFECTIVE TARGETS FOR LYSIS BY Ia-RESTRICTED, MBP-SPECIFIC T LYMPHOCYTES CAPABLE OF TRANSFERRING EAE, Michael K. Racke, Richard M. McCarron*, and Dale E. McFarlin, Neuroimmunology Branch and *LNNS, NINDS, NIH, Bethesda, MD 20892
In EAE, Ia antigen may form complexes with CNS antigens such as MBP, which are known to be present on EC surfaces in disease. Ia antigen expression by EC may function by promoting immune system-CNS interactions resulting in extravasation of lymphocytes and initiation of CNS lesion development. In experiments reported here, cerebral vascular EC isolated from SJL mice were lysed by encephalitogenic T cell lines from SJL mice. Both MBP and encephalitogenic peptide(89-101)-specific lines could function as cytotoxic T lymphocytes. The capacity of EC to serve as targets was dependent upon the expression of class II MHC antigens. The data suggest that the migration of immune cells across the blood brain barrier may occur by mechanisms involving CTL-mediated lysis of cerebral vascular EC.

**C 503** HLA-DQA1 AND HLA-DQB1 GENES MAY JOINTLY DETERMINE SUSCEPTIBILITY TO DEVELOP MULTIPLE SCLEROSIS (MS), Frode Vartdal, Anne Spurkland and Erik Thorsby, Institute of Transplantation Immunology, The National Hospital, 0027 Oslo 1, Norway.
Serological DR typing and oligonucleotide typing of PCR-amplified DRB1, DQA1, DQB1, DPA1 and DPB1 genes were performed in 69 MS patients and 181 healthy controls. The frequencies of DR2 and of the DR2 associated DQA1*0102 and DQB1*0602 alleles were increased, whereas the frequency of DR7 was decreased among patients. The distribution of DP allelles was similar in patients and controls. Comparisons of DR-DQ haplotypes with identical DRB1 and DQA1 genes and different DQB1 genes (e.g. some DR4 and DR7 haplotypes), revealed that the frequencies of haplotypes carrying DQB1 genes that share polymorphic stretches with the MS associated DQB1*0602 allele were increased compared to haplotypes that did not carry such DQB1 genes. Of the MS patients 97% compared to 72% of controls carried DQB1 alleles sharing long polymorphic stretches ($p<0.001$). Furthermore, 99% of patients compared to 79% of controls carried DQA1 alleles encoding glutamine at residue 34 ($p<0.001$). A combination of such DQA1 and DQB1 alleles was found in 95% of the MS patients and 59% of the controls ($p<0.0001$), suggesting an association between MS and a combination of particular DQA1 and DQB1 alleles.

**C 504** **A TRANSGENIC MODEL OF TOLERANCE TO MBP: DEVELOPMENTALLY DELAYED DELETION OF MBP-REACTIVE T-CELLS.**
*Zaller, Dennis M., and Hood, Leroy. California Institute of Technology. *present address: Merck, Sharp, and Dohme Research Laboratories.

A T-cell receptor transgenic mouse line has been established in which virtually all T-cells express a receptor specific for the self antigen, myelin basic protein (MBP). There is a developmentally delayed deletion of autoreactive T-cells in these mice. A skewing towards $CD4^+$ T-cells was observed in the thymuses of fetal and newborn transgenic mice. Within a week after birth, however, mature T-cells could no longer be found. We have thus observed deletion in the thymus of T-cells that are reactive to a brain-specific antigen. A dramatically reduced number of T-cells are found in the spleen and lymph nodes of these transgenic mice. These peripheral T-cells are capable of responding to MBP in vitro. Despite the presence of these autoreactive T-cells, no incidents of spontaneous autoimmune disease have been observed.

**C 505** EVALUATION OF ACETYLCHOLINE RECEPTOR-REACTIVE ANTIBODIES IN EXPERIMENTAL AUTOIMMUNE MYASTHENIA GRAVIS: BREAKTHROUGH OF DISEASE-CAUSING ANTIBODY IN "DISEASE-RESISTANT" RATS. T.E. Zoda and K.A. Krolick. Department of Microbiology, University of Texas Health Science Center at San Antonio, San Antonio, Texas 78284.

Induction of Experimental Autoimmune Myasthenia Gravis (EAMG) is mediated by the production of autoantibodies specific for the acetylcholine receptor (AChR) located within the neuromuscular junction. However, previous studies from this and other laboratories have indicated that not all AChR-reactive antibodies are equally capable of inducing neuromuscular disease. Thus, two rat strains, Lewis and Wistar Furth, have been used to examine the characteristics of anti-AChR antibodies with differing disease-causing potential. Following primary AChR immunization of each rat strain, resulting anti-AChR antibody titers, clonotypic heterogeneity, and relative antigen-binding avidity are similar (determined by isoelectric focusing). Contrasting the similarities of their antibody responses, however, is the clear difference in the ability to demonstrate detectable disease; Lewis rats are extremely susceptible to disease induction, while Wistar Furth rats are disease resistant. Interestingly, following multiple immunizations with AChR, Wistar Furth rats do not sustain an absolute resistance to EAMG, demonstrating a transient state of disease. It is believed that hyperimmunization of Wistar Furth rats results in the production of disease-causing antibodies not originally expressed as a significant proportion of the initial/early anti-AChR antibody response, and that may be down-regulated under some circumstances. The exact nature of this antibody(s) and the regulation of its production (via helper T cells) has been examined with respect to the breakthrough of neuromuscular disease symptoms.

*Diabetes*

**C 506** GLUTAMIC ACID DECARBOXYLASE (GAD/ 64kD)IS LOCATED IN THE SYNAPTIC LIKE MICROVESICLES (SLMV's) AND THE INSULIN SECRETORY GRANULES OF THE BETA CELL, Henk-Jan Aanstoot, Stephan Christgau, Debra Crumrine, Steinunn Bækkeskov, Hormone Research Institute, Depts of Microbiology, Immunology and Dept of Medicine, U.C.S.F. School of Medicine, San Francisco, Ca 94143-0534.

We have recently showed that the 64kD pancreatic islet beta cell autoantigen, which is the main target for autoantibodies associated with beta cell destruction and type-1 diabetes, is Glutamic Acid Decarboxylase (GAD). GAD is the key-enzyme in the synthesis of the neurotransmitter GABA. The beta cell appears to co-secrete GABA with insulin. In order to understand how this protein becomes an autoantigen and its possible role in beta cell destruction, we have analysed its subcellular localization using post-embedding immuno-electronmicroscopy of pancreatic beta cells. GAD is beta cell specific within the islet and GAD was found to be localized to the membrane of synaptic like microvesicles and to insulin containing secretory granules. It is conceivable that GAD may become exposed at the surface following fusion of vesicles with the plasma membrane and that this exposure is dependent on the functional status of the beta cell. Alternatively, if GAD remains confined to the cytoplasmatic space, peptides of GAD may become expressed at the surface associated with MHC-class I and thus be recognized by cytotoxic T-cells.

**C 507** CHARACTERIZATION OF THE ANTIGEN RECOGIZED BY THE ISLET-SPECIFIC T CELL CLONE BDC-2.5, Barbara Bergman and Kathryn Haskins, Barbara Davis Center for Childhood Diabetes, University of Colorado Health Sciences Center, Denver, CO 80262.

The islet-specific T cell clone BDC-2.5 was derived from a newly diabetic nonobese diabetic (NOD) mouse and is of the CD4 phenotype. BDC-2.5 proliferates and makes IL-2 in response to NOD antigen presenting cells and islet cell antigen isolated from a number of mouse strains or from beta tumor lines. Islet cell membranes, as well as whole islet cells and islet cell lysates, can stimulate BDC-2.5, suggesting a cell surface antigen. Preliminary data indicate BDC-2.5 recognizes distinct protein antigens in Nonidet P-40 (NP-40) islet cell extracts fractionated by SDS-PAGE and transferred to nitrocellulose membranes for use in T cell proliferation assays. In addition to the biochemical analysis, we are investigating the ability of anti-islet cell antibodies to block the response of BDC-2.5 to islet cell antigen. We have identified at least two antibody reagents that appear to react with the antigen recognized by the T cell clone.

**C 508** XENOGENEIC ENGRAFTMENT OF BB RAT LYMPHOHEMOPOIETIC CELLS IN *SCID* MICE: AN ANIMAL MODEL FOR INVESTIGATION OF XENOGENEIC TOLERANCE. Laura Crisá, Aldo A. Rossini, John P. Mordes, Una McKeever, Anna Tafuri, T.V. Rajan, Len D. Shultz, Dale L. Greiner. Dept. of Medicine, Univ. of Massachusetts Medical School, Worcester, MA 01655, Dept. of Pathology, Univ. of Connecticut Health Center, Farmington CT 06030, and The Jackson Laboratory, Bar Harbor, ME 04609

C.B-17 mice homozygous for the mutation severe combined immunodeficiency (*scid*) lack T and B lymphocytes due to a recombinase gene defect that prevents T and B cell receptor rearrangement. Peripheral but not thymic engraftment of human lymphohemopoietic cells has been reported in *scid* mice; we have previously shown that irradiated *scid* mice injected with LEW fetal liver cells develop rat thymocytes and peripheral T and B cells. We now extend these observations to the diabetes prone BB/Wor (DP) rat model of autoimmune diabetes. *Scid* mice were irradiated (300 rads) and injected with either DP or diabetes resistant BB/Wor (DR) fetal liver cells. After 8 wks, *scid* recipients of DP fetal liver had $9.2x10^6$ DP origin thymocytes, $1.1x10^6$ and $4.8x10^6$ DP origin splenic T and B cells, respectively. *Scid* recipients of DR fetal liver had $14.5x10^6$ DR thymocytes, and $8.8x10^6$ and $9.4x10^6$ DR splenic T and B cells. *Scid* recipients of DP and DR fetal liver had $<0.1$ and $2.0x10^6$ $RT6^+$ splenic T cells, respectively. This result traces the lymphopenia and lack of $RT6^+$ T cells in DP rats to fetal liver stem cells. DP and DR fetal liver recipient sera contained $2.2\pm1.9$ and $25.1\pm3.4$ mg/ml rat Ig. No mouse splenic T or B cells or Ig were found, and no diabetes or pancreatic insulitis was observed in *scid* recipients. The development of BB T cells in the *scid* thymus may have resulted in tolerance or clonal anergy to self pancreatic antigens, or in failure to "educate" rat T cells to recognize mouse MHC expressing self pancreatic antigens. Studies are underway to investigate these possibilities. Supported in part by NIH grants DK25306, DK36024, CA20408, and AI30046.

**C 509** THYMIC T CELL ANERGY IN PREDIABETIC NOD MICE, Terry L. Delovitch, Danny Zipris, Alan H. Lazarus and Andrew R. Crow, Banting and Best Department of Medical Research, University of Toronto, Toronto, Ontario, Canada M5G 1L6

Age-related alterations in thymic T cell function occur in prediabetic NOD mice. To study the mechanism involved, we examined whether a defect exists at the level of TcR mediated signaling after activation by Con A and anti-CD3. NOD $CD4^+$ $CD8^-$ and $CD4^-$ $CD8^+$ thymocytes responded weakly to Con A- and anti CD3-induced proliferation compared with mature thymocytes from several control strains including BALB/c and NON. Detection of this unresponsiveness (anergy) begins at 7-8 wk of age and correlates with the onset of insulitis. This T cell anergy is not due to the reduced expression of CD3, CD4, CD8 and TcR by NOD thymocytes. It can be partially reversed by the addition of IL-2 (but not IL-1, IFN-$\gamma$ or TNF-$\alpha$ or by an interaction with either syngeneic or allogeneic splenic APCs, and may arise from the inability of NOD thymic APCs to provide a costimultary signal to NOD mature thymic T cells. We also tested whether NOD thymocytes elevate their intracellular free $Ca^{2+}$ ion concentration ($[Ca^{2+}]_i$) in response to Con A. An equivalent Con A-induced increase in $[Ca^{2+}]_i$ in both NOD and BALB/c thymocytes was observed, suggesting a normal coupling between TCR and phospholipase C in NOD thymocytes. NOD thymocytes respond normally to PMA plus ionomycin and PMA plus Con A, but weakly to Con A plus ionomycin. Our data suggest that this age-related NOD mature thymic T cell anergy results from a defect in the signaling pathway of T cell activation that occurs upstream of protein kinase C activation. (Supported by Canadian Diabetes Association).

**C 510** DISEASE TRANSFER STUDIES WITH ISLET-SPECIFIC T CELL CLONES, Kathryn Haskins and Marcia McDuffie, Barbara Davis Center for Childhood Diabetes, University of Colorado Health Sciences Center, Denver, CO 80262.

We have shown that CD4+ islet-specific T cell clones isolated from NOD mice can accelerate the diabetes process in young, unmanipulated NOD recipients. Animals injected with the clones became overtly diabetic (hyperglycemic) or developed advanced insulitis within 2-4 weeks after treatment. Mice that recieved a control T cell clone, not specific for islet antigen, showed no signs of disease or intraislet infiltration. In order to determine the importance of the diabetes prone environment, we are investigating the disease transfer properties of the clones in NOD F1 animals. As most previous disease transfer studies have required the presence of both CD4+ and CD8+ T cells, we are also doing experiments to determine whether CD8+ T cells play a role in the disease process seen in young mice recieving CD4+ islet-specific clones.

**C 511** HLA ASSOCIATION IN BLACK PATIENTS WITH TYPE 2 DIABETES, A.J. Norin, M.A. Banerji, R. Chaiken, H.E. Lebovitz, Departments of Medicine and Anatomy & Cell Biology, SUNY Health Science Center, Brooklyn, NY 11203

Previous reports have not demonstrated an association between HLA and Type 2 diabetes. We have described insulin sensitive and insulin resistant Type 2 diabetic variants in black patients (Diabetes 38:784, 1989) as well as black diabetics who have nomoglycemic remission (Medicine 69:176, 1990). The 1 mu/kg/min euglycemic insulin clamp was used to measure peripheral insulin sensitivity in 24 near nomoglycemic Type 2 diabetic patients. Insulin response to oral glucose was measured using an oral glucose tolerance test. Fourteen individuals had normal peripheral insulin action (insulin sensitive) and 10 were insulin resistant. Insulin response to oral glucose was markedly lower in the insulin sensitive group compared to the insulin resistant group suggesting primary insulin deficiency. Islet cell antibodies were absent in all of these patients. HLA typing was performed and paired comparisons were analyzed using Fishers exact test. Black control subjects (n=20) without a family history of diabetes and black clinic patients with Type 2 diabetes (n=14) were studied for comparative purposes. The frequency of HLA A30 and DQW6 were significantly different in the insulin sensitive compared to the insulin resistant groups, ($p<0.05$). Significant differences ($p<0.05$) were found for frequencies of DQW7 and CW4 alleles of near normoglycemic Type 2 diabetics (patients in remission) compared to hyperglycemic Type 2 diabetics. These alleles were also significantly different between near normoglycemic Type 2 diabetes and normal black controls ($p<0.02$ & $0.05$), as were DQW6 and DRW52 ($p<0.05$). Our data suggest that black Type 2 diabetic patients may have an HLA association with DQW6 and that A30 may be a marker for insulin sensitivity in black Type 2 diabetics.

**C 512** CHARACTERISATION OF ISLET CELL ANTIGEN RELEVANT IN AUTOIMMUNITY RELATED DIABETES MELLITUS, R.Raju, S.Srikanta and N.Kochupillai, Department of Endocrinology and Metabolism, All India Institute of Medical Sciences, New Delhi 110 029, INDIA.

A long prodromal phase before the onset of Type I diabetes is evidenced by the presence of antibodies directed against islet cell differentiation antigens. These molecules include well defined ones like insulin and proinsulin and yet uncharacterised one(s) like islet cell autoantibody (ICAb) target antigen(s). Our objective was to attempt to characterise this autoantigen. Towards this we generated a series of murine monoclonal islet cell antibodies (n=18; immunogen=human insulinoma homogenate; screening=pancreas immunohistochemistry). Monoclonal antibody I-45 reactivity to pancreatic section was similar to the reactivity of ICAb positive patient sera, whereas I-39 reacted with all cells of the pancreas. I-45 as well as I-39 binding to pancreatic sections were not inhibited by ICAb positive patient sera. Preexposure to pH 2.0 abolished the immunoreactivity of the autoantigen; ICAgs recognised by MAbs I-45 was also sensitive to low pH. Sodium metaperiodate treatment abolished the reactivity of I-45 (similar to patient sera) but not I-39. Immunoreactivity of I-45 or I-39 was not abolished on preexposure of the sections to trypsin or protease demonstrating a non-protein epitope.

**C 513** TcR Vß8+ T CELLS PROTECT NOD MICE AGAINST PANCREATIC ß-CELL DESTRUCTION, Barbara Reitz, Dianne Schweiger and Marcia McDuffie, Barbara Davis Center for Childhood Diabetes, University of Colorado Health Sciences Center, Denver, CO 80262

As a precursor to experiments which will test the effects of varying thymic selection on insulitis and diabetes in the non-obese diabetic (NOD) mouse, we have prepared a profile of TCR Vß subsets in the NOD line being bred at the Barbara Davis Center. During this evaluation, we determined that variability of one T cell Vß subset (Vß8.3, one of 3 members of the Vß8 family) exceeded that routinely seen in inbred mouse strains. This variability results from a marked, transient decrease in circulating Vß8.3$^+$ T cells between 1 and 3 months of age. The decrease was most pronounced in the CD8$^+$ T cell fraction, the subset which binds to antigen in the context of Class I MHC molecules and contains a large fraction of cells which have been classified functionally as "killers" and "suppressors." Testing the hypothesis that the decrease in Vß8.3$^+$ cells in the circulating pool results from their participation in the early stages of pancreatic inflammation, we expanded T cell populations from diabetic NOD mice and enriched them for cells bearing Vß8 family TCR. These cell preparations delayed or prevented diabetes when injected into young NOD mice.

**C 514** THE EFFECT OF A MHC CLASS II TRANSGENE ON THE DEVELOPMENT OF TYPE I DIABETES IN THE NON-OBESE DIABETIC (NOD) MOUSE, Steven M. Singer*, Hans Acha-Orbea†, and Hugh O. McDevitt*, *Department of Microbiology and Immunology, Stanford University, Stanford, CA 94305 and †Ludwig Institute for Cancer Research, Lausanne, Switzerland

The NOD mouse is a model of the autoimmune disease, Type I diabetes. One of the features this model shares with the human disease is the presence of a recessive susceptibility gene which maps within the MHC. Studies in the NOD have shown that this gene is inherited recessively and also that the I-A $\beta$ gene in the NOD is unique among inbred mouse strains. To determine the role that this unique I-A molecule plays in diabetes pathogenesis we have introduced the I-A$\beta^d$ gene into the germline of NOD mice. Two independent lines of mice have been analyzed. One line carries 10-20 copies of the transgene and has severe developmental defects in the B cell, macrophage, and granulocyte lineages leading to death by four months of age. The second line has 1-2 copies of the transgene, and expresses both I-A$^{nod}$ and I-A$^d$ on B cells and macrophages. Expression of I-A$^d$ in the transgenics is roughly half the level found in a Balb/c X NOD F1. We are currently following these animals to determine the effect of this transgene on the development of insulitis and the progression to diabetes.

**C 515** MODULATION OF T CELL MEDIATED AUTOIMMUNITY TO HEAT SHOCK PROTEIN-65 (HSP-65) BY IMMUNIZATION OF NOD MICE WITH COMPLETE FREUND'S ADJUVANT, B. Singh, C. Hitchon, J. Lauzon, H.Y. Qin, E. Fraga, and M.W.J. Sadelain, Department of Immunology, University of Alberta, Edmonton, Alberta, Canada, T6G 2H7.

We have recently shown that a single injection of complete Freund's adjuvant (CFA) prevents the induction of diabetes in NOD mice (Diabetes 39: 583, 1990). Since CFA contains mycobacterial cell wall we have investigated the role of the *mycobacteria* derived antigen in the modulation of autoimmunity. Mice injected with *M. bovis* alone (BCG vaccine) at four weeks of age were also found to be protected from diabetes. Further investigation with purified protein derivative (PPD) and heat shock protein (HSP-65) derived from *mycobacterial tuberculosis* showed that the T cells from the unimmunized diabetic NOD mice proliferated in response to these antigens. In addition we have found that a synthetic peptide (P23127) of HSP-65, also stimulated T cells from unprimed diabetic NOD mice. Following immunization with the CFA, T cells from non-diabetic NOD mice responded to PPD, HSP-65 and two synthetic peptides (P23128 and P23129) of HSP-65 but not to P23127. Our results also indicate that T cells from the diabetic mice respond to different epitopes on HSP-65 than T cells from CFA protected NOD mice. Vaccination of NOD mice with the peptide antigens of *mycobacterial* HSP-65 provides strong evidence for the modulation of diabetes by immunotherapy. Cell transfer experiments further support the induction of regulatory cells following immunization with mycobacterial antigens and CFA in the prevention of autoimmune diabetes.

**C 516** T CELL HYBRIDOMAS REACTIVE WITH ISLET CELL ANTIGENS, Peter Stecha, Victor Ozols, Barbara Bergman and Kathryn Haskins, Barbara Davis Center for Childhood Diabetes, University of Colorado Health Sciences Center, Denver, CO 80262.

We have produced a panel of islet-specific T cell clones from NOD mice (PNAS 86: 8000, 1989). In order to obtain lines that would not require antigen stimulization, we made T cell hybridomas by fusing the islet-specific T cell clones to the T cell receptor-negative variant of BW 5147. The hybrids reflect the phenotypic and antigen-specific, MHC-restricted, properties of the clones in that they are all CD4+ and respond to islet cell antigen and NOD APC by producing IL-2. The response of the islet-reactive hybrids increases with antigen, which can be in the form of whole islet cells, freeze-thaw lysates, or islet membrane preparations. We are using these hybridomas as an alternate source of responder T cells to investigate T cell receptor gene usage by islet-specific T cell clones and to determine blocking effects of anti-islet antibodies.

**C 517** MONOCLONAL ANTIBODIES TO PANCREATIC ISLET CELLS, Patrick Supon, Peter Stecha, Barbara Bergman and Kathryn Haskins, Barbara Davis Center for Childhood Diabetes, University of Colorado Health Sciences Center, Denver, CO 80262. Insulin dependent diabetes mellitus (IDDM) is an autoimmune disease characterized by the specific destruction of the pancreatic β cells. Though this destruction appears to be predominantly a cell-mediated process, involvment of the humoral immune system is suggested by the presence of autoantibodies to islet cells in the sera of not only human IDDM patients but also of BB rats and NOD mice, the two common animal models of IDDM. In order to better characterize these autoantibodies and the β cell antigens bound by them, we have produced a panel of over 30 monoclonal anti-islet antibodies by fusing splenic B lymphocytes from unimmunized NOD mice with the P3X6.Ag8 myeloma. The majority of the monoclonal antibodies produced bind not only mouse islet cells but also human islet cells, rat islet cells and two murine β cell tumor lines. Only a small fraction bound non-islet cell lines. Immunohistochemical and two color fluorescent staining studies suggest some of the antibodies may be β cell specific. Biochemical characterization of the antigens bound by the antibodies is in progress, using immunoblotting and immunoprecipitation procedures.

*Thyroiditis*

**C 518 THYROGLOBULIN T CELL EPITOPES AND THEIR ROLE IN MURINE EXPERIMENTAL AUTOIMMUNE THYROIDITIS,** **Kim Dawe, Kevin R. Page, David C. Rayner, Paddy Hutchings, Anne Cooke, *Mario Geysen, Ivan Roitt and Brian R. Champion,** Department of Immunology, University College & Middlesex School of Medicine, London W1, UK; *Coselco Mimotopes Pty Ltd, Clayton, Victoria 3168, Australia.

Experimental models have been of great value in examining the role of autoreactive T cells in the development of autoimmune diseases. For example, immunization of a number of species with syngeneic thyroglobulin (Tg) in a suitable adjuvant leads to the development of autoimmune thyroiditis which can be transferred to naive histocompatible recipients with Tg-specific T cells. In these models, an understanding of the nature of autoantigenic T cell epitopes of Tg may give important insights into the disease mechanisms and their regulation.

Post-translational iodination of Tg in the thyroid by thyroid peroxidase is the basis for the formation of the thyroid hormone thyroxine. We have shown that this iodination of Tg is critical for its recognition by our murine Tg-specific T cell clones. Taking advantage of the cross-reactivity of our murine Tg-specific autoreactive T cell clones with human Tg, we have used affinity purified trypsin fragments of Tg and synthetic peptides covering the four major hormonogenic sites in Tg to show that the T cell clones recognize a 9 amino acid peptide containing a thyroxine residue. Further structural characteristics of this epitope will be discussed. That this observation may be relevant to the pathogenesis of thyroiditis is emphasized by the inability of non-iodinated Tg to elicit lesions and by the expression of the relevant epitope/MHC complex on the surface of cultured thyroid cells stimulated with interferon-γ. Experiments are in progress to evaluate the potential regulatory effects of this epitope in autoimmune thyroiditis.

**C 519** INDUCTION OF SEVERE GRANULOMATOUS EXPERIMENTAL THYROIDITIS (EAT) BY CELLS CULTURED WITH ANTI-IL2 RECEPTOR (R) MONOCLONAL ANTIBODIES (mAb). H. Braley-Mullen, G. Sharp, M. Kyriakos and J. Bickel. University of Missouri, Columbia, MO 65212.

Mouse thyroglobulin (MTg) primed spleen cells transfer EAT to syngeneic recipients after in vitro activation with MTg. Recipients of cells cultured with MTg and the anti-IL2R mAb M7/20 develop much more severe EAT and produce more anti-MTg autoantibody than do recipients of cells cultured with MTg alone. EAT induced by cells cultured with MTg and M7/20 was also qualitatively different; thyroids were enlarged and had granulomatous changes with multinucleated giant cells plus PMN's and mononuclear cells. Induction of granulomatous EAT lesions required addition of M7/20 during the first 8 hours of culture and was antigen MTg-dependent. CD4+ T cells were required to transfer granulomatous EAT; whether B cells are also required is currently under investigation. Although cells cultured with M7/20 had no detectable in vitro proliferative responses to MTg or Con A, the cells apparently had to be capable of proliferating in recipient mice since cells irradiated (2000R) just prior to transfer induced minimal EAT. Addition of other anti-IL2R mAbs (7D4, 3C7), anti-IL-2 (S4B6) and, to a lesser extent, anti-IFNδ (XMG-12) to cultures also resulted in activation of cells able to transfer granulomatous EAT and increased anti-MTg responses. An irrelevant rat mAb, anti-Forsmann IgM, had no effect on EAT induction while anti-IL-4 mAb (11B11) generally partially suppressed the transfer of EAT and anti-MTg. These results suggest that CD4+ EAT effector cells capable of inducing severe granulomatous EAT may be IL-4 producing ($T_{H2}$) cells while IL-2 and IFNγ-producing $T_{H1}$ cells may inhibit the activity of the former cells. (Supported by NIH Grant DK-35527.)

*Lupus/NZB/W*

**C 520** H-$2^z$ TRANSGENIC MICE FOR THE STUDY OF LUPUS-LIKE AUTOIMMUNITY, Virginia B. Appel[1], Andrea J. Bucci[1], Christophe O. Benoist[2], Diane J. Mathis[2], Brian L. Kotzin[1] and Edward Palmer[1], [1]Department of Pediatrics, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO 80206; [2]Laboratoire de Genetique Moleculaire des Eukaryotes du CNRS, Unite 184 de Biologie Moleculaire et de Genie Genetique de l'INSERM, Faculte de Medecine, 67085 Strasbourg Cedex, France.

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 tightly linked to the H-$2^z$ locus on chromosome I7. A genomic clone for I-$E^z$ has been isolated and used to produce transgenic mice carrying I-$E^z$ on a C57Bl/6 background. These transgenic animals are being used in breeding experiments to determine whether the NZW I-$E^z$ gene product regulates this lupus-like disease. In addition, we have isolated clones encoding I-$A^z$ in order to produce transgenic mice.

**C 521** IDENTIFICATION OF NEPHRITOGENIC ANTI-DNA ANTIBODIES, Hirabayashi Y. Sasaki,T. Osaki H. Shibata,S Yoshinaga,K. Sano H.
The Second Department of Internal Medicine, Tohoku University School of Medicine, Sendai,Japan.
Anti-DNA antibodies may play a role for the pathogenesis of systemic lupus erythematosus(SLE). Isoelectrofocusing studies revealed that human anti-DNA antibodies were heterogenous, but the populations of anti-DNA antibodies were restricted in circulating immune complexes(CIC) or in renal glomeruli-deposited immunoglobulins. Anti-DNA antibodies expressing O-81 idiotypes(Id) or NE-1 Id were specifically detected in CIC and in renal glomeruli of 74% of patients with active lupus nephritis. Thus, antibodies expressing O-81 Id or NE-1 Id are nephritogenic in humans. The analysis of nucleotide sequences of variable regions showed that NE-1 $V_H$ gene belongs to $V_H$ IV subgroup that share highest homology with a rearranged $V_H$ gene(Ab 44). NE-1 also utilized D21/7, J 21/7, JH6, V21b(k1) and Jk4. O-81 antibodies utilized a new $V_H$ III gene. This may be interesting in comparison with $V_H$ sequence studies for polyspecific autoantibodies originated from normal subjects.

**C 522 A NOVEL METHOD OF IMMUNOTHERAPY FOR AUTOIMMUNE DISEASES USING A V$\beta$ SPECIFIC T CELL MITOGEN**, Caius Kim and Atsuo Ochi, Department of Immunology, University of Toronto and the Samuel S. Lunenfeld Research Institute, Mt. Sinai Hospital, 600 University Ave. Toronto, Ontario, Canada. M5G 1X5

Animal models used in the study of Systemic Lupus Erythematosus (SLE) include the MRL/lpr mouse. This strain is characterized by a massive proliferation of an unusual subset of T cells that are Thy $1^+$, $CD4^-$, $CD8^-$, and $B220^+$. Interestingly, these T cells predominantly express receptors bearing V$\beta$8. Since the "superantigen", Staphylococcus enterotoxin B (SEB) reacts with T cells bearing V$\beta$3,7 and 8 TCR's, the effects of SEB were investigated in the MRL/lpr strain.

The results to date indicate that SEB is effective in ameliorating the various symptoms of SLE in the MRL-lpr/lpr strain. Specifically; the $\alpha$-DNA Ab titer decreased in a dose-dependent manner, the visible symptoms of SLE in this strain were reduced and delayed, and the proportion of $B220^+$ T cells which mediate the acceleration in disease onset were decreased in the treated animals.

**C 523** ANTICARDIOLIPIN COFACTOR AND DIFFERENTIAL DIAGNOSIS OF AUTOIMMUNE DISEASE, Takao Koike, Kenji Ichikawa, Takahiro Suzuki and Eiji Matsuura, Department of Internal Medicine, Chiba University, Chiba, Japan 280
Anticardiolipin antibodies (aCL) are frequently found in sera from patients with SLE who are liable to thromboembolic events and intrauterine fetal death. We recently found that aCL from these patients reacted with a complex of negatively charged phospholipids and cofactor derived from normal human sera. We purified cofactor by cellulose column chromatography and affinity chromatography, and further by a method based on the binding affinity of the cofactor on liposomes composed of CL. The cofactor selectively bound to liposomes composed of negatively charged phospholipids (ie, CL, PS, PA or PI), but not to liposomes composed of neutral phospholipids. The cofactor migrated with an apparent molecular mass of 50kD. The addition of human cofactor enhances the titer for SLE and depresses it for syphilis. These results suggest a simple method for the differential diagnosis of SLE and indicate that the complex of CL and human cofactor can be recognized only by the antibodies raised in sera of SLE patients. The antibodies induced by infectious diseases may react directly with CL and/or fee cofactor, but not with the complex.

**C 524** SPECIFIC MANIPULATION OF NEPHRITOGENIC ANTI-DNA ANTIBODIES BY ANTI-IDIOTYPIC ANTIBODY-CONJUGATED NEOCARZINOSTATIN,
Takeshi Sasaki, Shinobu Shibata, Naoko Harata, Tai Muryoi, Hirofumi Osaki and Kaoru Yoshinaga, 2nd Department of Internal Medicine, Tohoku University School of Medicine, Sendai, Japan.
Although anti-Id immunity may have an ability to regulate the production of autoantibodies associated with tissue injuries, the application in vivo has been hampered because of the complexity of the mechanism. We tried to manipulate the synthesis of spontaneously occurring anti-DNA antibody using monoclonal anti-Id antibodies (D1E2 and 1F5) conjugated with a cytotoxic agent, neocarzinostatin (NCS). In vivo administration of anti-Id antibodies conjugated with NCS brought about an improvement in the survival rate of female NZB/W $F_1$ mice. It also caused a retardation of development of lupus nephritis and decreased the numbers of anti-DNA producing cells. The suppression of anti-DNA antibody synthesis was specific and idiotype-mediated. The results indicate that the use of a limited number of anti-Id antibodies in combination with a cytotoxic agent may be applicable therapeutically to autoimmune diseases.

*Rheumatoid Arthritis and Joint Diseases*

**C 525** **T CELLS IN COLLAGEN-INDUCED ARTHRITIS IN MICE**, Mikael Andersson, Tom J. Goldschmidt, Erik Michaëlsson and Rikard Holmdahl, Department of Medical and Physiological Chemistry, Uppsala University, Uppsala, Sweden
Immunization with type II collagen (CII) leads to development of arthritis in certain mouse strains. We have studied the immune response to CII in mice with H-2q with special emphasis on the T cell reactivity to CII. The T cell response is strongly dependent on the source of CII. After immunization with heterologous CII a strong proliferative response against foreign determinants on the CII molecule can be demonstrated. These are located on the CB11 fragment. A part of this response is directed against an epitope present on the 26 aa peptide shown by others to vaccinate against the induction of CIA (Myers et al. J. Exp. Med. 170:1999, 1989). After immunization with autologous CII only a weak proliferative response is found. This is directed mainly against the CB10 fragment. No differences in T cell reactivity were found between the arthritis susceptible DBA/1 mouse and the resistant SWR mouse. (SWR x DBA/1)$F_2$ mice were studied for arthritis development and TCR Vß gene haplotype individually. No difference in arthritis susceptibility was found between mice carrying a complete Vß genome and those being homozygous for the SWR derived deletion.

**C 526** COLLAGEN-INDUCED ARTHRITIS (CIA) IN H-2 f/q MICE WITH FUNCTIONAL EXPRESSION OF THE HUMAN HLA TRANSGENE - $DQW6_\beta$. *Marie M Griffiths, Paul Zhou, Harvinder Luthra and Chella S. David, Departments of Internal Medicine and Immunology, Mayo Medical School, Rochester, MN 55905, *Univ. of Utah Medical School and Veteran's Administration Hospital, Salt Lake City, UT.

In $DQW6_\beta$ single transgenic mice, $DQ_\beta$ presents Mls-1a and causes clonal deletion of T-cells expressing several different T-cell receptor (TCR) molecules ($V_\beta$ 5.1, 5.2, 11, 8.1, 6), some of which are important in the arthritogenic response to native bovine type II collagen (BII). In initial studies, aimed at determining the role of these T-cell subsets in CIA, we mated H-2 f/f, transgenic B10.M($DQW6_\beta$) with H-2 q/q, Mls-1a strains DBA/1 (CIA susceptible) and SWR (CIA resistant). F1 progeny were tested for 1) the presence and expression of the DQ transgene by PCR and FACS analysis and 2) the development of CIA after intradermal injection of 100 ug BII emulsified in complete Freund's adjuvant. Although, the DQ transgene did not alter the incidence of CIA in B10.M($DQW6_\beta$) x DBA/1 F1 hybrids (75%), a trend toward less severe disease and increased anti-mouse collagen antibody (ELISA) was noted in the DQ positive F1 progeny when compared to their DQ-negative littermates. This is not surprising as DBA/1 mice likely respond to multiple collagen epitopes and utilize several different and alternative TCR molecules. A low incidence (14-22%) of mild, transient CIA developed in the B10.M($DQW6_\beta$) x SWR F1 hybrids which is similar to that previously found in B10 X SWR (b/q) F1 mice, thus showing that the DQ transgene does not directly present arthritogenic epitopes of BII in this milieu. We are now testing the effect of $DQW6_\beta$ in F1 hybrids derived from matings with other CIA-susceptible strains (B10.Q, B10.RIII) which are thought to use a more restricted number of TCR molecules in the development of CIA in response to immunization with porcine and chick type II collagens. These data will be presented.

**C 527** CHARACTERIZATION OF COLLAGEN-SPECIFIC T-T HYBRIDOMAS FROM PATHOGENIC AND NONPATHOGENIC RAT T CELL LINES

Grace Ku, Phuong Nguyen, Ernest Brahn, and Mitchell Kronenberg, UCLA School of Medicine, University of California, Los Angeles, CA. 90024

Collagen arthritis, induced in Louvain rats with the injection of native type II collagen (CII), is T cell dependent. T helper cell lines specific for CII have been derived from arthritic rats and a subset of these CII-reactive T cell lines can induce arthritis when transferred to naive, syngeneic recipients. Rat/mouse T-T hybridomas have been developed from an arthritogenic and a nonarthritogenic T cell line. CII reactivity was determined by an IL-2 release assay. These hybridomas express $\alpha\beta$ TCR and are $CD4^+$, $CD8^-$, and MRC $OX22^-$. Southern blot analysis of DNA extracted from these hybridomas demonstrated that each T cell line contains at least three different CII-reactive clones. The data suggest that the spectrum of TCR $\beta$ gene rearrangement is limited, as one hybridoma from the nonpathogenic line shares an identically-sized productive TCR $\beta$ gene rearrangement with two hybridomas from the pathogenic line. Unlike mouse collagen arthritis $V\beta 6$ and $V\beta 8$ gene segments are not rearranged in CII-specific T cells. Further evidence of limited TCR $\beta$ variable gene usage in the CII-specific hybridomas is provided by Northern blot analysis of hybridoma RNA, which demonstrated that 5 of 7 hybridomas use the $V\beta 4$ variable region in their expressed, TCR. The anti-CII responses of all the hybridomas are restricted to the rat class II MHC RT1.B gene loci. The hybridomas require antigen processing to respond to both native and denatured CII, and like their parental cell lines, do not respond preferentially to either native or denatured CII. These hybridomas recognize a specific CII epitope and not repetitive collagen-like sequence motifs. The immunogenic epitopes on CII have been determined using synthetic peptides and CII cyanogen bromide (CNBr) cleavage fragments. An immunodominant epitope is present on CNBr cleavage fragment 11 and a cross-reactive epitope is present on CNBr cleavage fragment 8. The lymphokine production profile and T cell receptor Vα gene usage of the hybridomas and cell lines are being ascertained and correlated with arthritogenicity.

**C 528** MULTIPLE EPITOPES ON CARTILAGE TYPE II COLLAGEN ARE ACCESSIBLE FOR ANTIBODY BINDING *IN VIVO*. John A. Mo*, Rikard Holmdahl*, Roland Jonsson#, Katarina Karlström" and Annika Scheynius".

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A panel of mouse antibodies specific for mouse type II collagen (CII) has been epitope mapped with inhibition ELISA and by binding to different CB-peptides (purified Cyano Bromide cleaved peptides of Collagen II). The V-regions of these anti CII antibodies, representing different distinct epitope specificities have been sequenced and will be presented. Biotinylated anti-CII antibodies, specific for four different epitopes on the CII molecule and representing different IgG subclasses, could be shown to bind specifically to joint surfaces in the paws of 2 days old or adult syngeneic DBA/1 mice after an intraperitoneal injection of 100 µg of biotinylated antibody. No signs of clinical arthritis was observed, but some of the monoclonals induced mild synovitis, increased expression of class II and infiltration of CD4+ T-cells. The biotinylated anti-CII antibodies, injected into neonates or adult mice, also bound specifically to most, but not all, tissues containing CII; including hyaline joint cartilage, fibrous sternal and costal cartilage, tracheal cartilage and fibrous cartilage in the spine but not to CII-containing tissue in the eye. The anti-CII antibodies did not bind *in vitro* to joint sections of cartilage from DBA/1 mice, unless the sections were pretreated with hyaluronidase or the specimens decalcified prior to freezing, showing that the epitopes are accessible *in vivo* but not *in vitro*. These results show that cartilage is normally exposed to the immune system since CII is accessible for specific antibody binding *in vivo* during the neonatal period as well as in adult mice.

**C 529** HLA ASSOCIATIONS IN SCLERODERMA PATIENTS WITH EITHER ANTI-CENTROMERE ANTIBODIES OR ANTIBODIES TO THE Scl70 ANTIGEN. Penelope A. Morel, Tina Chang David J. Tweardy and Thomas Medsger Jr. Dept of Medicine, University of Pittsburgh, Pittsburgh Cancer Institute, Pittsburgh , PA 15213

Systemic sclerosis (SSc, scleroderma) is a multisystem connective tissue disease of unknown etiology. Certain HLA alleles have been associated with SSc but these associations have all been weak. The strongest associations have been among patients with the autoantibodies to centromeres (ACA) to HLA-DR1, and to HLA-DR5 among patients with anti-Scl70 antibodies. SSc patients with ACA are clinically distinct from those with anti-Scl70 antibodies in terms of the degree of cutaneous involvement. The advent of molecular techniques in HLA typing has allowed a greater degree of precision in typing and also permits the identification of shared epitopes which could be responsible for disease association. We have studied 15 SSc patients with ACA and 15 SSc patients with anti-Scl70 antibodies. DNA was extracted from the peripheral blood lymphocytes of these individuals and the DRB1 and DRB3 genes were amplified using the polymerase chain reaction. Amplified products were analyzed using a panel of allele-specific oligonucleotide probes. We have observed that, among the SSc patients with anti-Scl70 antibodies 71% of them express either DR3,5 or 6. Interestingly, all of these individuals carry the DRw52b allele of the DRB3 gene. This allele is commonly associated with DR5 but in our series patients with DR3 and DR6 were also DRw52b positive. By comparison with published normal distributions of these alleles, these results give a chi-squared of 4.14 ($p < 0.05$, odds ratio 8.1). Among the ACA patients no particular DR allele has emerged to date, but the analysis is not yet complete. Thus it appears that the diffuse from of SSc with anti-Scl70 antibodies is associated with the DRw52b allele.

**C 530** CHARACTERIZATION OF THE T-CELL REPERTOIRE AND THE ARTHRITOGENIC EPITOPE(S) IN COLLAGEN-INDUCED ARTHRITIS, Gamal E. Osman, Mark Feild, K. C. Cheng and Leroy Hood, Division of Biology, California Institute of Technology, Pasadena, CA 91125

Collagen type II-induced arthritis (CIA) in animals has been shown to be an experimental animal model system of the human autoimmune disease, rheumatoid arthritis. CIA is induced in susceptible animal strains by immunization with collagen type II. Although a body of evidence indicates that collagen arthritis is associated with a high level of both cellular and humoral autoimmunity to collagen type II, the role of antibodies and T lymphocytes in the pathogenesis is poorly understood. To study the function of T cells in detail, our current approach is to dissect components of the trimolecular complex involved in triggering pathogenic T-cell subsets as outlined below. Over 100 collagen type II reactive T-cell hybridomas have been established. To estimate the size of the T-cell repertoire directed against collagen type II in afflicted mice, the nucleotide sequences of the TCR of some of these hybridomas are being determined. In addition, we have generated three collagen type II reactive T-cell lines. These cell lines will be used to examine their arthritis-inducing potential in naive mice. More recently, we generated cyanogen bromide collagen type II fragments and isolated a single fragment which is capable of stimulating the collagen specific cell lines in vitro. This fragment will be injected into normal mice to examine its ability to induce arthritis. Once the induction of arthritis is established, the amino acid sequence of this fragment will be determined and overlapping peptides will be synthesized to map the arthritogenic epitope(s). We are confident that this study will lead not only to different strategies to treat this autoimmune disease in mice, but also it will shed light on the possible approaches to treat rheumatoid arthritis in man.

**C 531** MYCOBACTERIUM TUBERCULOSIS SPECIFIC T-CELL CLONES FROM RHEUMATOID ARTHRITIS PATIENTS. A. Rijnders, P. Res*, R. de Vries* and W. Olijve, Department of Microbiology Organon Int. bv Oss and * Department of Immunehaematology, Bloodbank Leiden, The Netherlands.

Increased proliferation to mycobacterial antigens by T-cells from RA patients has been observed. To characterize this reactivity we have generated T-cell clones from the synovial fluid mononuclear cells of RA patients by stimulation with Mycobacterium tuberculosis extracts followed by cloning. Specific clones were generally but not exclusively of the $CD4^+$ $CD8^-$ phenotype. The clones were characterized with respect to the restriction element used. T-cell clones derived from a patient of the DR1 DR7 haplotype predominantly recognized Mycobacterium tuberculosis in the context of the RA associated DR1 restriction element (16 out of 18 clones), while only 2 clones were restricted by DR7. Similar analysis of T-cell clones from patients of the DR4 haplotype is in progress. The 65 kD heat shock protein has been implicated as a possible antigen for RA associated T-cell responses, however, none of the clones analyzed sofar is stimulated by this protein. We are currently investigating the Mw of the proteins from Mycobacterium tuberculosis that are recognized by our T-cell clones and in addition we determine the variable gene usage by PCR with Vα and Vß specific primers.

**C 532** **T CELLS IN THE IMMUNOPATHOGENESIS OF LYME ARTHRITIS**, Marie-Claude Shanafelt and Gary Peltz, Syntex, Palo Alto, CA.

Cloned T lymphocytes reactive with Lyme disease spirochete antigens were isolated from 5 patients with Lyme arthritis. All of the reactive clones were $CD4^+TCR\alpha\beta^+$. However, there was no evidence for limited clonality in the T cell response. The clones recognized a variety of spirochetal antigens, were restricted by distinct HLA-D region elements and expressed different TCR Vα and Vβ segments. One T cell clone exhibited an HLA-DR2 restricted response to an epitope located between amino acids 250-283 of the 60 kD spirochetal heat shock protein (HSP), but did not recognize the expressed recombinant human HSP60. However, antibodies in this patients serum, bound to expressed human and spirochetal HSP60. γδTCR T cell clones were isolated from 2 $HLA\text{-}DR2^+$ patients, but not from 3 $HLA\text{-}DR2^-$ patients nor from an $HLA\text{-}DR2^+$ healthy control. The γδTCR T cell clones expressed Vγ9Vδ2 variable regions, exhibited non-HLA restricted lytic activity against target cells in the absence of spirochetal proteins, and did not proliferate in vitro in response to spirochetal proteins.

## *Late Abstracts*

Molecular analysis of TCRs (αβ) expressed in human T-cell clones specific for a tetanus toxin immunogenic peptide. O.Acuto*, B.Boitel*, M.Ermonval*, P.Panina°, R.A. Mariuzza+ and A.Lanzavecchia°.Laboratory of Molecular Immunology*, and Laboratory of Structural Immunology+, Pasteur Institute, Paris and °Basel Institute for Immunology, Basel.

$CD4^+$ T-cell clones specific for the tetanus toxin immunogenic p2 peptide antigen (residue 830-843), which can be presented by several HLA-DR alleles, were isolated from tetanus toxoid primed donors. To ask whether TcRs recognizing p2 presented by the same HLA DR allele show structural conservation, DNA fragments corresponding to their Vα and Vβ regions were prepared by anchor PCR and sequenced. Out of 19 T cell clones obtained from two donors and specific for P2 presented by HLA-DR 6, 12 utilized the same Vβ2 family member: 7 clones out of 14 from one donor and 5 out of 5 from the other. Functional expression of Vβ2 was confirmed by the proliferative response to staphylococcal exotoxin toxic shock syndrome toxin-1 (TSST-1). In contrast, these (Vβ2+)TcRs utilized at least 7 different Vα family members and both Vα and Vβ junctional regions showed no apparent conservation in amino acid sequence and in length. Possible interpretations of these findings are that Vβ2 provides most of the contacts with the p2/HLA DR6 complex or that, in view of the proposed role of the junctional regions in contacting mostly the antigen, p2 peptide may be bound to the same HLA DR in different conformations. We then investigated whether the preferential usage of Vβ2 could be found in response to p2 presented by other HLA DR alleles. Premliminary results indicate that only one out of nine anti-p2 T cell clones isolated from non-HLA DR 6 donors (DR1/DR2, DR1/DR5, DR3/DR5 and DR7) express Vβ2. Studies are underway to extend this analysis to additional anti-P2 T cell clones from these and other donors.

**The Role of RIII S/Js T-Cell Receptor Vβ Deletion in its Resistance to Collagen Induced Arthritis**. G. D. Anderson*, S.Banerjee, H. S. Luthra, M. Griffith, C. S. David; Dept. of Immunology, Mayo Clinic, Rochester,MN.

Susceptibility to collagen induced arthritis (CIA) in mice is restricted to H-$2^q$ and H-$2^r$ haplotypes. Previously, the role of T-cell receptor Vβ gene deletions has been implicated in the resistance of SWR mice (H-$2^q$) to CIA. More recently, another even larger TCR Vβ deletion mutation has been described in the RIII S/J mouse (H-$2^r$). This strain has also been shown to be highly resistant to CIA, even though it has a susceptible haplotype. In order to determine if this deletion influenced susceptibility to CIA, gene complementation experiments were performed making several crosses with RIII S/J (H-$2^r$, $TCR^{mutant}$) and B10 (H-$2^b$, $TCR^{wild\ type}$). In these crosses the RIII S/J strain contributes the susceptible H-$2^r$ and the B10 strain would have the normal wild type TCR genes but a resistant H-$2^b$. As shown below, after injection with porcine type II collagen, we were able to induce arthritis in crosses between these two resistant strains.

(B10 x RIII)$F_1$ 7/7 (100%) (B10 x RIII) x B10 22/24 (92%)

(B10 x RIII)$F_2$ 16/22 (73%) (B10 X RIII) X RIII 19/29 (65%)

Furthermore, FACS analysis of TCR expression showed there was a correlation between the presence of wild type TCR genes and arthritis. This indicates that the TCR mutation in RIII S/J may play a role in its resistance to CIA, and that complementing these missing genes from the TCR in the B10 background results in CIA susceptibility.

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REGULATION OF ISLET ANTIGEN EXPRESSION, Michael C. Appel, Francesco Dotta, John J. O'Neil and George S. Eisenbarth, Univ. Mass. Med. School, Worcester, MA, and Joslin Research Laboratories, Boston, MA.

Implantation of insulin producing RINm38 tumors induces atrophy and functional impairment of host pancreatic B-cells. In diabetes prone BB rats these metabolically "suppressed" islets are less susceptible to spontaneous autoimmune insulitis. To evaluate whether the observed protection was accompanied by down regulated islet antigens we determined the relative expression of monoclonal islet reactive antigens (A2B5,R2D6,3G5) and human Type I diabetes associated islet cytoplasmic antigens in suppressed islets. Antigen expression was also monitored in recovering islets obtained 1,2, and 6 days following tumor excision during which time a synchronous burst of metabolic activity is seen. Antigens were revealed in frozen sectioned NEDH rat pancreas using indirect immunoperoxidase histochemistry and quantitated using densitometric image analysis. In all cases, antigen density was markedly decreased in suppressed islets and normalized within 1 day of tumor resection. Since these islet monoclonals are directed against ganglioside moieties, other pancreata were obtained at the above intervals (n=5 per group) and evaluated for ganglioside content. TLC and HPLC analyses revealed the presence of a monosialoganglioside with mobility between GM2 and GM1 in animals at days 1,2 and 6 following tumor ablation. However, this apparently islet specific ganglioside was not detectable in tumor bearing (suppressed) pancreata. Our data suggest that B-cell functional activity regulates the expression of ganglioside and perhaps other undefined antigens which may play an important role in determining susceptibility to autoimmune injury.

CYCLOSPORIN A INDUCED AUTOIMMUNE DISEASE IN MICE IS INHIBITED BY THE PRESENCE OF NORMAL T CELLS. R. Pat Bucy, Department of Pathology, University of Alabama at Birmingham, Birmingham, AL 35294

Cyclosporin A (CsA) inhibits thymic selection of the $\alpha\beta$ T cell receptor (TCR) repertoire, apparently as a result of blockade of TCR initiated signal transduction. These investigations have focused on the conditions under which these unselected $\alpha\beta$ T cells can lead to actual autoimmune lesions. Adult BALB/c mice treated with daily CsA have a deficiency of both positive and negative thymic selection, but no signs of active autoimmune lesions are detected. If the peripheral T cell compartment is abolished via lethal irradiation, the animal reconstituted with T cell depleted syngeneic bone marrow (BM) and treated with CsA during the re-growth of the T cell repertoire, multiple organs develop inflammatory lesions indicative of active autoimmunity. Lesions develop in the colon, liver, stomach, lung, pancreas, and skin, but unlike allogeneic graft-vs-host disease, do not involve the small intestine. Immunohistochemical characterization reveals that the lesions are composed of $\alpha\beta$ T cells of both $CD4^+$ and $CD8^+$ subsets, macrophage, and scattered B cell aggregates. If the BM is not depleted of T cells, CsA treatment does not lead to autoimmune lesions. Furthermore, as described in a similar system in rats, the development of these tissue lesions can be adoptively transferred to irradiated syngeneic mice with spleen and lymph node T cells from the diseased mice. The adoptive transfer is inhibited by co-transfer of normal syngeneic splenic T cells. Using Thy-1 congenic mouse strains, the recirculation patterns of the adoptively transferred cells has been examined *in situ*. These experiments demonstrate that same absolute number of autoreactive T cells persist in the recipient lymphoid tissues with or without co-transferred normal T cells, but do not accumulate in the target tissues resulting in active lesions in the presence of normal T cells. These results indicate a role for a peripheral immunoregulatory activity in the maintenance of tolerance by cells which escape clonal deletion in the thymus.

MOLECULAR CHARACTERIZATION OF IG GENES OF LYMPHOID CELLS ESTABLISHED FROM MOUSE EMBRYONIC STEM CELLS IN VITRO. Una Chen, Basel Institute for Immunology, Basel, Switzerland.

Mouse embryonic stem cells (ES) have retained their pleuripotency to differentiate in vivo and in vitro. I show that in an organ culture system, ES cells can differentiate to complicated embryonic bodies. In these bodies, lymphohemapoietic precursors equivalent to the fetal liver stage of embryogenesis can be found. These precursor cells have been characterized by several functional assays, including one culture system which is known for measuring the differentiation potency of pre B cells. In this system, cells are cultured in a serum free medium in the presence of thymic feeder cells. ES derived precursor are shown to be able to differentiate into Ig positive, LPS responding B cells. Molecular analysis of Ig genes in these cells shows a distinct pattern of V(D)J rearrangement, which contributes to the differentiation of these precursors to Ig-secreting plasma cells in vitro.

**PEPTIDE SPECIFICITY OF A T-CELL HYBRIDOMA THAT RECOGNIZES OVALBUMIN + I-$E^d$ AND A CROSS-REACTIVE CLONOTYPIC ANTIGEN ENDOGENOUSLY SYNTHESIZED BY A B-LYMPHOMA HYBRIDOMA**, W. Louis Cleveland, Jerzy D. Wloka, and Imre Bodo, Dept of Medicine, St. Luke's\Roosevelt Hospital Center and Dept. of Microbiology, Columbia University, New York, NY 10019.

Splenocytes from a mouse immunized with Texas Red-ovalbumin were directly fused to BW5147 cells without in vitro stimulation. Screening identifed a hybridoma specific for un-modified ova + I-$E^d$. In other experiments, this line was also found to recognize a clonotypic antigen ( + I-$E^d$) endogenously synthesized by a B-lymphoma hybridoma that was prepared by fusion of ova-immune splenocytes with a B-lymphoma fusion partner. To characterize the ova specificty, ovalbumin was treated with clostripain, fractionated by low pressure gel filtration, and reverse phase HPLC. An incomplete digestion product was identified as a source of activity. Molecular weight was determined by laser ionization mass spectroscopy and the N-terminus was sequenced. Synthetic peptides are being used to specify further the recognized sequence and v-regions of the B-lymphoma hybrid are being sequenced to determine if this line bears a T-cell recognized internal image of the recognized ova peptide.

CIRCULATING CD26 POSITIVE CELLS IN DYSTHYROID ORBITOPATHY. Milton H. Dalbow, John S. Kennerdell, Radmila B. Raikow, Deborah A. Scalise, Laurie Machen and Mary Jo Buffo. Department of Ophthalmology, Allegheny General Hospital and Allegheny-Singer Research Institute, Pittsburgh,PA 15212

Various cell membrane markers of activation were measured by flow cytometric analysis in peripheral blood specimens of patients with dysthyroid orbitopathy. Grossly abnormal elevations in CD-$26^+$ circulating lymphocytes, monocytes and/or granulocytes were observed in 65 of 130 patients with stable disease. $CD26^+$ cells were serially monitored in 19 patients with initially active disease through transition to stable disease. Fifteen of these patients demonstrated significant increases (10 to 30 fold) in their circulating $CD26^+$ cells over their active disease baseline levels. CD26 is the cluster designation for dipeptidylpeptidase IV (DAP IV), a putative surface activation marker for lymphocytes involved in anamnesis. These data suggest a suppressor regulatory function for DAP IV in autoimmune disorders.

T CELL REPERTOIRE SELECTION: DO MATURE CTL RETAIN THE ABILITY TO BIND TO THE SELF MHC COMPLEXES THEY ENCOUNTERED DURING MATURATION?, Richard C. Duke and Paul B. Nash,. Immunothanatology Unit, Department of Microbiology and Immunology, University of Colorado School of Medicine, Denver, CO 80262

Triggered cytotoxic T lymphocytes (CTL) can kill any cell to which they are bound (A. Lanzavecchia, *Nature* 319:778). Based on this observation, we have developed an assay system to determine whether CTL have the ability to recognize cells bearing the self-MHC antigens expressed in the thymus in which they matured. When triggered by interaction with their specific targets or with anti-CD3 antibodies, 22 alloreactive CTL clones of 5 different specificities and origins, killed bystander targets bearing syngeneic (to the CTL) but not third-party MHC antigens. Using target cells derived from MHC-recombinant animals, syngeneic bystander killing was found to be restricted to a single self-MHC-encoded molecule. In addition, triggered, but not unmanipulated, alloreactive CTL (H-$2^d$ anti-H-$2D^b$) killed L cells (H-$2^k$) transfected with mutated H-$2D^b$ lacking the putative CD8 binding site (T. Potter, *Nature* 337:73). Our data are consistent with the idea that the mature CTL repertoire arises from positive selection of precursor cells having low but real affinity for self-MHC.

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DIFFERENTIAL ACTIVATION OF $CD4^+$ T CELL SUBSETS BY MURINE BRAIN CAPILLARY ENDOTHELIUM VS. SMOOTH MUSCLE. Zsuzsanna Fabry, Matyas Sandor, Tom Gajewski*, Richard G. Lynch and Michael N. Hart. University of Iowa, Iowa City, IA 52242, and *University of Chicago, Chicago, IL 60610.

Murine brain microvessel smooth muscle cells (SM) activate $CD4^+$ lymphocytes. This activation is surprisingly more pronounced in a syngeneic system than under allogeneic conditions and can be abrogated by anti MHC II mAb. Sorted activated $CD4^+$ cells upon adoptive transfer induce granulomatous vasculitis reactions in recipient mice. In contrast to SM, brain microvessel endothelial cells (En) activate $CD4^+$ lymphocytes in allogeneic co-cultures better than in syngeneic cultures. The supernates of syngeneic co-culture in the two systems differ in their lymphokine profile: SM-Ly 52±3 U/ml IL2; 4±0.5 U/ml IL4 while En-Ly 2.5±0.1 U/ml IL2; 41±4 U/ml IL4. When a well characterized panel of $CD4^+$ T cell clones was tested it was clear that SM preferentially activated $Th_1$ clones, however, En cells activate the $Th_2$ clones better. That preference was demonstrated with clones which were recognizing the same peptides on the same antigen in context of the same class II allotype. The role of different interleukins (IL1, IL3, IL4, IL5, IL6) and adhesion molecules (ICAM-1, ICAM-2, LFA-1, CD2) was studied in these systems. At this point, we do not have an explanation for differences in SM-Ly: En-Ly syngeneic activation but these findings may have implications concerning the mechanism of autoimmune vasculitis reactions.

LACK OF N REGIONS IN FETAL AND NEONATAL IMMUNOGLOBULINS AND T CELL RECEPTORS Ann J. Feeney, Medical Biology Institute, La Jolla, CA 92037

Much of the diversity in T and B lymphocyte receptors is derived from the addition of non-templated N region nucleotides by TdT at the junctions of the receptor V, D and J gene segments during somatic rearrangement. I have amplified genomic DNA from murine fetal and newborn liver and spleen cells by PCR and sequenced the IgH chain variable regions. These junctional sequences showed a virtual absence of N regions, in contrast to adult splenic DNA, where 83% of sequences contained N regions. It has been shown that fetal $\gamma\delta$ T cells lack N regions. Since the level of TdT is slow to reach adult levels in the thymus, and since $\alpha\beta$ T cells are the predominant T cells in the thymus after birth, I analyzed junctional sequences of TCR $\beta$ chains from DNA of fetal and newborn thymuses to determine if they also lacked N regions. Few N regions were detected in junctional sequences from fetal d. 18 DNA, and 29% of sequences from newborn thymuses had N regions. In contrast, 88% of sequences from adult thymus or spleen contained N regions. Since it has been proposed that TCR CDR3, which contains all of the N regions, is the region of the TCR which recognizes peptides, the lack of N regions in TCR should restrict the repertoire of the fetal/neonatal T cells even more drastically than the repertoire of fetal/neonatal B cells. These data show that N region insertion is a developmentally regulated process in all lymphocytes. They also show that the fetal/neonatal repertoire of all lymphocytes is more restricted than, and largely different from, the adult repertoire. Data will be presented to explain why the idiotypic composition of the neonatal Ig repertoire can significantly affect the adult repertoire to that antigen.

INAPPROPRIATE EXPRESSION OF CLASS II MHC BY NOD MOUSE SINGLE-CELL CELL ISLET CELLS: IS IT THE INITIAL EVENT ? Bent Formby, Laboratory of Immunology, Sansum Medical Research Foundation, Santa Barbara CA 93105

The expression of class II MHC molecules by viable single-cell islet cells (SCICs) prepared from male and female 4- and 10-week-old nonobese diabetic (NOD) mouse islets was investigated by flow cytometric analysis. With one mAb and two alloantisera specific for I-A(NOD), and FITC-conjugated goat anti-mouse IgG as second step antibody, it was found that SCICs aberrantly expressed class II molecules. Double-indirect immunofluoroscence of male SCICs indicated that expression of class II MHC molecules was a property of beta cells. Control experiments documented that macrophages and mononuclear cells did not contaminate the SCIC preparations. Coculture experiments with responder splenic CD4 T cells isolated from diabetic NOD mice and stimulator male SCICs indicated a recognition event evidenced by a 12-fold increase in proliferative response. The three antibodies to class II MHC and anti-CD4 mAb blocked the proliferative response. Results from autologous and allogeneic MLRs suggest that the responder CD4 T cells are autoreactive self-class II MHC restricted. This was further evidenced from experiments where 4-week-old female NOD mice were injected with 10 million splenic CD4 T cells isolated from diabetic NOD mice. By the age of 7 weeks overt diabetes developed in 4 of 7 experimental animals, which all showed severe pancreatic infiltration.

CHARACTERIZATION OF ALLORESPONSE REGIONS OF T CELL ANTIGEN RECEPTOR, Soon-cheol Hong, Rong-Hwa Lin, Linda Klein, Adina Chelouche, Charles A. Janeway Jr. HHMI and Section of Immunobiology, Yale University School of Medicine, New Haven, CT 06510

$\alpha$ and $\beta$ chains of T cell antigen receptor from two different T cell lines, D10.G4.1 (D10) and AK8 have been cloned and sequenced. Both clones are AKR/J-derived, antigen-specific, CD4 positive helper clones, and specific for hen's egg conalbumin:I-$A^k$. However, variances in alloreactivity distinguish the D10 from AK8 T cell clone. Whereas D10 T cell clone is stimulated by allostimulators such as I-$A^b$, I-AP, I-$A^v$, I-$A^q$, and I-$A^d$, AK8 is not. DNA sequencing results reveal that both T cell clones used identical $\beta$ chains, but different $\alpha$ chains of the T cell antigen receptor. These differences, which are present only in the CDR1, CDR2 and CDR3 regions of the $\alpha$ chain, may explain the lack of alloreactivity in AK8.

PRESENTATION OF AUTOANTIGEN BY HUMAN T CELLS, Janine M. LaSalle, Kohei Ota, and David A. Hafler, Center for Neurologic Diseases, Division of Neurology, Department of Medicine, Brigham and Women's Hospital, and Harvard Medical School, Boston, MA 02115

Activated human T cells express MHC class II and have been previously shown to present foreign antigen to other T cells. We now demonstrate that T cells generated against the autoantigen myelin basic protein (MBP) are capable of presenting this self antigen to autologous T cells with both HLA-DR and -DP as class II MHC restricting elements. While T cell clones can present peptide antigen without the need for processing, they can minimally process and present whole MBP. T cell clones presenting MBP peptides specifically stimulated autologous T cell clones to flux calcium and proliferate. These activation responses were peptide specific and blocked by monoclonal antibodies (MAb) to MHC class II, indicating a T cell receptor (TcR) mediated response. In addition, MAbs to the adhesion molecules CD4, LFA-3, CD2, LFA-1, 4B4, and to the tyrosine phosphatase CD45 also blocked the interaction. These results raise the possibility that the interaction between activated autologous T cells involving the TcR reacting to peptide antigen presented by class II MHC may be important in the immunoregulation of responses to self antigen.

ACTIVATION OF T CELLS REQUIRES COEXPRESSION OF TCR-LIGAND AND COSTIMULATORY SIGNAL ON THE SAME ACCESSORY CELLS. Yang Liu and Charles Janeway, Jr, Section of Immunobiology, Yale University School of Medicine and Howard Hughes Med Institute, 310 Cedar Street, New Haven, CT06510

Costimulatory signals play a critical role in switching the consequences of TCR engagement from progra cell death or clonal anergy into clonal expansion. Because nonhemopoetic tissues are usually devoid of costimulatory activity, it has been proposed that interaction of T cells with tissue-specific antigens activel induce T cell tolerance to tissue-specific antigens. This hypothesis is plausible only if activation of T ce requires coexpression of the T cell ligand and the costimulatory activity on accessory cells. The major ol to formally demonstrating such a requirement has been the obscure nature of costimulatory signals and t consequent difficulty in obtaining cells expressing one or the other of these two signals. In this study we anti-TCR/CD3 mAb as ligand and measure proliferation of normal murine CD4 T cells as a model systen analyse this question. The formation of an effective TCR ligand in this system depends strictly on functi FcR on accessory cells that could be blocked anti-FcR mAb. This treatment does not affect the costimula activity of LPS-activated B cells, as the treated cells still have costimulatory activity if we use an alternat pathway to cross-link TCR. We studied the cooperation of fixed, anti-FcR-treated LPS-activated B cells fixed B lymphoma A20 which express high level of FcR yet lack of costimulatory activity in anti-CD3 in proliferation of CD4 T cells. Our results demonstrate that these two types of cells can not cooperate to in proliferation of CD4 T cells in the presence of anti-CD3 mAb. This provide direct evidence that activatic CD4 T cells requires coexpression of the TCR ligand and a costimulatory signal on the same accessory c

A POSSIBLE ROLE FOR THE TRANSCRIPTION FACTOR-ENCODING GENE EGR-1 IN THE TRANSLATION OF sIgM SIGNALS INTO ACTIVATION VERSUS TOLEROGENIC RESPONSES IN IMMATURE AND MATURE B LYMPHOCYTES, John G. Monroe, Vicki Seyfert, Vikas Sukhatme, and Xinman Cao. Dept. Pathology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

Egr-1 is an immediate/early transcription factor encoding gene whose inducible expression is associated with positive activation responses to sIgM signalling in mature B lymphocytes. In contrast, bone marrow-derived immature B cells do not express Egr-1 following sIgM crosslinking. Using the phenotypically immature B cell lymphoma WEHI-231 as a model system for these cells, we have investigated the regulation of Egr-1 induction and have determined its association with the differential processing of sIgM signals into positive versus negative activation responses. Our studies indicate that Egr-1 is methylated in WEHI-231 and that this methylation accounts for its lack of inducible expression in these cells. Under conditions where Egr-1 can be induced in WEHI-231, we observe a reversal in the normal negative growth response to sIgM induced signalling. We propose that methylation of Egr-1 during early B cell development may account for the lack of antigen responsiveness of immature B cells and lead to tolerance rather than activation of those B cells encountering antigen during this stage of development.

THE CONTROL OF PROLIFERATION IN TH0 T CELLS

Daniel L. Mueller, the Department of Internal Medicine, University of Texas Southwestern Medical Center at Dallas, Dallas, Texas 75235

Immune responsiveness to foreign antigen, in the face of a requirement for tolerance to self-antigen, may depend on the tight control of CD4+ T-cell proliferation. Clonal anergy is one mechanism by which self-reactive T cells could be rendered incapable of proliferating in response to peripheral self-antigens. *In vitro* models of anergy predict that recognition of self-antigen by naive T cells *in vivo* would not result in proliferation; instead, an IL-2 production defect would be induced in the cells.

Naive T cells, however, may be capable of producing both IL-2 and IL-4 upon antigen stimulation. The work presented here, therefore, examines the control of antigen-induced proliferation in Th0 (IL-2- and IL-4-producing) T-cell clones, *in vitro*. Both IL-2 and IL-4 are shown to be important in the autocrine proliferative response of these cells. In addition, proliferative anergy can be induced in Th0 cells, and the anergy is associated with the development of an IL-2 production defect. Remarkably, antigen-induced IL-4 production remains relatively intact in these anergic cells. The results support the hypothesis that clonal anergy can be important in the control of T-cell proliferative responses to self-antigen *in vivo*, even in cells capable of producing both IL-2 and IL-4. Furthermore, the data suggest a physiological mechanism whereby the pattern of lymphokine secretion (IL-2 versus IL-4) by T cells can be regulated.

**IL-10 Acts on the Antigen Presenting Cell to Inhibit Cytokine Production by Th1 Cells** A. O'Garra, A. Zlotnik, P. Vieira, T. R. Mosmann, M. Howard, K. W. Moore, and D. F. Fiorentino· Department of Immunology, DNAX Research Institute, 901 California Avenue, CA 94304

Murine IL-10, which is produced by Type 2 (Th2) helper T cell clones and by B cells, inhibits cytokine production by Type 1 (Th1) helper T cell clones when they are activated under conditions requiring the presence of antigen presenting cells (APC). We show that IL-10 inhibits IFNγ and IL-2 production by Th1 clones by impairing APC function and using FACS purified cells, as well as macrophage and B lymphoma cell lines, we show that IL-10 inhibits the ability of purified macrophages, but not B cells, to stimulate Th1 clones. Our data suggest that IL-10 does not interfere with the T-cell-receptor-MHC-antigen complex but inhibits the APC function of macrophages by an unknown mechanism. It is possible that IL-10 induces production of an inhibitory factor by the macrophage which then acts on the T-cell to inhibit cytokine secretion. However, we have been unable to identify such an activity. Alternatively, IL-10 may down-regulate a costimulatory activity needed for optimal Th1 cytokine secretion, and hence proliferation, in response to macrophages and antigen. We show that IL-10 down-regulates the production of IL-1, IL-6 and TNF-α by activated macrophages, but neither these cytokines nor supernatants from activated macrophages could overcome the ability of IL-10 to down-regulate APC function. Our data are reminiscent of systems previously described whereby T-cell receptor occupancy alone, without the delivery of an accessory-cell derived costimulatory signal, gives a signal to down-regulate further antigen responsiveness and possibly to maintain self tolerance. The possibility that the costimulator involved in these systems is regulated by IL-10 is under investigation.

RHEUMATOID SYNOVIAL FLUID DERIVED T CELL CLONES RESPONSIVE TO MYCOBACTERIAL ANTIGENS INCLUDING THE 65KD HEAT SHOCK PROTEIN.

Alison J. Quayle, Jens Kjeldsen-Kragh, Shu-Guang Li, *Fredrik Oftung, #Thomas M. Shinnick, Øystein Førre and Jacob B. Natvig. Institute of Immunology and Rheumatology, University of Oslo, 0173 Oslo, Norway, *Department of Immunology, Radium Hospital, 0310 Oslo, and #Hansens Disease Laboratory, Center for Disease Control, Atlanta GA 30333.

The response of synovial compartment T cells to BCG and to a recombinant preparation of M.bovis 65kD heat shock protein (65kD HSP) is substantially higher than peripheral blood T cells in over 80% of the BCG responding rheumatoid arthritis patients examined in our laboratory.

A panel of clones was raised from the synovial fluid of one of these patients (HLA DR4,5) using BCG. All 26 clones were αβTCR$^+$ CD4$^+$, but used a selection of V gene segments. The Vβ6$^+$ and Vβ8$^+$ clones (12) were studied in more detail; all were DR restricted, did not respond to 65kD HSP, and all so far examined exhibit specific cytotoxicity against BCG pulsed autologous macrophages.

Two clones were also raised from the synovial fluid using 65kD HSP. Using a series of overlapping synthetic peptides corresponding to the M.tuberculosis 65kD HSP, the specificity of one of the clones was mapped to the amino acid sequence 241-255, a region almost identical to the human 65kD HSP.

The significance of these results will be discussed.

ALTERED EXPRESSION OF THE TYROSINE KINASE $p56^{lck}$ IN ANERGIC TH1 CLONES, Helen Quill and Eun Ah Cho, Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA 19104

Long-lived proliferative nonresponsiveness (anergy) was induced in cloned TH1-type cells by culture with immobilized anti-CD3 antibody. Resting anergic and control cells were compared for expression of the T cell tyrosine kinase $p56^{lck}$ (*lck*) by immunoblotting with specific anti-*lck* antiserum. Anergic cells expressed decreased amounts (40-70% decrease) of detectable *lck* protein as compared with control levels. Decreased *lck* expression correlated with continued nonresponsiveness in anergic cells. Furthermore, inhibition of the induction of anergy also inhibited the loss of *lck* protein. Activation of control cells by antigen or soluble anti-CD3 antibody in the presence of APC resulted in the tyrosine phosphorylation of an as yet unidentified 38kd protein, whereas activation of anergic cells under the same conditions resulted in greatly decreased phosphorylation of this substrate. These data indicate that tyrosine kinase expression is altered in anergic T cells.

IgE INVOLVEMENT IN GRAVES' ORBITOPATHY Radmila B. Raikow, John S. Kennerdell, Milton H. Dalbow and Deborah A. Scalise. Department of Ophthalmology, Allegheny General Hospital and Allegheny-Singer Research Institute, Pittsburgh, PA 15212

IgE was detected in extraocular rectus, Muellers and levator muscles from Graves' orbitopathy patients and in orbital adipose tissue hypertrophied due to Graves' orbitopathy. Different distributions of reactivity were observed using monoclonal antibodies from different sources. Thus, anti-IgE No. I 6510 from Sigma was most reactive with material associated with muscle fibers, both skeletal and smooth; and anti-IgE No. 411500 from Cal Biochem was most reactive with infiltrating lymphocytes and with hypertrophied connective tissue. Similar patterns were obtained using either a streptavidin-biotin or a peroxidase-anti-peroxidase secondary system. Isotype and concentration matched reagent controls were negative in the case of nonspecific mouse immunoglobulin and positive only on myelomonocytic cells in the case of anti CD15. Ocular tissues from non-Graves' patients with congenital esotropia or ptosis were IgE negative or had positivity on mast cells only. However, ocular tissue from a patient with eye phthisis and another patient with esotropia due to scarring from accidental trauma had some IgE reactivity around muscle fibers indicating that IgE may also be involved in these ophthalmopathies. Serum IgE levels were found elevated by ELISA in some Graves' patients, independent of allergies and associated with severe or progressing Graves' orbitopathy. Tests to ascertain the antigen specificity of the serum IgE are in progress.

**T Cell Clones: A Probe for Understanding Murine Diabetes**

Eva-Pia Reich, Eric Rashba, Deborah Webb, Robert S. Sherwin and Charles A Janeway, Jr. Section of Immunobiology, Howard Hughes Medical Institute,and Department of Medicine, Yale University School of Medicine, New Haven, Connecticut.

Insulin dependent diabetes mellitus (IDDM) is believed to be caused by a specific T lymphocyte-mediated autoimmune attack against pancreatic beta cells. We have isolated and cloned T-lymphocytes from islets of newly diabetic NOD mice, some of which are diabetogenic in irradiated NOD mice and islet specific and others which are autoreactive to syngeneic NOD spleen cells and prevent disease in normal NOD mice. In the present study, we asked if islet derived T cells can down-regulate activated diabetogenic NOD spleen cells in an adoptive transfer system. Eight weeks old \ irradiated (700R) NOD female mice were treated i.v. with saline, PR-3 (a CD4+ autoreactive T cell), 2E9 (a CD4/CD8 islet specific T cell clone) or with a combination of these T cell clones ($10^6$ cells/animal). All mice except a control group 24 hrs later received fresh spleen cells from newly diabetic NOD female mice. Mice were screened for diabetes by urine and blood analysis.

| Treatment Groups-day 0 | Transfer of diabetic spleen cells-day 1 | #Diabetic mice/ total # mice |
|---|---|---|
| PBS | - | + 0/7 |
| PBS | + | + 7/7 |
| PR-3 (CD4) | + | + 4/7 |
| 2E9 (CD4/8) | + | + 2/5 |
| PR-3 and 2E9 | + | + 1/9 |

A combination of the islet-derived CD4 and CD4/CD8 T cell clones appear to be able to down regulate activated diabetogenic spleen cells and thereby prevent ß cell destruction and diabetes in the NOD mouse. Islets of NOD mice appear to contain both diabetogenic and regulatory T cells. This may explain the chronicity of ß cell destruction in IDDM.

**CELLULAR AND MOLECULAR ANALYSES OF SCID B CELLS RESCUED BY ADOPTIVE TRANSFER OF NEONATAL T LYMPHOCYTES**, James E. Riggs, Ann J. Feeney, Marybeth Kirven, Donna Thuerauf, and Donald E. Mosier, Division of Immunology, Medical Biology Institute, La Jolla, CA 92037

SCID (severe-combined immune-defective) mice lack mature lymphocytes due to an autosomal recessive mutation manifest as defective joining of T cell receptor and immunoglobulin (Ig) genes. Penetrance of this mutation is incomplete as 10-25% of SCID mice spontaneously produce serum Ig. We have recently shown that all SCID mice can be induced to produce Ig by provision of neonatal, but not adult, T cells. The IgM produced was that of the SCID recipient ($Igh^{b+}$) as shown by allotype-specific ELISA. SCID B cells can class switch as evidenced by production of all Ig isotypes. Allotype-specific spotELISAs revealed small numbers of SCID B cells in the spleen, bone marrow, and peritoneal cavity. We have initiated molecular analyses of these B cells using the polymerase chain reaction. Two of three SCID mice analyzed to date exhibit rearrangement of the recently described Vh11 gene family. Another SCID mouse had an expanded set of VhS107 (V1) sister clones with somatic mutations. These results will be discussed in regards to the *scid* defect and lymphocyte development (supported by RO1 A1-22871, PO1 A1-24526, and T32-AI-07259).

PRESENTATION OF Ig EPITOPES IN THYMUS, Alexander Rudensky*, Svetlana Mazel† and Vitalij Yurin†, *Section of Immunobiology and Howard Hughes Medical Institute Yale University School of Medicine, 310 Cedar Str., New Haven, CT 06510, †Laboratory of Immunology Institute for Genetics and Selection of Microorganisms, Moscow, USSR .

The presentation of endogeneous Ig epitopes in thymus was studied in the experimental system utilizing allelic differences in Ig kappa chain in inbred rats. There are two allelic variants of rat Ig kappa chain - Igk-1a and Igk-1b which differ by 11 amino acid substitution in Ck domain. Using Ig-recognizing Th clones the expression of Ig peptide /MHC class II complexes derived by the processing of endogeneous Ig in the thymus was demonstrated. It was found that thymic B cells but not "professional" thymic APC (dendritic cells and MØ) represent the major APC type of endogeneously synthesized sIg (Igk-1b) and idiotype of anti-sIg (Igk-1b) antibodies . Similar results were previously obtained in the study of Igk-1b and anti-Igk-1b idiotope presentation by spleen cells. The Igk-1b-presenting activity is expressed in thymus relatively late, only after 3 weeks of postnatal life , while in the spleen an efficient presentation of endogeneous Ig epitope is observed very early after birth. The observed difference between thymic and peripheral presentation of endogeneous Ig epitopes may be important for understanding of T cell tolerance to self Ig.

HUMAN IMMUNOGLOBULINS THAT BIND TO STAPHYLOCOCCAL PROTEIN A CONTAIN $V_H$III H CHAINS. Sasso, E.H., Silverman, G.J., and M. Mannik. University of Washington, Seattle, WA 98195, and University of California, San Diego, La Jolla, CA 92037.

Staphylococcal Protein A (SPA) possesses, in addition to its well-characterized Fc-binding sites, distinct sites that bind to the Fab region of some IgM, IgA, IgG, and IgE. The Fab site bound by SPA has been localized to the V region of the immunoglogulin (Ig) H chain. This study provides a genetic basis for the SPA-Fab interaction by demonstrating an association between $V_H$ subgroup and ability to bind to SPA. Binding of 24 human monoclonal IgM was measured in a solid phase radioimmunoassay. The $V_H$ subgroup of each IgM was determined by immunoblotting, and detection with rabbit antisera to synthetic peptides specific for first framework regions of $V_H$ subgroups I, II, and III. Ten IgM bound well to SPA, 14 bound SPA poorly or not at all. Binding did not correlate with IgM binding to goat $F(ab')_2$ anti-human Fc, or with rheumatoid factor activity. All ten SPA-binding IgM belonged to the $V_H$III subgroup. Only one $V_H$III IgM failed to bind SPA, whereas none of 7 $V_H$I or 6 $V_H$II proteins bound to SPA. Fractionation of polyclonal human IgM, IgA, and IgG $F(ab')_2$ on SPA-agarose columns yielded SPA-binding fractions of 6%, 22%, and 15%, respectively. The SPA-binding fractions of IgM and IgA were dominated by $V_H$III, and depleted of $V_H$I and $V_H$II protein. The SPA-binding fraction of IgG $F(ab')_2$ contained only $V_H$III molecules. Furthermore, the $V_H$III molecules contained in the SPA-binding fractions comprised from half (IgG) to about 90% (IgM) the total $V_H$III protein detected. We conclude that $V_H$III H chains are a property of most SPA-binding human IgM and IgA, and essentially all SPA-binding human IgG, and that within the $V_H$III subgroup, SPA-binding Ig are highly prevalent.

THYMUS SELECTION AS A REGULATOR OF AUTOIMMUNITY IN AN AUTOMATON MODEL OF THE IMMUNE SYSTEM, Philip E. Seiden, IBM Research Center, Yorktown Heights, NY 10598 and Franco Celada, Hospital for Joint Diseases, New York, NY 10003

We have constructed a "cellular automaton" model of the immune system for the purpose of examining the importance of interactions between the various components of the system. The model includes B cells (both plasma and memory cells), T cells, APC, antigens and antibodies. Eight bit binary sequences are used to represent the receptors (for B and T cells), the MHC (for APC and B cells), and the epitopes, paratopes and idiotopes. The B and T cells initially span the complete repertoire possible (256 types for an 8 bit system). However, the model includes a "thymus" which provides both positive and negative selection on the T cell repertoire. Given a series of rules directing the interactions of antigen with antibody, B cell receptors and APC, and T cell receptors with MHC, the progress of the model is self-sustained and self-regulated. The system allows us to design experiments to test any aspect of the simulation and weight their importance. For example by varying the numbers of MHC and "self-peptides" we investigate how these influence both the efficiency of the immune system and autoimmunity.

MOLECULAR ANALYSIS OF V REGIONS ENCODING PATHOGENIC AUTOANTIBODIES TO THE RELATED I AND i RED BLOOD CELL ANTIGENS, Leslie E. Silberstein, Gregg J. Silverman, David F. Friedman, June Goldman, Jonni Moore, Peter C. Nowell, and Leigh C. Jefferies, Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA 19104 and Department of Medicine, University of California San Diego, La Jolla, CA 92093

The I and i antigens constitute a complex system of oligosaccharide determinants on the red blood cell membrane. At the end of fetal development, there is a regulated transition from the predominant expression of i antigen on fetal red blood cells to mostly I antigen on adult red blood cells. Autoantibodies to the I - and rarely to the i - carbohydrate antigen on red blood cells are readily detected in neonatal serum and persist throughout adult life. In some individuals, however, these autoantibodies cause autoimmune hemolytic anemia and are derived from clonal B-cell populations which may progress to frank lymphoma. Previous serologic studies have demonstrated a preferential $V_H$IV-$V_K$III gene family use by pathogenic anti-I, suggesting that this autoimmune response may be influenced by antigen selection. To further understand the molecular basis of this autoimmune response, we established B-cell clones from two patients with anti-i and anti-I induced hemolysis. Sequence analyses indicate that both anti-i and anti-I specificities are encoded by the same $V_H$4.21 gene, whereas the anti- i use $V_K$I and the anti-I use $V_K$III genes. When compared to their relevant germline sequences, the anti-i secreting B-cells express germline encoded $V_H$ and $V_L$ region gene sequences; in contrast, the $V_H$ and $V_L$region genes of the anti-I secreting B-cells harbor numerous non-random base pair changes, typical of antigen driven immune responses. Taken together, these sequence analyses suggest that the pathogenic immune response to the related i and I autoantigens is restricted to a single gene of the $V_H$4 family and further support a role for antigen mediated selection.

A MONOCLONAL CROSS-REACTIVE IDIOTYPE LINKED TO THE HUMAN VH4.21 GENE IDENTIFIED RHEUMATOID FACTOR AND ANTI-RED CELL MEMBRANE AUTOANTIBODIES, Gregg J. Silverman and *Leslie E. Silberstein. Dept. of Medicine, University of California, San Diego, La Jolla CA, 92093, *Dept. of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA 19104

Rheumatoid factors (RF), and cold reactive autoantibodies to cell surface glycoconjugates, cold agglutinins (CA), are the most common types of monoclonal human antibodies to spontaneously arise during lymphoproliferative syndromes. These anti-self antibodies may be produced as part of physiologic responses, or may be pathogenic when they take part in autoimmune attack, causing vasculitis or severe hemolysis. To assess the structural basis of autoreactivity, serologic reagents specific to diagnostic primary sequences in VH and VL regions have been created. We have applied these anti-peptide antibodies to the characterization of the V region diversity of circulating antibodies of known and unknown sequence, and used them to define the binding specificity of conventional monoclonal anti-idiotypes. A panel of 12 monoclonal IgM CA with predominant reactivity with the I determinant, and 9 monoclonal IgM CA reactive with the structurally and developmentally related i determinant were found to use exclusively H chains that derive from a VH4 subfamily, but L chains derived from diverse VL families. These CA were also identified by a previously described rat monoclonal anti-idiotype, termed 9G4. This anti-idiotype inhibits CA activity, and anti-IgG binding by the VH4-derived IgM RF, Les. By sequence comparison the 9G4 idiotope is restricted to H chains that derive from the VH4.21 germline gene. Taken together, the data suggest that the binding activities of pathogenic anti-Ii CA require the expression of a single VH4 gene, in combination with a permissive L chain that may derive from diverse VL genes.