THE T CELL RECEPTOR

John Kappler and Mark Davis, Organizers
April 26-May 1, 1987

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The T Cell Receptor

T Cell Receptor Gene Structure and Organization

T 001 The organization and expression of T-cell receptor genes in mouse and man

L. Hood, B. Arden, R. Barth, P. Concannon, N. Costlow, W. Funkhouser, J. Goverman,
J. Kobori, D. Korn, C. Kuo, E. Lai, N. Lan, N. Shastri, J. Urban, R. Wilson

In order to obtain probes for T-cell receptor (Tcr) gene mapping we have sequenced 27 human and 15 murine α cDNAs and 27 human and 8 murine β cDNAs. Statistical analyses of these results suggest that there may be approximately 100 Vα and 25 Vβ murine gene segments and ~100 Vα and 60 Vβ human gene segments. Using the V-gene segments as probes, we have employed the deletional analyses of cloned T-cells, pulse gradient gel elecrophoresis and the restriction mapping of cosmid clones to construct physical maps of the β-gene families. The β-gene family of mouse has 19 Vβ gene segments and the Dβ-Jβ-Cβ clusters encoded over about 450 kb of DNA whereas the human β-gene family (>35 Vβ gene segments and two Dβ-Jβ-Cβ clusters) encompasses more than 600 kb of DNA. Similar mapping studies are now underway of the α-gene families of human and mouse. In addition we have analyzed the Vβ gene segment polymorphisms in DNAs isolated from more than 100 humans.

To produce relatively large amounts of T-cell receptor molecules for further study we have utilized a baculovirus expression system for the Tcr. Genes encoding the α and β chains of a murine alloreactive Tcr recognizing the K^b class I MHC molecule were isolated from a cytotoxic T-cell cDNA library and separately cloned onto the polyhedron gene promoter of the Autographa californica nuclear polyhedrosis virus. Sf9 insect cells infected with either the α- or β-chain recombinant virus produce high amounts of a novel 3.6 kb RNA transcript consisting of the α- or β-chain coding sequences and polyhedron 5' and 3' untranslated regions. The level of message expression is more than 1,000 times that found in murine T-cells on a per cell basis. Furthermore, this high level of expression does not decrease when Sf9 cells are co-infected with both the α- and β-chain recombinant viruses. We are currently analyzing the nature of the proteins produced in these cells using 2-D gel electrophoresis and are exploring the possibility of producing large amounts of secreted Tcr by introducing stop codons in the α and β chain transmembrane regions.

In efforts to understand factors which regulate the expression of T-cell receptor genes, we have 1) constructed transgenic mice with murine β genes, 2) transferred α and β genes from a T_H cells specific for cytochrome C and lysozyme and into a T-cell tumor line, and 3) examined the Jα-Cα intron of a rearranged Cα gene for the presence of enhancer-like sequences. The results of these expression studies will be summarized.

T 002 ORGANIZATION AND EXPRESSION PATTERN OF T CELL RECEPTOR β-CHAIN GENES, Dennis Y. Loh, Hubert Chou and Mark Behlke, Howard Hughes Medical Institute, Departments of Medicine, Microbiology and Immunology, Washington University School of Medicine, St. Louis, MO 63110.
We have focussed our studies on the murine T cell receptor (TCR) β-chain locus. Our studies reveal that there are only 20 V_β genes and most of these genes are very closely linked on the chromosome. One consequence of this close linkage is that functional β-gene messages can be created by novel RNA splicing of the upstream leader exon to the V_β coding exon of the downstream rearranged gene. In addition, possible implications of the close linkage of the V_β genes on development of the TCR repertoire will be discussed.

The T Cell Receptor

Structure of the Receptor Complex and Signal Transduction

T 003 THE T CELL ANTIGEN RECEPTOR AND SIGNAL TRANSDUCTION. Lawrence E. Samelson and Richard D. Klausner. Cell Biology and Metabolism Branch, National Institute of Child Health, National Institutes of Health, Bethesda, Maryland 20892.

The murine clonotypic T cell antigen receptor consists of multiple components. The heterodimeric α and β chains responsible for MHC-restricted interactions are non-covalently associated with the γ, δ, ϵ, ζ and p21 subunits. Receptor engagement results in multiple rapid biochemical events occurring at the receptor. Phosphoinositide hydrolysis is coupled to the receptor, and subsequent protein kinase C activation results in serine phosphorylation of the γ chain. Similarly receptor occupancy leads to activation of a tyrosine kinase which phosphorylates the p21 chain and additional cellular substrates. Both kinase events are regulated by the activation of a third kinase, cyclic AMP dependent protein kinase. Signal transduction mediated by the antigen receptor thus involve the interplay between several stimulatory and inhibitory kinases.

T 004 CELL SURFACE MOLECULES AND MOLECULAR EVENTS INVOLVED IN HUMAN T CELL ACTIVATION. Arthur Weiss, Dan Littman and Mark Goldsmith. Howard Hughes Medical Institute, Dept. of Medicine and Microbiology/Immunology, U. of Ca., San Francisco, Ca., 94143.

Two stimuli are required for the transcriptional activation of the IL-2 gene in resting T cells. One of these stimuli can be provided by ligands which interact with the CD3/antigen receptor (Ti) complex and the other may involve interactions with accessory molecules expressed on the T cell. Although the transmembrane signal of these accessory molecules is unclear, stimulation of the CD3/Ti complex results in phosphoinositide (PI) hydrolysis with consequent increases in cytoplasmic free calcium ($[Ca^{2+}]_i$) and activation of protein kinase C.

The relative function of the components of the CD3/Ti complex in transmembrane signalling is not clear. Of particular interest is the CD3+ T cell leukemic line PEER. In marked contrast to other T cell lines or most peripheral T cells, no PI turnover or changes in $[Ca^{2+}]_i$ were observed in response to the lectins PHA or Con A or to anti-CD3 in PEER. Activation of PI turnover could be induced in response to AlF_4^-, an agent known to activate G-binding proteins, suggesting defective coupling of the antigen receptor to the PI pathway. The CD3 containing complex on PEER was found to be unusual; on this cell, CD3 is associated with a non-disulfide linked 55 kD protein instead of a $Ti_{\alpha/\beta}$ heterodimer. This 55 kD protein has been identified as a product of the Ti_γ locus using an anti-peptide antiseras and by the presence of a functional Ti_γ chain rearrangement in PEER. The expressed Ti_γ chain utilizes the $C_\gamma 2$ gene but contains an unusual triplication of 48 bp. The unusual structure of the antigen receptor complex on PEER may be responsible for its failure to function in signal transduction.

In a second approach to understanding the function of the CD3/Ti complex, signalling mutants of the Jurkat leukemic line have been isolated utilizing the cell sorter and the calcium sensitive fluor indo-1. These mutant cells, unlike the wild-type cells, fail to accumulate the products of PI hydrolysis or manifest increases in $[Ca^{2+}]_i$ in response to anti-CD3 or anti-Ti antibodies despite the ability of these antibodies to bind to these cells. The response of these mutants to AlF_4^- suggests a proximal defect in the CD3/Ti complex. These cells together with PEER should provide useful models to address the relative function of the components of the CD3/Ti complex in signal transduction.

The T Cell Receptor

The Role of the Receptor in T Cell Ontogeny

T 005 CLASS I-RESTRICTED CYTOTOXIC T CELL RESPONSES TO A SOLUBLE PROTEIN ANTIGEN,
Michael J. Bevan, Francis R. Carbone, James M. Sheil and Dale R. Wegmann, Research Institute of Scripps Clinic and Lilly Research Laboratories, La Jolla CA 92037.
Recent evidence from a number of laboratories has demonstrated that cytotoxic T lymphocytes (CTL) recognize peptide fragments of antigen in association with Class I MHC molecules. This finding has led to the postulate that both helper T cells (T_H) and CTL respond to a degraded form of antigen expressed on the cell surface following antigen processing. We reasoned that, if such is the case, it should be possible to generate CTL which are specific for peptides derived from soluble antigens in the context of Class I MHC molecules. We primed H-2^b mice with an unfractionated tryptic digest of chicken ovalbumin (cOVA), and following in vitro stimulation with the same antigen, obtained cOVA digest-specific CTL activity. At least two discrete HPLC-purified fractions of the cOVA digest are able to sensitize EL4 (H-2^b) target cells for lysis by the CTL. One peak of activity has been positively identified by amino acid analysis and synthesis as the 323-339 peptide which is also recognized by Class II-restricted T_H in H-2^b mice. The majority of effector CTL are Lyt-2^+, L3T4^- and are restricted to the H-$2D^b$ molecule. We are currently attempting to identify the other active peptides, to clone the CTL, and to determine what this has to say about Class I versus Class II antigen presentation.

T 006 T CELL RECEPTOR GENE REARRANGEMENT AND EXPRESSION DURING THYMUS DEVELOPMENT <u>IN VIVO</u> AND <u>IN VITRO</u>.
E.J. Jenkinson,+ J.J.T. Owen,+ R. Kingston,+ G.T. Williams+ and M.J. Owen° +Department of Anatomy, Medical School, University of Birmingham, U.K. and °Department of Zoology, University College, London, U.K.
From a predominantly germ line configuration at d14 of gestation in the mouse, T-cell receptor β chain genes undergo rearrangement and expression during thymus ontogeny. We have investigated these processes during <u>in vivo</u> development and in fetal thymus organ cultures. In contrast to Thy-1^+ cells developing outside the thymus we have found that T-cell precursors developing in thymus organ cultures undergo receptor gene rearrangement and expression comparable to that <u>in vivo</u> suggesting an important role for the intra-thymic environment in triggering these processes. These thymus organ cultures can be manipulated by culture in the presence of deoxyguanosine which eliminates the indigenous lymphoid cells leaving an epithelial rudiment. We have shown that such alymphoid lobes can be recolonised by a single micromanipulated precursor selected from another 13-14d thymus lobe, allowing the clonal progeny of a single T-cell precursor to be investigated. Using this system we have shown that a single precursor can generate multiple β chain gene rearrangements providing definitive evidence that these events are intra-thymic. In normal development we have found that receptor expression detected with monoclonal antibody F-23.1, is first seen at d.15 of gestation in a few cells which show cytoplasmic β chain labelling but lack surface β chains ($C\beta^+$ cells). These are followed in development by cells with both surface and cytoplasmic β chain labelling and, just before birth, by cells with surface β chain but no detectable cytoplasmic expression ($S\beta^+$ cells). Both $C\beta^+$ and $S\beta^+$ cells are generated in thymus organ culture with $S\beta^+$ cells accumulating with time whilst $C\beta^+$ cells eventually decline suggesting a precursor product relationship between these two populations. In contrast to $S\beta^+$ cells, $C\beta^+$ cells enter mitosis more frequently. Thus cells already committed to a particular β chain rearrangement may, after proliferation, undergo further diversification by associating with various α chain rearrangements occuring at a later stage. Finally, we have observed that additions of antibody F-23.1 to organ cultures results in the disappearance of F23.1 positive $S\beta^+$ cells. On antibody removal $S\beta^+$ cells reappear although these may be newly generated from $C\beta^+$ cells that are unaffected by antibody treatment rather than representing recovery of modulated $S\beta^+$ cells. Thus addition of anti-receptor antibody may mimic receptor/antigen interactions involved in intra-thymic selection of the repertoire, providing a model to investigate this process.

T 007 THE T CELL REPERTOIRE, Philippa Marrack, Neal Roehm, Terri Wade, Marcia McDuffie, Marcia Blackman, Willi Born, Ella Kushnir, Janice White, Jerry Bill and John Kappler Natl, Jewish Ctr. for Immunol. and Resp. Med., Denver, CO 80206.
Both T and B cells bear antigen binding receptors, but these molecules are constructed from different gene families. Thus it is likely that the germ line repertoires of T and B cell receptors have been selected to bind MHC Class I and Class II molecules. Recent findings on a new mouse V protein, V 17, which is expressed in C57BR, C572, SJ2 and SWR mice support this idea. T cells with receptors which include V 17 react with IE products with unexpectedly high frequency. This reactivity occurs regardless of the other variable components of the receptors suggesting that V 17 itself has a high affinity for IE molecules. VB17 is found on the immature thymocytes of all mice expressing the gene. It's expression is severly reduced, however, on mature thymocytes and peripheral T cells of development at which tolerance to self MHC is induced.

T 008 THE THYMUS AND T CELL TOLERANCE, J. Sprent, IMM4A, Research Institute of Scripps Clinic, 10666 N. Torrey Pines, Rd., La Jolla CA 92037.

Mature post-thymic T cells generally recognize exogenous antigen X in association with self H-2 determinants while maintaining unresponsiveness to self H-2 determinants per se. It seems highly likely that these two properties of T cells - H-2 restriction and self H-2-tolerance - are both imposed in the thymus during early T cell differentiation. Precisely how T cells are imbued with these two properties is poorly understood, nor is it clear which thymic stromal cells are involved.

In the case of H-2-restricted specificity, some workers argue that restriction is imposed by a class of intrathymic bone-marrow-derived cells with features of macrophages or dendritic cells (MØ/DC). However, studies from this laboratory suggest that restriction is imprinted not by MØ/DC but by thymic epithelial cells. Our view is that the prime function of MØ/DC in the thymus is to impose self tolerance. Experimental evidence on this issue will be discussed.

Expression of Cloned Receptor Genes

T 009 EXPRESSION AND ORGANIZATION OF THE T CELL RECEPTOR γ, Nobuki Nakanishi, Keiji Maeda, Koichi Ito, Mark Heller, Lars Hellman, Joseph Heilig, Yohtaroh Takagaki and Susumu Tonegawa, Center for Cancer Res.,M.I.T., Cambridge, MA 02139. During the search for the cDNA clones coding for the α and β subunits of the antigen-specific T cell receptor (TCR) we encountered a third gene, γ, that shares a number of structural properties with α and β genes, including the specific V-J rearrangement in T cell development (Saito et al., Nature 309:757-762 1984). In order to identify the poulation of cells that express the γ chain we prepared antibodies using as the immunogen γ polypeptide chains synthesized in E. coli by genetic engineering. The γ chain was found to be expressed on the surface of a subpopulation of murine fetal thymocytes as well as the $Lyt2^-$ $L3T4^-$ population of adult thymocytes. In both cases the γ chain is disulfide-linked to another subunit of about 45 kd (δ chain). By contrast the CTL clone, 2C, from which the original in-frame γ cDNA was cloned does not bear a detectable level of such a heterodimer. We are in the process of investigating several other cell populations for the expression of γ chains and γ genes. On the basis of these studies we will discuss the possible function of the γ-bearing cells.

T 010 TRANSGENIC MICE PRODUCED WITH CLONED REARRANGED T-CELL RECEPTOR GENES, Michael Steinmetz, Yasushi Uematsu, Stefan Ryser, Judy Ways, Zlatko Dembic, Anton Berns*, Horst Bluthmann** and Harald von Boehmer; Basel Institute for Immunology, Basel, Switzerland; *The Netherland Cancer Institute, Amsterdam, the Netherlands; **Central Research Unit, Hoffmann-La Roche, Basel, Switzerland. We have isolated the productively rearranged T cell receptor -and -chain genes from a cytotoxic T cell clone specific for the male antigen H-Y and $H\text{-}2D^b$. The cariable region of the B chain is encoded in the part by the $V_\beta 8.2$ gene segment and is therefore recognized by the isotypic antibody F23. Five transgenic mice were obtained by injecting the chain gene including 1 kb of 5′ and 3 kb of 3′ flanking sequence into the male pronucleus of fertilized eggs obtained from matings of (C57BL/6 x $SJL)F_1$ mice. The transgenic mice contained between 1/4 to 20 copies of the rearranged gene as determined by tail DNA Southern blot hybridization. Four of the five transgenic mice and their offspring were analyzed for expression of the transgene in Con-Astimulated, peripheral T cells using the F23 antibody. No evidence for expression of the transgene was found in mice that were either homozygous or heterozygous with respect to the presence or absence of endogenous V_8 genes. We are currently testing various alternative constructs of the cloned -chain gene for expression in transfected cells and transgenic mice.

The T Cell Receptor

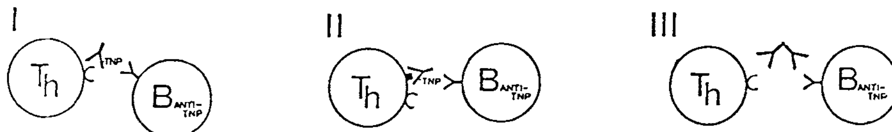
T Cell Effector Functions

T 011 ANALYSIS OF THE ACTIVATION AND FUNCTION OF A CLONED HELPER T CELL LINE USING MONOCLONAL ANTI-T CELL RECEPTOR ANTIBODIES, Charles A. Janeway, Jr., Jose Rojo, Kaj Saizawa, Wen-jen Poo, and Sakhina Haque, Section of Immunobiology, Yale University School of Medicine and Howard Hughes Medical Institute, New Haven CT 06510.
Monoclonal anti-T cell receptor antibodies have allowed the analysis of the structure and functions of T cell receptors. We have used a collection of such antibodies, specific for the receptor of a single cloned T cell line, to perform several different studies relating to the structure and functional role of the T cell receptor. The antibodies have been used to define at least three structurally distinct epitopes on the receptor molecule, and to relate the epitope recognized to the functional activity of the particular antibody. There are wide disparities between the amount of an anti-receptor antibody bound and its ability to activate the cloned T cell line. The degree of activation obtained was related to the epitope recognized by the monoclonal antibody, and was independent of affinity for antibodies directed at the same epitope. This suggested that simple cross-linking of the T cell receptor was necessary but not sufficient for T cell activation. To confirm this, a poorly activating antibody was used in combination with the Fab fragment of a potent activating antibody, to demonstrate that the T cell receptor requires both cross-linking and conformational change when activation is driven by anti-T cell receptor antibodies. We also noted that antibodies to the T4 molecule differentially affected responses to anti-T cell receptor antibodies directed at distinct epitopes. This suggested that T4 is part of the receptor complex, and this was supported by two other findings. First, antibodies that are most potent in activating this cloned T cell line cause T4 molecules to comodulate with the T cell receptor. Second, anti-T4 antibodies, combined with the Fab fragment of a potent activating anti-T cell receptor antibody, will cause activation, suggesting that anti-T4 can, under these conditions, provide the necessary cross-linking of the receptor. Natural activation of $T4^+$ cells may involve the cross-linking of T4 to the T cell receptor by means of binding to class II MHC molecules. Finally, the role of the T cell receptor in mediating helper function has been examined in several systems. Anti-T cell receptor antibody can bypass the need for antigen and MHC recognition in T dependent B cell activation. However, receptor-mediated intimate contact of the helper T cell and the B cell may promote B cell activation by several means. First, it causes stable association of T cells and B cells; second, it plays a role in cytoskeletal reorientation; and third, it directs the secretion of B cell activating lymphokines by the cloned T cell line, especially under conditions of limiting stimulation. Supported by NIH grants CA-29606, AI-14579 and the Howard Hughes Medical Institute.

T 012 AN ANALYSIS OF MOLECULAR AND CELLULAR INTERACTIONS LIMITING B CELL ACTIVATION, Michael H. Julius, Hans-Georg Rammensee and Georges Köhler. Dept. of Microbiology and Immunology, McGill University, Montreal, Quebec H3A 2B4, Basel Institute of Immunology, Basel CH-4005, Switzerland, Max Planck Institute for Immunobiology, Freiburg, D-7800, FRG.

A cognate lymphocyte interaction limits T helper cell (Th) dependent induction of resting B cells to high rate Ig secretion. The question follows whether the surface molecules on Th and B mediating the interaction are integral, having been specifically designed for the transduction of signals resulting in B cell activation. Alternatively, Th/B membrane juxtaposition mediated by any molecular interaction might be sufficient. To determine whether the "cognate" aspect of Th-B interaction is critical, we assessed the consequences on B cell physiology of Th-B conjugate formation with the inclusion/exclusion of membrane molecules known to be involved during a physiological interaction.

The sucessful engineering of the $V_L\kappa$ and $V_H\mu$ encoding TNP specific Ig into the genome of mice provided an unlimited source of antigen specific, idiotypically characterized resting B cells. In conjunction with clones of idiotypically characterized Th, we have organized Th-B conjugates of three general categories pictorally represented below:



Category I is designed to assess the role of B cell MHC class II molecules. The Th used are functionally restricted by IA^d and the TNP specific B cells are from transgenic mice backcrossed onto the A/J background (IA^k). Any specific interaction with B cell surface molecules, other than mIg, is thus excluded in these conjugates. The anti-Th receptor (TCR) antibody is conjugated with TNP, providing the focus for cell interaction. Category II was organized to exclude the involvement of TCR. Category III provided the most versatile approach in generating conjugates of varying architecture.

T 013 CELL BIOLOGY OF THE SPECIFIC INTERACTION OF CLONED HELPER T CELLS AND ANTIGEN PRESENTING B CELLS, A. Kupfer, S.L. Swain, C.A. Janeway, Jr. and S.J. Singer, Department of Biology, University of California, San Diego, La Jolla, CA 92093 and Department of Pathology, Yale University School of Medicine, New Haven, CT 06510. Using immunofluorescence microscopy, we present direct evidence for the formation of specific cell couples of cloned antigen (Ag)- specific helper T (Th) cells and Ag-presenting B cells. These cell couples were formed within minutes after the mixing of equal numbers of the two cell types. The binding of either one of two cloned helper T cells (D10,D8) or of a T-T hybridoma (2H10) to either the LK B hybridoma or the CH12 B lymphoma, both of which were pulsed overnight with the appropriate specific Ag, resulted in the rapid reorientation of the microtubule organizing center (MTOC) inside the effector cell (but not the B cell) to face the cell:cell contact region. The processing of the specific Ag by the B cells, an event which is inhibitable by chloroquine, was essential for triggering the Th cells which either did not present the specific Ag or expressed the wrong Ia determinants, but the Th MTOC remained randomly oriented in these cell couples. In specific T:B cell couples we observed also that the cytoskeletal protein talin became highly concentrated in the effector cells under the plasma membrane along the cell:cell contact region. This redistribution of talin was also Ag-specific and Ia restricted. The chelation of the free extracellular Ca^{+2} by EGTA prevented the Th-MTOC reorientation but did not significantly interfere with redistribution of talin. These intracellular rearrangements were observed previously also in cell couples of cytotoxic T lymphocytes bound to their target cells. The Ag- and Ia-dependent intracellular redistributions of the MTOC and talin inside the Th cell represent the first demonstration of a specific direct interaction of the Th and Ag-presenting B cells. We propose that the polarization of the MTOC, which is accompained by a coordinate reorientation of the Golgi apparatus (GA), serves the function of directing the secretion of GA-derived B-cell growth and differentiation factors from the Th to the bound B cell.

T 014 ANTIBODIES AS ANTIGENS: DELINEATION OF THE ANTIGEN PROCESSING PATHWAY AND TARGETING OF CTL TO TUMOR B CELLS, Antonio Lanzavecchia, Basel Institute for Immunology, 4005 Basel, Switzerland.
When injected into humans, mouse monoclonal antibodies (Mab) are recognized as foreign antigens and elicit a strong T cell response. Because Mabs can bind selectively to cell surface structures, they can be used to send the same antigenic determinant (mouse Ig) to any preselected cell surface molecule. Using T cell clones specific for mouse Ig, we have begun to analyze which cell surface structures can serve as targets for the internalization, processing and presentation of the mouse Ig; in other words, which structures are involved in the normal Class II-linked internalization pathway.
We have isolated, from patients injected with mouse Mab, T cell clones that recognize processed mouse Ig in association with Class II molecules. These T cell clones have been used in combination with different sources of HLA-matched antigen presenting cells to: i) Identify which cell surface molecules are in the pathway of internalization, processing and Class II restricted presentation of soluble antigens, and in particular we have looked at Surface Ig, Class II, Transferrin Receptor and T cell receptor; ii) Assess the capacity of different cell populations, such as B cells, macrophages and activated T cells to process and present antigen; iii) Evaluate the capacity of a cell to present its own endogenous Ig, using somatic cell hybrids between a human B cell tumor and mouse spleen cells; iv) Demonstrate that patients injected with mouse Mab have Class II-restricted CTL that can specifically recognize and kill autologous fresh B cell tumor targets pulsed with anti-Idiotypic antibody.

Accessory Molecules

T 015 THE COMPLETE PRIMARY STRUCTURE OF THE SHEEP ERYTHROCYTE BINDING PROTEIN: MOLECULAR CLONING AND EXPRESSION OF T11 cDNAs REVEALS A NOVEL RECEPTOR LIKE STRUCTURE ON HUMAN T LYMPHOCYTES. P.H. Sayre, H.C. Chang, R.E. Hussey, N.R. Brown, N.E. Richardson, G. Spagnoli, L.K. Clayton and E.L. Reinherz, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA 02115.

The T11 (CD2) sheep erythrocyte (SRBC) binding protein is a T cell surface molecule involved in activation of T lymphocytes and thymocytes including those lacking the T3-Ti antigen/MHC receptor complex. The primary structure of T11 was deduced from protein micro-sequencing and cDNA cloning. The mature human protein appears to be divided into three domains: a hydrophilic 185 amino acid external domain bearing only limited homology to T4 and T molecules; a 25 amino acid hydrophobic transmembrane segment; and a novel 126 amino acid cytoplasmic domain rich in prolines and basic residues. Transfection of cDNAs encoding either the 1.7 or 1.3Kb T11 mRNAs into COS cells results in expression of surface T11 epitopes as well as SRBC binding capacity. The predicted structure is consistent with the possibility that T11 represents a primitive activation system, perhaps having evolved prior to development of other T lineage surface structures with specialized binding (Ti α, Ti β, T4 and T8) or signal transduction functions (T3 γ, δ and ε). The role of T11 in facilitating T3-Ti triggered responses will also be discussed with reference to analysis of T11 mutant cell lines.

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T 016 HUMAN CYTOTOXIC T CELLS ADHERE TO POTENTIAL TARGETS BY TWO ANTIGEN-INDEPENDENT PATHWAYS: CD2 BINDING TO LFA-3 OR LFA-1 BINDING TO AN UNDEFINED LIGAND.
Stephen Shaw, Gale G. Luce, Timothy A. Springer*, Marian L. Plunkett*, Ralph Quinones, Ronald E. Gress, Martin E. Sanders. Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892 and *Department of Membrane Immunochemistry, Dana Farber Cancer Institute, Boston, MA 02115
T-cell-mediated cytotoxicity has been separated into 3 sequential steps: 1) formation of a stable adhesion between effector and target (conjugate formation); 2) triggering of effector to damage target (lethal hit); 3) target cell lysis and effector recycling. Studies from murine model systems have indicated that conjugates are formed principally with targets which express the relevant antigen. In contrast, our studies demonstrate marked antigen-independent conjugate formation by human T cells (1). The model system in which we have studied conjugate formation most extensively is the interaction of human cytotoxic T lymphocyte (CTL) clones with in vitro cell lines. However, findings have been confirmed in studies using peripheral blood T cells as effectors or using subsets of peripheral blood cells as targets. Conjugate formation with antigen-positive or antigen-negative targets reaches maximal levels within 3-6min (after centrifugation and incubation at 37C); such conjugates are strong since they resist the shear of vigorous vortexing. Monoclonal antibody (MAB) inhibition studies with individual MAB and mixtures of MAB demonstrate that LFA-1 (CDw18) is involved in one pathway of adhesion, which is functional at 37C but not 4C and requires Mg^{++}. Effector CD2 (T11, LFA-2, E-rosette receptor) binding to target LFA-3 is a distinct adhesion pathway which functions at 4C as well as at 37C and is independent of divalent cations. Both pathways are susceptible to inhibition by other agents known to inhibit CML such as: TLCK, cytochalasin E and cytochalasin B. Although both pathways function in conjugate formation with a variety of in vitro cell lines, they can apparently function independently. The CD2/LFA-3 pathway mediates rosetting of T cells with human erythrocytes. The LFA-1 pathway is the primary mode of interaction of T cells with macrophages. Our working hypotheses are: 1) that initial conjugate formation may be principally mediated by antigen-independent mechanisms; and 2) that antigen-independent adhesion may provide optimal alignment of membranes for a time long enough to allow the T cell's antigen receptors to encounter specific antigen on the target, even when the antigen is present at low concentration.

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The T Cell Receptor

T 017 THE MOLECULAR BASIS OF ANTIGEN-INDEPENDENT LYMPHOCYTE ADHESION, Tim Springer, Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA 02115.

An overview of the molecular and functional characterization of the lymphocyte function-associated molecules LFA-1, CD2, (LFA-2, T11), and LFA-3 will be presented. Studies with purified molecules show that LFA-3 is the ligand of the CD2 T lymphocyte glycoprotein. The purified molecules mediate adhesion, and LFA-3 incorporated into planar membranes mediates CD2-dependent T lymphocyte adhesion. This receptor-ligand interaction is important in T lymphocyte adherence to target and antigen-presenting cells and also mediates rosetting of activated T lymphocytes with human erythrocytes and of thymocytes with thymic epithelial cells. The LFA-1 molecule appears to interact with the intercellular adhesion molecule 1 (ICAM-1) ligand. The N-terminal sequence of the LFA-1 alpha subunit, and the complete sequence of its cloned beta subunit, show 30 and 45% identity, respectively, with extracellular matrix (e.g. fibronectin) receptors. The matrix receptors recognize the core sequence RGD within their ligands. Our studies define a novel family of leukocyte adhesion and RGD receptors. The structural and functional implications will be discussed.

The Receptor Ligand

T 018 THE INTERACTION BETWEEN Ia AND PEPTIDES IMMUNOGENIC FOR T CELLS, Howard M. Grey, Allessandro Sette and Soren Buus, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO.

Using gel filtration to study the formation of complexes between an immunogenic Ova peptide323-339 and $I\text{-}A^d$ we have demonstrated that the complexes once formed are extremely stable (kd = $3 \times 10^{-6} s^{-1}$) but the rate of complex formation is very slow (ka ≈ $1\ M^{-1}s^{-1}$) explaining the overall low equilibrium constant of ~ 2×10^{-6} M. The relevancy of such complexes to T cell stimulation was documented in a planar membrane system in which it was shown that 2×10^4 less antigen was required in the form of a peptide-Ia complex than when uncomplexed peptide was used. By studying a panel of 12 immunogenic peptides and their capacity to interact with Ia, it was found that in every instance, a specific saturable binding to the relevant restriction element occurred. Most of the peptides bound relatively weakly or not at all to Ia molecules not used as a restriction element, with one exception in which such binding was stronger. The data further suggest that a single binding site for immunogenic peptides is present on each Ia molecule, and glutaraldehyde crosslinking of the peptides to Ia suggest that this binding site is composed of both the α and β chain of Ia.

T 019 HIGH AFFINITY FLUORESCENT PEPTIDE BINDING TO I-A^d IN LIPID MEMBRANES,
Harden M. McConnell and Tania H. Watts, Physical Chemistry Laboratory, Stanford University, Stanford CA 94305.
Phospholipid vesicles containing I-A^d were pulsed with the fluorescinated peptide OVA(323-339) and then extensively dialyzed. Supported planar membranes prepared from these vesicles retain a fluorescence corresponding to about 1 peptide per 100 I-A molecules. These planar membranes as well as pulsed and washed cell membranes retain the ability to trigger 3DO-54.8 helper T-cells. About 1000 I-A-associated peptides are sufficient to trigger the response of a single 3DO-54.8 cell. Peptide competition experiments indicate that the on- and off-rate constants for peptide-I-A^d binding are low, and raises the possibility of kinetic control for selective antigen presentation.

T 020 RECOGNITION AND EARLY MOLECULAR EVENTS IN THE INDUCTION OF A NONRESPONSIVE STATE IN IL-2 PRODUCING T CELL CLONES.
Ronald H. Schwartz, Marc K. Jenkins, Helen Quill, Drew M. Pardoll, Junichiro Mizuguchi, and Thomas Chused., Laboratory of Immunology, NIAID, NIH, Bethesda, MD 20892
Antigen stimulation of normal T cell clones with antigen-presenting cells (APC) chemically modified with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (ECDI) or with purified Ia molecules in planar lipid membranes resulted in unresponsiveness to subsequent stimulation with untreated APC and antigen as measured by both thymidine incorporation and interleukin-2 production. The unresponsive state lasted for more than one week, although the cells remained viable as manifested by their ability to respond to exogenous interleukin-2. Induction of unresponsiveness required new protein synthesis and was accompanied by the production of IL-3, increases in TCRβ mRNA, and partial increases in IL-2 receptor expression; however, no IL-2 was produced. The addition of IL-2 did not prevent the induction of nonresponsiveness. The critical biochemical event for the induction of unresponsiveness appeared to be a rise in intracellular calcium, as measured with indo-1. Entry into the unresponsive state was blocked by EGTA and the state could be chemically induced by the addition of the calcium ionophore, ionomycin. These observations suggest that occupancy of the antigen-specific receptor on IL-2 producing T cell clones, in the absence of any second signals, leads to an increase in intracellular calcium, activating a biochemical program that eventually prevents the cell from producing IL-2 in response to normal activation signals.

T 021 ANTIGEN PROCESSING, Emil R. Unanue and Paul M. Allen, Department of Pathology, Washington University School of Medicine, St. Louis MO 63110

We have examined the requirements for antigen processing of the protein hen-egg lysozyme (HEL). HEL in its native form is not immunogenic but requires the uptake by antigen-presenting cells and its processing in their intracellular acid compartment. HEL is recognized by some T cell clones as an undenatured protein and by others as a small proteolytic fragment. The immunodominant epitope recognized by $H\text{-}2^k$ mice resides in peptide fragment 46-61. Peptide fragment 46-61 associates with purified $I\text{-}A^k$ molecules in a saturable process with an affinity consistent in the µM range. Such a binding is competed by autologous lysozyme peptide as well as by other structurally different HEL peptides such as HEL(34-45). The competition is also reflected at the level of physiological antigen presentation. The major contact site of the $I\text{-}A^k$ is the alpha chain. Using various derivatives of 46-61 we have been able to map the amino acid residues that contact the $I\text{-}A^k$ and those that contact the T cell receptor. Peptide 34-46 also binds to $I\text{-}A^k$ in a saturable process. The binding of both peptides is haplotype specific. We postulate that denatured proteins or fragments from proteolytic digestion have sites that allow for physical interaction with $I\text{-}A^k$. The physical interaction creates the antigenic determinant recognized by the T cells. We view the class II MHC proteins as a transport system with broad discrimination for proteins and peptides.

The T Cell Receptor

The Gamma Chain

T 100 γ Chain Associated Functional T-Cell Receptors on Cerebrospinal Fluid Derived NK-like T cells, Siew-Lan Ang, J.G. Seidman, Gary Peterman, Allan D. Duby, Deborah Benjamin, Soon Jin Lee and David A. Hafler, Department of Genetics, Harvard Medical School, Boston, MA 02115.
We have derived 33 independent T cell clones from the cerebrospinal fluid (CSF) of a patient with subacute sclerosing panencephalitis (SSPE) using a very efficient single cell cloning method. Six percent (2/33) of these clones express the T-cell receptor gamma (TCR-γ) protein. Phenotypic analyses of these clones indicated that they were WT31-,CD3+, CD4- and CD8-. The TCR-γ protein exists on the cell surface as 80 kilodalton disulphide-linked dimers associated with the CD3 polypeptides. The TCR-γ protein bearing T cells have NK-like activity and can cause a calcium flux upon trigerring by anti-CD3 monoclonal antibody. The identification of significant numbers of functional T cells expressing the TCR-γ protein in the CSF during an inflammatory response suggests that these cells are mature and play a role in the immune response.

T 101 ONTOGENY, PHENOTYPE AND FUNCTIONAL CHARACTERIZATION OF MURINE THYMOCYTES POSSESSING T3-ASSOCIATED RECEPTOR STRUCTURES. Jeffrey A. Bluestone, R. Cron, B. J. Fowlkes, S. O. Sharrow, D. Pardoll, D. H. Sachs, and L. E. Samelson. NIH, Bethesda, MD 20892

A monoclonal antibody specific for murine T3 complex has been derived by immunizing Armenian hamsters with a murine CTL clone. The antibody is specific for a 25kd component of the antigen specific T cell receptor (TcR) (designated T3-ε). It reacts with all mature T cells and can both activate and inhibit T cell function. The T cell ontogeny of T3 expression in the developing thymus has been examined. In fetal development, $T3^+$,$Lyt2^-$,$L3T4^-$ cells were observed as early as day 15. These cells can be functionally activated with anti-T3 antibody plus IL2, to proliferate and develop cytolytic activity. The $T3^+$,$Lyt2^-$,$L3T4^-$ cell population is also present in the adult thymus in small percentage (0.5% of the whole thymocyte population). The $T3^+$ cells in the fetus do not express TcR α-specific RNA and express low or undetectable levels of TcR β-specific RNA. However, all cells express T cell receptor γ specific RNA. Immunoprecipitation studies using the anti-T3 monoclonal antibody have shown that these early fetal thymocytes express a novel heterodimer of 45kd and 35kd molecular weight. Preliminary studies suggest that the $T3^+$,$Lyt2^-$,$L3T4^-$ cells also exist in the periphery of normal and athymic nude mice. These cells maintain a somewhat different cell surface phenotype in that a significant percentage of the cells express Ly6.2C, a marker present on extrathymically derived peripheral $Lyt2^+$ cells (Leo et al., submitted). Thus, it would appear that at least a portion of these cells were derived from a different lineage.

T 102 CLONED γ-CD3 T CELLS FROM HUMAN PERIPHERAL BLOOD EXERT MHC NONRESTRICTED CYTOLYSIS R.L.H. Bolhuis, J. Borst, J.W. van Oostveen, J.G. Seidman and R.J. van de Griend Rotterdam Radiotherapeutic Institute, Dept. of Immunology, Rotterdam, the Netherlands.
Human T cells express T cell receptor glycoproteins comprised of polymorphic disulphide linked heterodimers of αβ chains which are coexpressed with the CD3 antigen. Hence, $CD3^+$ $\alpha\beta^+$ T cells are clearly distinct from natural killer (NK) cells which lack productive TCR-gene rearrangements,and hence TCR. Functionally the differences are reflected by the antigen specific MHC-restricted regulatory and cytolytic functions by $CD3\alpha\beta^+$ T cells in contrast to the MHC nonrestricted cytolytic activity by NK cells. Along with the TCRαβ genes, the putative TCR γ gene was identified with rearranges specificly in T cells being either cytotoxic or helper cells. This γ gene is organized in the same fashion as the TCR genes although its diversity may be more limited. We have identified the TCR γ gene protein product to be present on $CD3^+$ 4^- 8^- $\alpha\beta^-$ lymphocytes, a subpopulation of T cells found in the peripheral blood and the thymus. The γ chain forms part of a disulphide-linked dimer which can also comprise a protein not reactive with anti-γ antiserum. The subpopulation of $CD3^+$, 4^-, 8^-, γ^+, T cells exert MHC nonrestricted cytolysis express IgG-Fc receptors, consequently exert antibody dependent cellular cytolysis. Our analysis of the lytic mechanism of these cells suggest that the TCR γ chain is involved in MHC nonrestricted cytolysis by T cells which use the CD3 antigen for signal transduction. The lytic activity of these cells can be modulated by use of monoclonal antibodies directed against either the CD2; the CD3 and/or CD16 cell surface molecules. Moreover their lytic activity is also enhanced by IL2 and Interferon β which already are known to enhance lytic activity of (cloned) NK cells.

T 103 TRG γ GENE REARRANGEMENTS IN *IN VIVO* ALLOSENSITIZED HUMAN T CELL CLONES.
M. Bonneville, J.P. Moisan, J.F. Moreau, J.P. Soulillou, M.-P. Lefranc*
Laboratoire d'Immunologie Clinique, I.N.S.E.R.M. U211, Faculté de Médecine,
1 rue G. Veil, 44035 Nantes Cedex, France
*Laboratoire d'Immunogénétique, UA CNRS 1191, USTL, Place E. Bataillon,
34060 Montpellier Cedex, France.

Twenty four human alloreactive T cell clones (ATLC) derived from cells invading one irreversibly rejected kidney were analyzed for the rearrangement of the T-cell rearranging γ genes (TRG γ). These ATLC were specifically commited against kidney donor derived B lymphoblastoïd cell lines, and could be subdivided in three phenotypic/functional subpopulations (i.e. $CD4^+$ killers, CD4 non killers and $CD8^+$ killers). All these ATLC recognized either HLA class I or class II specificities. ATLC DNA_s were digested with BamH I, Hind III or Eco RI restriction enzymes and hybridized with the pH60 probe containing the J γ 1 segment (1, 2). All the clones exhibited at least one TRG γ rearrangement. Several patterns of rearrangement were found indicating that different Vγ genes were used. Correlations between ATLC function or recognition pattern and the presence of given rearranged bands will be discussed.

1- LEFRANC, M.-P. and RABBITTS, T.H. (1985) Nature (London) 316, 464-466.
2- LEFRANC, M.-P., FORSTER, A. and RABBITTS, T.H. (1986) Nature (London) 319, 420-422.

T 104 MOLECULAR ORGANIZATION OF T CELL RECEPTOR GAMMA/T3 COMPLEX ON CYTOLYTIC PERIPHERAL BLOOD LYMPHOCYTES AND ON LEUKEMIC CELLS.
J. Borst[1], R.v.d.Griend[2], J.v.Dongen[3], H.v.Oostveen[1], S.-L.Ang[4], J.Seidman[4], C.Melief[1], R.Bolhuis[2].
[1]The Netherlands Cancer Institute, Amsterdam; [2]TNO, Rijswijk; [3]Erasmus University, Rotterdam, The Netherlands; [4]Harvard Medical School, Boston, MA, USA.

Until very recently, the protein product of the third T cell specific rearranging gene, γ, was unidentified. We have found that a small population of 1-3% of PBL from normal donors express the T3 molecule, but lack the common determinant of the α,β T cell receptor, as identified by a monoclonal antibody WT31. Cloned cells from this population acquire T3 mediated cytotoxicity upon activation in vitro, which is MHC non-restricted. These clones lack α mRNA, express immature β mRNA, but do express γ mRNA. The γ gene shows a monoallelic rearrangement to Cγ1 in all clones tested. A 36-40 kDa γ protein occurs on these clones, in association with T3, and is involved in the formation of a disulfide-linked dimer. In most clones, a second, non-γ chain was detected, which participates in the dimer formation. PBL from a patient with acute leukemia expressed no α mRNA, 1.0 and 1.3 kB β mRNA, and γ mRNA. The γ gene was rearranged to Cγ1 as well as Cγ2. On these cells a 40-44 kDa γ protein is expressed is association with T3. No evidence for either covalent or non-covalent association with additional polypeptides was found. The possible implications of this difference in molecular organization of the γ protein for its putative role in cytolytic T cell function and/or T cell differentiation will be discussed.

T 105 IDENTIFICATION AND BIOCHEMICAL CHARACTERIZATION OF γδ T CELL RECEPTORS ON THY-1^+ DENDRITIC EPIDERMAL CELL LINES. J. Coligan, F. Koning, W. Yokoyama, A. Lew, W. Maloy, G. Stingl, and E.Shevach, Labs Immunogen. and Immunol. NIAID, Bethesda, MD 20892

Recently, Thy-1^+ dendritic epidermal cell (DEC) lines have been established whose surface phenotype (Thy-1^+, asialo GM1 , Ly-1 , Lyt-2 , L3T4 , Ia , sIg) distinguishes them from Ia dendritic cells, B cells and mature T cells. Expression of T cell receptors (TCR) by these cell lines has been examined by immunoprecipitating ^{125}I-surface-labeled and metabolically-labeled cell lysates with anti-peptide sera against the CD3 δ (anti-CD3δ) and TCRγ (anti-TCRγ) chains. Three different patterns of TCR expression were observed. (1) Two cell lines (245NCM, T195) expressed CD3 in association with TCR αβ like heterodimers. (2) One cell line (Y245) expressed a CD3-associated TCR αβ-like heterodimer but, in addition, CD3 was associated with a non-α/non-β chain that could be precipitated with anti-TCRγ. Off-diagonal gels indicated that the γ chain had a disulfide linked partner but no δ chains could be detected. (3) Two cell lines (T245, T93) expressed a non-α/non-β CD3-associated TCR composed of a disulfide linked 35kD-45kD heterodimer. Removal of N-linked sugars reduced the molecular weights to 32kD-35kD, respectively. This receptor from the T245 cell line, but not the T93 cell line, could be precipitated with anti-TCRγ. Thus, the T245 and T93 cell lines express γδ chain TCR similar to those expressed by a subpopulation of murine thymocytes (dLy1, Lyt-2 , L3T4). Moreover, the fact that anti-TCRγ reacts with Cγ1, Cγ2 and Cγ3 but not Cγ4 suggests that the T93 cell line expresses Cγ1 whereas the T245 cell line expresses Cγ4 chains. These cell lines are being used to study the biosynthesis and molecular properties of the γδ TCR.

T 106 T LYMPHOCYTES THAT LACK BOTH CD4 AND CD8 ANTIGEN EXPRESSION: REARRANGEMENT AND TRANSCRIPTION OF T CELL ANTIGEN RECEPTOR GENES. N.A. Federspiel, J.J. Ruitenberg, J.H. Phillips, A. Weiss, and L.L. Lanier. Becton Dickinson Monoclonal Center, Inc., Mountain View, CA 94043 and Department of Medicine and the Howard Hughes Medical Inst., Univ. California Medical School, San Francisco, CA 94143

A subpopulation of $CD3^+$ T lymphocytes lacking CD4 and CD8 was purified from peripheral blood using a FACS. After culturing in IL-2 (in which the $CD3^+4^-8^-$ phenotype was maintained), these cells were shown to contain rearranged T cell receptor β and γ chain genes. Like normal peripheral blood T cells, the $C_{\beta 1}$ germline fragment is preferentially lost in the $CD3^+4^-8^-$ population, and the population is polyclonal. Similarly, polyclonal rearrangements of the γ chain genes were observed, and discrete rearranged fragments could be seen due to the more limited repertoire of V_γ genes. The $CD3^+4^-8^-$ cells exhibited all of the rearranged fragments which were found in the normal peripheral blood T cell population. No α or β T cell receptor transcripts could be detected in the $CD3^+4^-8^-$ populations. In contrast, full-length transcripts of the T cell receptor γ chain gene were abundant in these $CD3^+4^-8^-$ cells.

T 107 EXPRESSION OF THE T CELL RECEPTOR GAMMA CHAIN IN MATURE T CELLS, Barry Jones, Charles A. Janeway, Jr., and Adrian Hayday, Department of Biology, Yale University and Section of Immunobiology, Howard Hughes Medical Institute at Yale University School of Medicine, New Haven, CT, 06510.

The T cell receptor on most T cells consists of an alpha and a beta chain non-covalently associated with the molecules of the CD3 complex. The discovery of a third rearranging gene family highly homolgous to the alpha and beta genes, and selectively expressed in the T cell lineage, raises interesting questions about the function of the products of this gene. Furthermore, the finding that gamma mRNA is not expressed in mature cells expressing alpha and beta mRNA, and that cloned T cell lines expressing gamma mRNA generally have rearranged gamma genes in a non-functional fashion have led to the proposal that gamma is only expressed as a protein on immature T cells.

To examine this question, we have measured mRNA content by RNA blotting, and also sequenced cDNA isolated from mature peripheral T cells activated in various fashions in vitro. These freshly isolated, uncloned T cells do express abundant gamma mRNA, provided they are activated in mixed lymphocyte culture, and not by concanavalin A or anti-Thy-1 stimulation. The initial cDNA sequences have shown that at least some of this mRNA is functional, being joined in frame. We are currently determining the frequency of in-frame joins in gamma cDNA isolated from such cells, and the cellular distribution of these mRNAs amongst the responding cells. Supported by the Howard Hughes Medical Institute and NIH grants CA-29606 and AI-14579.

T 108 STRUCTURE OF T-GAMMA CONTAINING T CELL RECEPTORS. Michael Krangel, Peter Devlin, Barbara Bierer, Janice Riberdy, Joanne McLean and Michael Brenner. Dana-Farber Cancer Institute, 44 Binney Street, Boston MA 02115.

We have identified on PBLs from an immunodeficiency patient (IDP2) a 55kD T-gamma peptide (40 kD nonglycosylated) and a 40kD peptide (38kD nonglycosylated) termed T-delta, which are associated with T3. Gamma and delta are unrelated based upon tryptic peptide mapping comparisons. T3 is glycosylated differently than in T3-TCR $\alpha\beta$, but is otherwise functionally quite similar. T3 delta carries two complex oligosaccharides, as opposed to one high mannose and one complex oligosaccharide. Nevertheless, anti-T3 mAb induce an increase in cytoplasmic Ca++ and rapid modulation of T3 and T-gamma from the surface, and T3 gamma is phosphorylated in response to phorbol esters. We have also identified PBL clones from a normal adult which express a 40kD (34kD nonglycosylated) disulfide-linked T-gamma peptide. T-gamma transcripts from this clone are shorter than those on other cells examined so far. The structures of the T-gamma peptides in the disulfide-linked and nondisulfide linked forms are being compared by the analysis of T-gamma encoding cDNA clones.

T 109 T LYMPHOCYTES THAT LACK BOTH CD4 AND CD8 ANTIGEN EXPRESSION: STRUCTURE OF THE CD3/T CELL ANTIGEN RECEPTOR COMPLEX. L.L. Lanier, N.A. Federspiel, J.J. Ruitenberg, J.H. Phillips, and A. Weiss. Becton Dickinson Monoclonal Center, Inc., Mountain View, CA 94043 and Department of Medicine and the Howard Hughes Medical Inst., Univ. California Medical School, San Francisco, CA 94143

CD3+ T lymphocytes that express neither CD4 nor CD8 antigen are present in normal thymus (<0.2-1%) and peripheral blood (~3%). CD3+,4-,8- T cells failed to react with WT31, an antibody directed against a framework determinant of α/β T cell antigen receptor heterodimer. CD3+,4-,8- T cells were isolated to >95% purity using a FACS and cultured in medium containing IL-2. 85-99% of these cells maintained the phenotype, CD3+,4-,8- and >95% remained unreactive with WT31. CD3+,4-,8- T cells mediated non-MHC restricted cytotoxicity against a broad panel of tumor cell targets. The CD3 complex from these cell lines was immunoprecipitated with anti-Leu 4, under conditions that allowed co-immunoprecipitation of the antigen receptor. A 90 kd protein was co-immunoprecipitated with the 20-29 kd CD3 complex from three independent CD3+,4-,8- cell lines. Reduction of the 90 kd structure revealed three proteins of ~46, 42, and 37 kd. Analysis by diagonal 2-D SDS-PAGE revealed that the 90 kd structure dissociates into 3 proteins below the diagonal, indicating the presence of two disulfide-linked hetero-dimers sharing a common chain. Further biochemical analysis of these proteins is under way.

T 110 ORGANISATION AND REARRANGEMENT OF HUMAN T CELL REARRANGING γ GENES, M.-P. Lefranc*, A. Forster and T.H. Rabbitts
Medical Research Council, Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH, England.
* Present address : Laboratoire d'Immunogénétique, UA CNRS 1191, Université des Sciences et Techniques du Languedoc, Place E. Bataillon, 34060 Montpellier Cédex, France.

We have identified twelve Vγ genes and two Cγ genes in human DNA. The two Cγ genes have been linked (they are 16 kb apart) and each has at least one Jγ segment. The exon structure shows that Cγ1 has three exons, but Cγ2 has four, due to a duplication of exon 2. The group of Vγ genes we sequenced so far includes five pseudo-genes, and six potentially active genes. These fall into three distinct subgroups VγI (including Vγ1 to Vγ8 previously described), VγII (probably consisting of a single V gene, Vγ9) and VγIII (also probably consisting of a single Vγ gene, Vγ10, rearranged in the cell line RPMI 8402). The Vγ11 gene we identified by rearrangement in the JM cell line seems to belong to a fourth subgroup, VγIV. The structural analysis of these Vγ genes allows assignment of most of the TRGγ rearrangements observed in whole thymus or cell lines to particular Vγ genes.

T 111 INDEPENDENT ASSOCIATION OF TWO DISTINCT T CELL RECEPTOR CHAINS WITH CD3 IN THE SAME CELL. W. L. Maloy, F. Koning and J. E. Coligan, LIG, NIAID, NIH, Bethesda, MD 20892

The antigen receptor (TCR) on effector T lymphocytes was initially characterized as an αβ-chain heterodimer that is associated with the CD3 (T3) complex on the cell surface. Recently, a second CD3-associated heterodimer composed of a γ-gene product and a putative δ-gene product, has been identified on a subset of human lymphocytes, human thymocytes, and murine thymocytes. Because β and γ chain mRNA, in contrast to α chain mRNA transcripts, are abundant in immature murine thymocytes it has been hypothesized that such thymocytes could express βγ receptors which are replaced by αβ receptors after translation of α chain mRNA. However, no evidence exists that β and γ protein can be synthesized by a single cell. The human T-cell line PEER expresses CD3 in association with a non-α/non-β 55-60 kD molecule which is apparently a γ-gene product. Furthermore, this cell line has been reported to express β chain mRNA. Using a TCR β chain specific antiserum, we found that PEER synthesizes a 40 kD β-chain product that associates intracellularly with CD3, but that this complex is not found on the cell surface. This β chain is not a precursor of the cell surface CD3 associated 55-60 kD molecule. We conclude that β and γ proteins can be produced within the same cell and that CD3-TCRβ chain and CD3-TCRγ chain complexes can occur independently with only CD3-TCRγ being expressed on the cell surface. In addition, we could find no evidence for CD3-TCRβγ complexes in this cell line.

T 112 STRUCTURE AND EXPRESSION OF THE T CELL RECEPTOR GAMMA LOCUS IN PRE-B CELL LINES AND THE GERMLINE OF MICE, James McCubrey and John McKearn, E. I. DuPont, 500 S. Ridgeway Ave., Glenolden, Pa. 19036. We have examined the genetic structure and expression of the T cell receptor (TcR) alpha, beta and gamma loci in Abelson MuLV (A-MuLV) transformed pre-B B, IL-3 dependent myeloid, macrophage and fibroblast cell lines. The TcR beta locus is in the germline configuration in all non-T cell lines examined and is transcriptionally silent. This is consistant with our data examining the TcR alpha locus as well. In contrast, thirty-eight percent of A-MuLV transformed pre-B cell lines are rearranged at the TcR gamma locus. Moreover, of the pre-B cell lines that are rearranged at the V-gamma locus, 60% are rearranged at a non-cross hybridizing V-gamma region. By Southern blot analysis, the gamma rearrangements in the pre-B cell lines appear similar to the gamma rearrangements in either the thymoma EL4 or T lymphoma BW5147 and the rearranged gamma gene structure depends on whether the cell line was derived from BALB/c or C57L mice due to RFLP at the TcR gamma locus. Two pre-B cell lines produce gamma RNA (V2C) that is indistinguishable in size from that detected in two T cell tumors. In addition, many pre-B and IL-3 dependent myeloid lines produce gamma RNA that is smaller than full length gamma RNA and lacks V-gamma sequences.

T 113 TRGγ and TCRβ GENE REARRANGEMENT IN T and NON-T ACUTE LYMPHOBLASTIC LEUKEMIA Nicola Migone, Giulia Casorati and Robert Foa, Istituto di Genetica Medica e Centro CNR Immunogenetica ed Istocompatibilità, Clinica Medica A, University of Torino, Italy. The DNA configuration at the T-cell rearranging gene (TRG)γ, T-cell receptor (TCR)β and Immunoglobulin (IgH,κ,λ) loci was analyzed in 35 T- and in 57 non-T(15 "null", 42 "common")Acute Lymphoblastic Leukemias(ALL) whose phenotype had been assessed by means of T- and B-cell related monoclonal antibodies. The major findings are summarized as follows:
a) The TRGγ and TCRβ loci appeared concordantly rearranged in most T ALL (29/35). The five cases with a germ line pattern at both loci showed a more immature T-cell phenotype.
b) A significant proportion of non-T ALL carried signs of TRGγ or TCRβ gene involvement (40% and 30%, respectively). The pattern of TRGγ rearrangement appeared qualitatively and quantitatively different in non-T versus T ALL: in the former a preferential rearrangement of Jγ1, in the latter of Jγ2 (P<.001); in apx. 1/3 of the TRGγ-rearranged non-T ALL the novel bands appeared anomalous both in intensity(fainter) and/or in number(more than two). The coexistence of a monoclonal rearrangement in both IgH-carrying chromosomes and no IgH germ line bands suggests that the TRGγ involvement may have occurred after the establishment of the neoplastic process. The absence of TRG rearrangements in 30 B Chronic Lymphoid Leukemias tested so far supports the view that, if Jh-Jγ double rearrangements occur at an analogous rate in normal hemopoiesis, a selection mechanism against such aberrant cells should exist at an early stage of development. c) The apparent preference to rearrange functional Vγ genes over those pseudo Vγ which have no obvious defect in the "epta-nonamer" regions might suggest a selection preceding the neoplastic event.

T 114 CHARACTERIZATION OF T CELLS BEARING TCR γδ, Drew M. Pardoll, B.J. Fowlkes, Ada Kruisbeek, Andrew Lew, Lee Maloy, Jeffrey Bluestone, Ronald Schwartz and John Coligan, LI, LMI, LIG, NIAID; IB, NCI and BRMP, National Institutes of Health, Bethesda, MD 20892. Using an antiserum specific for the carboxy-terminus of the murine gamma chain and a monoclonal anti-murine T3 antibody, we have identified a $T3^+$ $Lyt2^-$ $L3T4^-$ cell in the thymus and in the periphery which bears a novel T cell receptor consisting of a 35K gamma chain disulfide linked to a 45K delta chain. All of the cell surface γδ is T3 associated. Both chains are N-glycosylated. The δ chain is acidic and has a core M_r of 37 kD while the γ chain is basic and has a core M_r of 32 kD. These cells are not precursors to αβbearing cells but rather, appear to represent a separate lineage of cell differentiating within the thymus. We plan to present results of studies aimed at determining: (1) whether these cells are selected intrathymically, (2) whether these cells are selected extrathymically, (3) the relationship between γδbearing cells in the thymus and in the periphery and (4) the ligand for this receptor.

The T Cell Receptor

T 115 A NEW MURINE Vγ GENE, Jukka Pelkonen, André Traunecker and Klaus Karjalainen, Basel Institute for Immunology, CH-4005 Basel, Switzerland.
The second T cell receptor (TCRII), a γ/δ heterodimer, is encoded by γ chain and still "hypothetical" δ chain genes. The function of TCRII bearing cells is obscure but these cells can constitute up to 10 percent of human peripheral T lymphocytes.
We have identified a new murine Vγ gene segment which undergoes rearrangements with high frequency in AKR thymomas, and also in fetal thymocytes but with lower frequency. This is the fourth Vγ gene undergoing rearrangements to Jγ4, therefore named Vγ4.4. Vγ4.4 is the most 5' V gene in γ4 cluster and it was found to be 7.3 kb away from Vγ4.3. Strikingly, Vγ4.4 has much higher nucletide and amino acid sequence homology to eight human Vγ genes than to murine ones. Because of the homology to human Vγ genes, Vγ4.4 could code for a conserved specificity which might be important for the function of cells expressing TCRII.

T 116 IDENTIFICATION OF A PUTATIVE SECOND T-CELL ANTIGEN RECEPTOR IN MOUSE AND MAN BY AN ANTI-PEPTIDE GAMMA CHAIN SPECIFIC MONOCLONAL ANTIBODY. Chris D. Platsoucas, Kyogo Itoh, Rajenda Pahwa, Robert Good and Constantin Ioannides, M. D. Anderson Hospital and Tumor Institute, Houston, TX 77030 and All Children's Hospital, St. Petersburg, FL 33701.
We developed a monoclonal antibody (mAb) against a synthetic peptide (P13K, amino acids 118-130) selected from the deduced amino acid sequence of the constant region of the gamma chain of the murine T-cell antigen receptor. This mAb immunoprecipitated from cultures of non-adherent spleen lymphocytes from nude mice expanded in recombinant IL2, a 57 kd molecule under non-reducing conditions, and a 31 kd polypeptide chain under reducing conditions. This anti-P13K mAb also recognized a putative second T cell antigen receptor expressed on T lymphocytes from a patient with severe combined immunodeficiency propagated in culture with ConA, OKT3 and rIL2. Experiments using chemical crosslinking (DTSP) of ^{125}I-labeled cells, followed by immunoprecipitation with the anti-Leu4 monoclonal antibody under both reducing and nonreducing conditions, revealed that this molecule is associated with the T3 antigen. This putative T-cell antigen receptor had a molecular weight of approximately 100 kd and comprised of two polypeptide chains with molecular weights under reducing conditions of 56 kd and 41 kd and under nonreducing conditions of 62 kd and 41 kd. In contrast with the mouse, these two polypeptide chains were not disulphide-linked. This putative T-cell antigen receptor is absent from human peripheral blood T lymphocytes from normal donors cultured for 5 days with PHA.

T 117 EVIDENCE FOR DEFECTIVE REARRANGEMENT OF TCRγ GENES IN A MOUSE MUTANT (*scid*) WITH SEVERE COMBINED IMMUNE DEFICIENCY. Walter Schuler, Amelie Schuler and Melvin J. Bosma, Institute for Cancer Research, Philadelphia, PA 19111

We recently reported (CELL 46, 963 1986) that rearrangement of the genes encoding antigen-specific receptors on T and B cells is defective in *scid* mice. Specifically, in *scid* bone marrow cells transformed by Abelson murine leukemia virus, the majority of rearranged *Igh* alleles delete their entire Jh region. Similarly, most rearranged TCRβ alleles in spontaneous *scid* thymic lymphomas delete their entire Jβ2 region. These J-region deletions appear to result from faulty D-to-J recombination.

We now have evidence for defective rearrangements of TCRγ genes in *scid* thymic lymphomas. In all lymphomas studied, rearrangement of TCRγ genes was accompanied by large deletions affecting Vγ genes and extending into the flanking region of Cγ genes. No such deletions were found in thymic lymphomas of control mice. Our data suggest that these deletions resulted from attempted site-specific recombination. Since D elements have not been found at the TCRγ locus we interpret our results to indicate faulty V-to-J recombination. Thus, the defective gene rearrangements seen in transformed lymphocytes of *scid* mice appear not to be limited to D-to-J recombination. This gives further support to our hypothesis that the *scid* mutation may affect a component of the recombinase system used in common by B and T cells to assemble antigen-specific receptor genes.

T 118 A NEW $V\gamma 2$-CROSSHYBRIDIZING REARRANGEMENT IN MRL THYMOCYTES, Jeffrey N. Siegel$^+$, David H. Raulet*, and David I. Cohen$^+$, $^+$Laboratory of Chemical Biology, NIDDK, National Institutes of Health, Bethesda MD 20892 and *Department of Biology, MIT, Cambridge, MA.

The described γ gene locus in the Balb/C mouse consists of four related constant regions, one of which is apparently a pseudogene. These constant regions are associated with at least 6 variable region genes, 3 of which are members of a gene family and the other three possess a wide degree of diversity. Probes specific for these V regions were generated (Garman et al., Cell, 1986) and utilized to study the pattern of γ gene rearrangement in T cells of the MRL mouse (H-2^k). We found that $V\gamma 1$, $V\gamma 3$, and $V\gamma 4$ showed a pattern similar to that described in the Balb/C mouse. Interestingly, Eco RI digested thymic, peripheral lymph node (LN), and splenic DNA probed with the $V\gamma 2$ gene displayed a new 7.6Kb rearranged band in addition to the 5.0Kb germline band and the 17Kb rearrangement seen in Balb/C DNA. This new rearrangement does not hybridize to a $C\gamma 1$ probe, suggesting either that it utilizes a new J separated from $C\gamma 1$ by an Eco RI site, or that it utilizes a $C\gamma$ distinct from $C\gamma 1$. This rearrangement was never seen in DNA from liver or kidney, although an additional band distinct from this band and probably representing partial digestion was occasionally seen. The rearrangement is most prominent in thymic DNA but is seen in lesser amounts in LN and spleen. The finding of a new $V\gamma 2$ cross-hybridizing rearrangement in thymus adds to the potential diversity of the γ gene family.

T 119 T CELL RECEPTOR γ CHAIN EXPRESSION IN Thy-1$^+$ DENDRITIC EPIDERMAL CELLS. G. Stingl, H. Yamada, F. Koning, A. Lew, J. Bluestone, W. Yokoyama, G. Steiner, J. Coligan, and E. Shevach. LI and LIG, NIAID, Immunol. Br., NCI, NIH, Bethesda, MD and Dept. of Derm I., Univ. of Vienna, Vienna, Austria.

Thy-1$^+$ dendritic epidermal cells (Thy-1$^+$ DEC; Thy-1$^+$, Ia$^-$, L3T4$^-$, Lyt-2$^-$, SIg$^-$) are murine bone marrow-derived leukocytes of unknown function. Cell lines derived from Thy-1$^+$ DEC by Con A/IL-2 stimulation contain either full-sized or incomplete transcripts of the various chains of the T cell antigen receptor (TCR) complex suggesting that Thy-1$^+$ DEC belong to the T cell lineage. We studied Thy-1$^+$ DEC in situ and freshly isolated Thy-1$^+$ DEC-enriched (80-90%) epidermal cells for their reactivity with mAbs directed against the murine T3 complex (clone 145-2C11), against a determinant of the TCR β chain (F23.1), and with an antiserum against the murine TCR γ chain. Analysis of NH_4-SCN-separated, acetone-fixed epidermal sheets from adult C3H/HeN mice and of Thy-1$^+$ DEC-enriched epidermal cells revealed that anti-T3 selectively reacts with a population of dendritic cells which, by double-labeling, were uniformly Thy-1$^+$ and Ia$^-$ and that most Thy-1$^+$ DEC bore T3 antigens. While mAb F23.1 failed to react with any epidermal cells, the anti-γ serum reacted specifically with a sizable portion of both Thy-1$^+$ DEC in situ and of ethanol-permeabilized Thy-1$^+$ DEC-enriched EC. The demonstration of T3 antigen on virtually all Thy-1$^+$ DEC now definitively proves the T cell nature of this population. The reactivity of the anti-γ with a major portion of Thy-1$^+$ DEC strongly suggests that T3 on the surface of Thy-1$^+$ DEC is linked to the product of the TCR γ chain genes.

T 120 TARGET CELL SPECIFICITY AND γ CHAIN DIVERSITY IN CYTOTOXIC CD3$^+$ TCR$\alpha\beta^-\gamma^+$ T CELL CLONES.

R.J. van de Griend, J. Borst, S.-L. Ang, J. Seidman and R.L.H. Bolhuis. Rotterdam Radiotherapeutic Institute, Dept. of Immunology, Rotterdam; The Netherlands Cancer Institute, Amsterdam, The Netherlands; and Harvard Medical School, Dept. of Genetics, Boston, U.S.A.

CD3$^+$4$^-$8$^-$ T cell receptor $\alpha\beta^-\gamma^+$ cloned T cells like CD3$^-$ NK cells but in contrast to CD3$^+$ TCR$\alpha\beta^+$ T cells, were found to exert MHC nonrestricted cytolytic activity against a variety of tumour target cells including freshly derived tumour cells. Functional TCRγ genes, producing mRNA and γ proteins were identified on these CD3$^+$TCR$\alpha\beta^-$ clones, which were derived from normal human PBL. These cells seem to form disulphide-linked dimers in which the γ chain participates. We have analyzed now twenty of such CD3$^+$4$^-$8$^-$ TCR$\alpha\beta^-\gamma^+$ clones derived from different individuals, all of them exerting lytic activity and related their target cell specificity (pattern) to the quarternary organization of this "alternative" T cell receptor. Eight CD3$^+$TCRγ^+ clones, derived from a single individual, were also studied for lytic activity, γ chain rearrangement and γ chain diversity. Clones from different individuals could exert different target cell specificity patterns. Cytolytic activity of these clones was regulated by various monoclonal antibodies (MAb). Enhancement of cytolysis was obtained by MAb directed against either CD3 or CD2 antigens or by both MAb, depending on the target cell, possibly reflecting the state of involvement of the TCRγ chains. Finally, evidence was obtained that some other MAb (non-anti-CD3) raised against TCR$\alpha\beta^+$ cells, could also modulate cytolysis in CD3$^+$ TCR$\alpha\beta^-\gamma^+$ clones.

T 121 T CELL RECEPTOR GENE EXPRESSION OF NOVEL T CELL SUBSETS IN AUTOIMMUNE MICE. Katsuyuki Yui, Yasuhiro Hashimoto, and Mark I. Greene. Univ. Pennsylvania Philadelphia PA 19104.

A spontaneous recessive mutation, generalized lymphoproliferative disease (gld), in C3H/HeJ mice determines the development of age-related massive lymph node enlargement with autoimmunity. Accumulating lymphocytes include Thy-1$^+$ Lyt-2$^-$ L3T4$^-$ Ia$^-$ cells (Double-negative cells) and Thy-1$^-$ Ia$^-$ Ig$^-$ cells (Null cells).

We have analyzed T cell receptor gene expression in these cell subsets. Both Double-negative and Null cells are of the T cell lineage, because they uniformly express T cell receptors on their cell surface which are detected by KJ16 and F23 antibodies. The analysis of the T cell receptor expression of these T cell subsets at the mRNA level revealed that the expression of T alpha, T beta and T3 mRNA is enhanced compared with that of normal peripheral T cells. The enhanced level of mRNA expression was not reflected by increased expression of T cell surface receptors. Interestingly, the mRNA expression of T gamma was not detected in Null cells. These results suggest a possible relationship of the expression of Thy-1 to the developmentaly related expression of T cell receptor genes.

Antigen Binding T Cell Factors

T 122 THE ANTIGEN SPECIFIC HELPER FACTOR - A SECRETED FORM OF THE T CELL RECEPTOR Ben-Sasson, S.Z., Kagan, Y., Azar, Y., Falek, P., Guy, R., Haion, K., and Nahor, O. The Lautenberg Center for General and Tumor Immunology, The Hebrew University-Hadassah Medical School, Jerusalem, Israel.

Immortalization of antigen specific T cells was accomplished through viral transformation of immunocompatent cells. Some of the immortalized clones perform different immune activities upon stimulation with the relevant antigen where every clone has a distinctive immune function. The MHC restricted helper clone triggers B cells both in vivo and in vitro, via an antigen bridge, to secrete antibodies of the IgG subclasses. The helper clone secretes an antigen specific helper factor (ASHF). The factor is carrier specific and activates B cells both in vivo and in vitro, via antigen bridge, to secrete antibodies of the IgG subclasses. Monoclonal antibodies were raised against clone specific determinants on the cell surface of the helper clone. Some of the monoclonal antibodies, that recognize a unique cell surface component of the helper clone, bind the ASHF. The affinity purified helper factor is very similar to the specific surface molecule isolated from the helper clone. The structural and functional similarities between the helper clone receptor and the ASHF suggest that the latter is a secreted form of the T cell receptor. Thus, it is postulated that the helper T cell, like the B lymphocyte, has an antigen binding site in two molecular forms: a membrane integrated structure and a soluble secreted product. The implications of these findings to the mechanism of T-dependent B-cell activation will be discussed.

T 123 ANALYSIS OF T-CELL RECEPTOR GENES IN A SUPPRESSOR CLONE, Michael Darsley, Luciano Adorini* and Ettore Appella, Lab of Cell Biology, N.C.I., Bethesda MD 20892, *Lab of Pathology, ENEA C.R.E. Casaccia C.P. 2400, 00100 Rome, Italy.

LH8 is a suppressor/inducer T cell clone, immortalised by in vitro infection with radiation leukemia virus. It constitutively secretes an antigen binding factor capable of inducing effector T_S cells when injected in vivo at the time of antigen priming into I-J and IgH compatible mice. The factor will suppress the immmune response to HEL or a synthetic peptide representing residues 1-18 of HEL, to HuL but not to RNPEL, implicating Phe at position 3 in the epitope. LH8 has previously been shown to express an α/β T cell receptor; we show here that this receptor is associated with the T3 polypeptides, as is the case for T_H and T_C cells. Full length cDNA clones encoding the two receptor chains have been isolated and sequenced. The α-chain uses the same Vα gene segment as found in the thymic clone TA65 and a new Jα, while the β-chain uses a Vβ segment closely related, but not identical, to the 3 members of the Vβ5 family. Thus this T_S clone appears to use the same pool of gene segments as do T_C and T_H cells to produce a surface receptor. The α- and β-chain cDNAs have been subcloned into a vector to allow in vitro transcription of mRNA which will be translated in oocytes in an attempt to confirm the role of the α and β chains in T_SF production, since it has already been shown that total mRNA from LH8 or mRNA adsorbed separately to filters containing Cα and Cβ sequences and subsequently eluted and recombined can be translated in oocytes to produce suppressive molecules (De Santis et al. Abstract no. 2.21.8, 6th International Congress of Immunology, Toronto, Canada, 1986).

T 124 CLONING AND EXPRESSION OF cDNA ENCODING AN IMMUNE SUPPRESSOR OF AN IN VITRO GAT-SPECIFIC PFC RESPONSE. *Chris Funckes-Shippy, #Craig Sorensen, #Carl Pierce and *Alan Levine, *Monsanto and #Jewish Hospital, Washington University, St Louis, MO 63198.

Studies with the model antigen GAT indicate that a single chain protein, GAT-TsF1, is synthesized by suppressor T lymphocytes and initiates a regulatory cascade of antigen-specific suppressor T cells. A recombinant plasmid, pMON7503, containing 1400 bp cDNA specific for the hybridoma synthesizing GAT-TsF1, has been isolated by subtractive hybridization. Lysates of E. coli expressing the protein encoded by pMON7503 demonstrate dose dependent suppression of only a GAT-PFC response. This recombinant suppressor activity binds to antigen and bears the epitopes for $I\text{-}J^b$, the cGAT idiotype, IgH-linked determinants, and Tsu. pMON7503 hybridizes specifically to 1600 and 2000 b mRNAs from the T cell hybridoma that synthesizes GAT-TsF1 and not to RNA isolated from other GAT-specific supressor T cell hybridomas or a B cell myeloma. pMON7503 is encoded by a single copy integrated gene in the GAT-TsF1 producing hybridoma, but no genomic signal is detected in murine DNA isolated from the fusion partner, liver, spleen or kidney. There is no significant homology of pMON7503 at the amino acid or nucleotide level to any sequence contained in the EMBL or Genbank data banks.

T 125 β-CHAIN GENES IN GAT-HELPER AND SUPPRESSOR T CELLS, Ellen Kraig, Carol Kannapell, and Anne Plessis, Univ. of Texas Health Sci. Center, San Antonio TX 78284.

The genes encoding the β-chain of the T-cell antigen receptor have been examined in murine helper (T_H) and suppressor (T_S) cells specific for GAT. The immunoglobulin response to GAT is very homogeneous; the V_H and V_κ gene segments have been cloned and sequenced. Therefore, we can compare directly the variable region genes used by T cells with those used by B cells in recognizing the same antigen. We have cloned and sequenced the V_β, D_β, and J_β gene segments from a GAT-specific T_H clone and analyzed its expression in other independently derived GAT-T_H cell clones. There is no region of increased homology apparent between this β-chain gene segment and the GAT-V_H and V_κ gene segments. Additionally, we have shown that the β-chain genes deriving from the normal T-cell fusion partner are deleted in numerous functional GAT-T_S cell hybridomas. Therefore, either suppressor T cells do not use the β-chain genes or the β-chain from BW5147, the thymoma fusion partner, imparts the necessary functions. To date we have found no evidence of a unique α chain transcript in these cells. We are currently attempting to identify the genes encoding the receptors on T_S cells using serological approaches.

We thank Drs. Webb, Kapp, Abruzzini, Sorensen and Pierce for providing the cells and Drs. Barth, Kronenberg, Kobori, Malissen, and Hood for the probes. This work has been funded by Welch Foundation Grant AQ-985 and by NIH Grant AI-22181.

T 126 BIOCHEMICAL ANALYSIS OF A Ts1 SUPPRESSOR HYBRIDOMA:, Jing Liu, David B. Weiner, Jeffrey A. Bluestone, Ralph T. Kubo, Mark I. Greene, University of Pennsylvania, School of Medicine, Department of Pathology and Laboratory Medicine, Philadelphia, Pa 19104.

Previously we have reported on a Ts1 suppressor hybridoma constructed by the fusion of first order ABA induced T suppressor spleen cells, taken from A/J mice, with the murine thymoma BW5147. From this fusion we have isolated a T cell hybridoma designated F12.23 which continuously secretes an intact TsF1 molecule capable of mediating suppression of inflammatory and cytotoxic T cell responses in an identical manner to the primary spleen cells produced TsF1. Immunofluorescence analysis by FACS of the F12.23 hybridoma demonstrates that this hybridoma is stained positively by anti Thy 1.2 as well as anti T3 epsilon chain antibody but is negative for expression of L3T4 and LFA-1 surface antigens. The hybridoma fails to react with KJ-16, KJ1-26 as well as 23F12 antibodies, all three of which recognize different epitopes of the murine T cell receptor chains. Furthermore diagonal SDS-PAGE of surface radioiodinated F12.23 hybridoma cells identifies a disulfide lined heterodimer which falls below the diagonal running in the first dimension at 80KD and resolving in the second dimension as two spots of between 35KD and 40 KD. This unusual heterodimer was clearly absent from the BW5147 control samples. Taken together these results demonstrate that F12.23 is a functional Ts1 hybridoma that expresses many T cell properties. This suppressor hybridoma expresses a unique heterodimeric molecule on it's surface that fails to react with a panel of antibodies which detect several different allotypes of the traditional alpha and beta T cell receptor heterodimer. The relationship between this molecule and the gamma and delta chains of the murine T cell receptor will be presented.

The T Cell Receptor

T 127 ISOLATION AND CHARACTERIZATION TIMOTHY GRASS POLLEN ANTIGEN B T SUPPRESSOR FACTORS, A. Malley and P. Perry, Oregon Primate Res. Ctr., Beaverton,Oregon 97006

Antigen B (AgB) - specific T suppressor (Ts) cells hybrids were obtained by fusions of BW514T cells with Lyt 1^-2^+ enriched T cells from photo-oxidized AgB primed spleen cells of (CBA/J x C57BL/6 F_1 mice. The Ts cells were characterized by their cell surface antigens, lack of IL-2 production, and the secretion of a soluble antigen binding T cell factor. AgB-specific T suppressor factor (Tsf1) was isolated from the cell free supernatants of 5 Lyt 1^-2^+I-J^+ T cell hybridomas cloned twice by limiting dilution. Purification of Tsf1 was achieved by preparative DEAE-HPLC and affinity methods on antigen and anti-idiotypic antibody (anti-Id) adsorbents. The Tsf1 fractions were analyzed by SDS-PAGE for purity, and by ELISA for their ability to inhibit AgB-monocloned anti-AgB antibody interaction. Factors showing good inhibition of AgB-anti-AgB interaction induced significant levels of idiotype-binding Ts cells in vitro. The induced Ts cells suppress AgB specific IgE responses, but not the IgG response to AgB. The interaction of Tsf1 with anti-Id in an ELISA is specifically inhibited by cellbiose, the determinant on AgB that induces IgE. SDS-PAGE analysis of Tsf1 shows two major bands (53 and 25Kd) that bind anti-Id on Western blots. AgB-specific Tsf1 is a glycoprotein containing 5% sugars, I-J^+, and does not bind with anti-mouse IgG, anti-mouse IgM, or anti-light chain enzyme conjugates in an ELISA.

T 128 I-J EPITOPES AND T CELL RECEPTOR (TcR) ON THE MHC-RESTRICTED CLONED T CELLS.
Toshinori Nakayama, Masato Kubo, Isao Fujisawa, Junko Shimura, Yoshihiro Asano, and Tomio Tada. Dept. of Immunol., Fac. of Med., Univ. of Tokyo, Tokyo, Japan.

Anti-I-J^k monoclonal antibodies (mAb) originally established as those reactive with antigen-specific T suppressor factor (TsF) of H-2^k mice have been found capable of inhibiting H-2^k-restricted, but not H-2^b-restricted, T cell functions of H-2^{kxb} F_1 T cell populations. Since the MHC-restricted recognition is the property of authentic T cell receptor heterodimers, we examined the expression of I-J epitopes on H-2-restricted T cell clones with rearranged α and β genes established from H-2^k, H-2^b, H-2^{kxb} F_1 and semiallogeneic radiation bone marrow chimeras. It was demonstrated that anti-I-J^k mAb inhibited the antigen-induced proliferative responses of both I-A^k- and I-E^k-restricted Th and Ts clones derived from F_1 mice. I-J^k was also positive on I-A^k-restricted Ts clones of the B6 → F_1 chimera origin. None of H-2^b-restricted clones derived from F_1 and chimeras expressed the I-J^k epitope. The in vitro secondary antibody response induced by I-A^k- or I-E^k- restricted Th clones were also inhibitable by a low concentration of anti-I-J^k mAb. The I-J epitopes were demonstrable on several H-2^k-restricted T cell clones by the microimmunofluorocytometry. Conclusions derived here are: 1) I-J epitopes are associated with the class II MHC restriction site associated with T cell receptor regardless of their functions, 2) I-J^k epitopes are expressed on both I-A^k- and I-E^k-restricted clones, and 3) the I-J phenotype is not primarily determined by MHC genes of stem cells but is somatically and clonally acquired by T cells. Our anti-I-J mAb do not precipitate the authentic αβ heterodimer.

T 129 HYPOTHETICAL TUMOR ASSOCIATED ANTIGEN T-CELL RECEPTOR DEBLOCKING BY IMMUNE MODULATING MECHANISMS OF UKRAIN. J.W. Nowicky, M. Greif, F. Hamler, W. Hiesmayr, W. Staub, Laimgrubengasse 19/5, A - 1060 Vienna, Austria.

T-Lymphocytes predominantely recognize antigen in the context of membrane bound products of the major histocompatibility complex. T-cell receptors may even identify altered cell membrane structures of various malignancies. There are many theories why, in the course of a progressive oncological disease, these recognition and defence mechanisms are blocked off, so that finally the complete immune system becomes entirely exhausted. Clinical studies on the immunomodulating agent Ukrain have shown that it exerts some unblocking and/or unmasking effect on the relationship between tumor and defense mechanism. The increase of the T-Helper/T-Suppressor ratio and the amelioration of NK-cell activity in oncological patients treated with Ukrain give the strong evidence, that, as to immunocompetent regulation, not only quantitative but also qualitative changes must have taken place in tumor associated recognition of antigens. Local signs like inflammatory and demarcation processes in the tumor areas may indicate such surface dependent destructive mechanisms. It must be added that Ukrain exerts a regulative effect on data conflict phenomena in autoagressive diseases, maybe by normalising the transmission of signals between biological receptor- and transmitter interludes.

T 130 SUPPRESSION OF IgE SECRETION FROM HYBRIDOMA CELLS IN ALLOTYPE CONGENIC MICE: SUPPRESION OF ALLOTYPE 7 a BY T CELLS IN ALLOTYPE b MICE. Zoltan OVARY and Soichiro MAEKAWA, New York University Medical Center, Department of Pathology New York, NY 10016

Anti-2,4-dinitrophenyl (DNP) IgE producing hybridoma B 53 when injected subcutaneously is established equally well in syngeneic BALB/c (heavy-chain allotype a) and congenic CB20 (heavy-chain allotype b) mice. However, secretion of anti-DNP IgE monoclonal antibody is greatly suppressed in CB20 mice. B 53 cells taken from the subcutaneous tumors of CB20 mice produce anti-DNP IgE in vivo in BALB/c mice and in vitro. No difference was observed in IgE production between these cells and the controls taken from BALB/c mice. The suppression of IgE production was due to T cells and/or their product(s) of CB20 mice. Supported by National Institutes of Health Grant AI-03075.

T 131 HTLV-1 INFECTION OF A HUMAN ANTIGEN SPECIFIC PROLIFERATING-CYTOTOXIC T CELL CLONE. H. Spits, H. Yssel L. Gazollo and J. de Vries. Unicet, chemin des peupliers 27, 69572 Dardilly, France.

An antigen specific proliferating-cytotoxic T cell clone, HY-827, (CD3+CD4+) was obtained by stimulating the T cells of a normal donor with Tetanus Toxoid (TT). HY 827 reacted with a series of monoclonal antibodies (mabs) detecting different epitopes on the variable part of the T cell receptor (Tcr). Thirty days after the infection with HTLV-1 the cells started to grow independently from antigen restimulation but IL-2 was still required. The cells then expressed the HTLV-1 structural protein p19 and contitutively expressed the IL-2 receptor and HLA-DR. Between day 30 and day 200 the Tcr phenotype did not change and the cells were unable to lyse TT preincubated HLA-DR 3+ target cells in contrast to the uninfected HY 827 cells. In the absence of exogeneously added IL-2 TT could induce proliferation. This induction of proliferation was TT specific and did not require the presence of antigen presenting cells and could be blocked by the anti Tcr mabs. Irradiated HTLV-1 infected cells could present TT to the uninfected cells. Our data indicate that HTLV-1 infection leads to an irreversible loss of cytotoxic activity which is not due to an inability to recognize antigen with the Tcr. In addition the apparent unrestricted response of the virus infected T cells is probably caused by the aquired ability to present antigen to itself.

T 132 CHARACTERIZATION OF A D^b-SPECIFIC HELPER FACTOR, Hung-Sia Teh and Pearl C. Kwong, Department of Microbiology, University of B.C., Vancouver, Canada V6T 1W5.

A T helper clone (clone 9), isolated from a H-2^d anti-H-2^b mixed lymphocyte culture, was previously found to produce an antigen-specific helper factor (ASHF) that could be specifically absorbed out with B10.A(2R)($K^kA^kE^kD^b$), but not B10.A ($K^kA^kE^kD^d$), spleen cells. To further characterize this ASHF, we have constructed T cell hybridoma lines by fusing clone 9 cells with the AKR thymoma, BW5147. One of these hybridoma clones, referred to as clone 25, produced an ASHF that was specific for the D^b alloantigen. Immunization of allogeneic C57BL/6 mice with clone 9 cells and subsequent fusion of these immune spleen cells with non-secreting myeloma cells led to the isolation of a monoclonal antibody (mAb) (clone 30 IgM) that was capable of neutralizing the helper activity of clone 25 ASHF. Clone 30 IgM affinity column was found to retain clone 25 ASHF; clone 30 IgM column eluates augmented the cytotoxic responses of CBA/J thymocytes to B6 (H-2^b), but not D2 (H-2^d), alloantigens. Preabsorption of clone 25 ASHF with D^b-bearing spleen cells prior to affinity purification over a clone 30 IgM column resulted in the abrogation of D^b-specific helper activity as well as the loss of a 50 kDa band in SDS polyacrylamide gels ran under reducing conditions. Clone 25 ASHF was also retained by immunoadsorbents made with an IgG2a mAb (F23.1) whose reactivity is against the β chain of the T cell receptor. Furthermore, affinity purification of clone 25 ASHF over a F23.1 affinity column, but not an irrelevant mAb column, also yielded a 50 kDa molecule. These findings suggest that this particular ASHF may be intimately related to the T cell antigen receptor.

The T Cell Receptor

T 133 STRUCTURAL RELATIONSHIPS AMONG ANTIGEN-SPECIFIC SUPPRESSOR FACTORS AND T-CELL ANTIGEN RECEPTOR SPECIFIC FOR THE TERPOLYPMER L-GLUTAMIC AUD^{60}-L-ALANINE^{30}-L-TYROSINE^{10} (GAT), D.R. Webb[1], C.M. Turck[1], N. Mensi[1], J.A. Kapp[2] and E. Kraig[3], [1]Roche Institute of Molecular Biology, Nutley, N.J. 07110, [2]Dept. of Pathology and Laboratory Medicine, The Jewish Hospital of St. Louis, St. Louis, MO. 63110, [3] Dept. of Cell and Structural Biology, University of Texas Health Science Center, San Antonio, TX 78229.
The immune response to GAT-MBSA in non-responder mice (H2^{p}, H-2^{q}, H-2^{s}) primed with GAT is suppressed via a 2 cell suppressor circuit. The suppressor inducer cell (Tsi) produces a suppressor inducer factor (TsF_1) that is a monomeric, antigen-specific, I-J^{+} and non-MHC restricted. The TsF_1 activates a suppressor effector cell (Tsi) that produces a suppressor effector factor (TsF_2) that is a disulfide linked heterdimer consisting of a basic, antigen binding chain and an acidic, I-J^{+} chain that is MHC restricted and acts directly on GAT-specific T helper cells to prevent specific help. Although structurally, TsF_1 and TsF_2 are different, they both react with selected monoclonal anti-TsF_1 or anti-TsF_2 antibodies. The TsF_2 which bears a striking resemblance to the T-cell receptor, is currently being analyzed serologically and by peptide mapping to establish its structural relationship to GAT-specific T-cell receptor on T-H cells. This may help to define the genetic relationship since it has been shown that the GAT-specific T-H β chain is not utilized by the Tse cell in coding for TsF_2.

Receptor Genetics and Phylogeny

T 134 T-CELL RECEPTOR VARIABLE GENE POLYMORPHISM IN MICE. Virginia L. Barr, Jordi Yague and Ed Palmer. National Jewish Center for Immunology and Respiratory Medicine, Denver, CO 80206.

We have used cloned variable (V) region genes from α and β chain cDNA clones to examine the extent of V-gene polymorphism in 19 inbred strains of mice. The presence of restriction fragment length polymorphisms (RFLPs) in the germline DNA of different mice was taken as as indication of V-gene polymorphism. The data indicate that there are 3 different β-chain haplotypes among these inbred strains. One haplotype (TCβ^{a}) is shared by BALB/c, C57BL6/J, AKR and numerous other strains; a second haplotype (TCβ^{b})is found in SJL, SWR, C57L and C57BR mice; while a third haplotype (TCβ^{c}) is found only in NZW mice. Examination of the same inbred stains with V_α gene probes indicates the presence of at least 1 distinct α-chain haplotype. All strains within the C57BL/C58 lineage carry a distinct α-chain locus as defined by RFLPs. Many other V_α RFLPs exist but their distribution among the inbred strains is not straightforward, indicating a history of recombination between different α chain alleles in the mouse population.

T 135 CHROMOSOMAL ORGANIZATION OF THE MURINE V_β GENE SEGMENT FAMILY, Richard K. Barth*, Eric Lai† and Leroy Hood†, *University of Rochester, Rochester, NY 14642, and †the California Institute of Technology, Pasadena, CA 91125.
An unusual feature of the β - chain genes of the murine T-cell receptor is their use of few V gene segments relative to the other T-cell receptor and immunoglobulin gene families. Twenty different V_β gene segments, distributed into sixteen subfamilies, have been identified. We have combined three different methods, deletion mapping in T-lymphoma lines, field-inversion gel electrophoresis of genomic DNA and the analysis of cosmid clones, to study the chromosomal organization of the entire V_β gene segment family in the mouse. Our analysis has allowed us to map the relative chromosomal order of the V_β gene segments and has permitted us to estimate the size of the murine β -gene locus.

T 136 ANALYSIS OF THE GERMLINE V BETA AND V ALPHA GENES IN PATIENTS WITH MULTIPLE SCLEROSIS, S.S. Beall*, P. Concannon**, W.E. Biddison*, H. McFarland*, L. Hood**, D.E. McFarlin*. *NIB/NINCDS/NIH, Bethesda MD 20892 and **Division of Biology, Cal Tech, Pasadena Ca 91125

Studies in twins with multiple sclerosis (MS) have led to the hypothesis that this disease has a multigenetic predisposition. Animal models of the disease such as experimental allergic encephalomyelitis and Theiler's murine encephalomyelitis utilize the SJL mouse, a strain known to delete a large portion of its germline V beta repertoire. These observations have prompted the consideration that an analogous defect in the T-cell repertoire may occur in MS. The hypothesis that patients with MS may have certain V beta and/or V alpha gene segments deleted from their germline repertoire was tested by Southern analysis with a series of restriction enzyme digests, using as probes, V beta gene segments representative of 14 subfamilies of the human genome as well as human V alpha gene segments. Additionally, restriction fragment length polymorphisms were identified with V beta and constant region gene probes and compared to those known to exist in normal individuals as a prerequisite for future molecular genotyping studies of large families of patients with MS.

T 137 GENES ENCODING THE RAT T CELL RECEPTOR, E. P. Blankenhorn, Dept. Micro. & Immunology, Hahnemann University, Philadelphia, PA 19102

Inbred rats have long served as good experimental models for human immunological disorders, especially autoimmune syndromes. Recently, with the advent of DNA cloning techniques and probes specific for T cell receptors (TcR) from other species, it has been possible to examine the genetics and structure of TcR-homologous genes in rats. Several interesting results have emerged from experiments conducted in my lab on genomic DNAs from a variety of genetically defined inbred rat strains: (1) Rats have a TcR genetic repertoire which is very similar to that of mice in both sequence and number; (2) two major alleles of rat TcR-β constant region locus have identified; (3) these two alleles are quite different from each other, indicating a complex allotype much like rat Ig-kappa; (4) differences at rat TcR-β are found in pairs of congenic rats which also differ for susceptibility to EAE (Hickey and Blankenhorn, this volume).

T 138 ANALYSIS OF THE GUINEA PIG T CELL RECEPTOR USING EXPRESSION VECTORS
R. Burger, R. Schäfer, H. Schäfer, S. Koch, D. Cohen, J. Schenkel, Institutes for Immunology of the Univ. of Heidelberg and the DKFZ, Heidelberg, FRG, and NCI, NIH, Bethesda.
The production of useful antibodies to constant region determinants of the T cell receptor (TCR) subunits proved to be difficult. Few antibodies to non-clonotypic determinants of the murine or human TCR were obtained. We described in the guinea pig an antibody which might detect such a determinant (Ag 188). For further analysis, gene segments coding for the constant region of the α- or β-subunit of the murine TCR were cloned in the expression vectors pEX 1,2,3 or pEX 31 a,b,c. The corresponding hybrid proteins were isolated from E. coli lysates and used for immunization of rabbits or rats. Antisera were obtained which reacted with the hybrid proteins. They detect also the β-galactosidase- or the MS2 polymerase moieties, respectively, as shown by their reactivity with several, unrelated hybrid proteins. However, the antisera failed to react with murine or guinea pig T cells in indirect binding or cytotoxicity assays and did not inhibit antigen-induced T cell proliferation. Immunoprecipitation experiments are performed with radiolabeled mouse T cell hybrids or guinea pig T cell clones in order to demonstrate a potential reactivity with the solubilized TCR. A cDNA library was prepared from guinea pig T cell blasts using the λ gt 11 expression system. Several cDNA clones were identified by cross-hybridization with the mouse β-chain probe. The cDNA clones hybridized in Northern blot analysis with poly (A) RNA isolated from T cell blasts but not with liver RNA. In Southern blot analysis of genomic DNA a rearrangement of the corresponding genes was shown in guinea pig T cell clones compared to liver cells. A heteroantiserum to immunoprecipitated Ag188 was produced and is tested for reactivity with the hybrid proteins produced by these cDNA clones.
Supported by DFG 400/1-2 and G. Hagemann Stiftung.

T 139 CHROMOSOMAL ORGANIZATION OF HUMAN V GENE SEGMENTS, P. Concannon, E. Lai, and L. Hood, Division of Biology, CalTech, Pasadena, CA, 91125

We have isolated probes corresponding to 14 different human T-cell receptor V_β gene segment subfamilies. These probes detect approximately 50 V_β gene segments in human germline DNA. An analysis of germline DNA from 100 individuals detected only minor differences in the repertoire of these V gene segments present in the population. We have used field inversion gel electrophoresis (FIGE), analysis of the V_β gene rearrangements in T-cell leukemias, and cosmid walking to map 36 of these V_β gene segments. Approximately 350 Kb of cosmids have been isolated which map into 6 clusters. These clusters contain 26 V_β gene segments from 8 subfamilies. They can be localized by FIGE into a region of less than 700 Kb that contains 10 additional V_β gene segments (members of 5 additional subfamilies) and both constant region genes. V_β gene segments of widely varying degrees of homology are interspersed on these cosmids. Members of different subfamilies (31-75% nucleotide homology) can be separated by as little as 5 Kb. Members of the same subfamily (75-100% nucleotide homology) may lie as close together as 2 Kb or be separated by stretches of greater than 200 Kb containing more than 20 other V_β gene segments. In general, the organization of the human V_β gene segment family is more similar to the human V_H and V_K families than to murine V gene families.

T 140 ANALYSIS OF C57Bl.10 T CELL RECEPTOR GENE USAGE IN RESPONSE TO AN ALLOANTIGEN. David DiGiusto and Ed Palmer. National Jewish Center for Immunology and Respiratory Medicine, Denver, CO 80206.

The high frequency of T cells that are alloreactive remains an enigma. To study this phenomenon we have generated a panel of hybridomas from C57BL.10/J mice that are specific for the $I\text{-}A^{bm-12}$ alloantigen. In order to determine the receptor repertoire to $I\text{-}A^{bm-12}$ alloantigen we have performed Northern analysis with specific Vα and Vβ probes. Vα4 and Vβ6 are expressed in three different $I\text{-}A^{bm-12}$ reactive hybridomas. To determine the influence of particular α -and β-chain gene segments and the contribution of junctional and N region diversity to allorecognition we have made a cDNA libraries from these hybridomas and are in the process of generating sequence information to address these issues.

T 141 UNUSUAL T CELL RECEPTOR PHENOTYPE IN A T HELPER HYBRIDOMA, Julie P. Deans, Bhagirath Singh, Arun Fotedar and Linda M. Pilarski, University of Alberta, Edmonton, Alberta CANADA T6G 4R7.

The Balb/c-derived T helper hybridoma Poly-18.68 (specific for the synthetic polymer poly 18 and Ia^d) has been examined by FACS analysis for the expression of T cell receptor determinants defined by the monoclonal antibodies KJ16.133 and F23.1. We find that this hybridoma is reactive with the KJ16 antibody, but negative for the F23.1 determinant. KJ16.133 and F23.1 apparently detect determinants on the T cell receptor which are associated with the Vβ8 family of V genes. KJ16+ and F23+ peripheral T cell populations are largely overlapping, with the F23+ population being slightly larger. It has been suggested that F23.1 recognizes determinants associated with all three members of the Vβ8 family, while KJ16.133 recognizes determinants associated with only two. It is therefore surprising to find that the Poly-18.68 hybridoma is KJ16+ but F23-. In addition there is an unusual degree of heterogeneity with KJ16 on this hybridoma, with a FACS staining pattern which is dissimilar from that obtained with a polyclonal anti-clonotypic serum. Implications and follow-up of these observations will be presented.

T 142 RECEPTOR DIVERSITY IN MHC-SPECIFIC AUTOREACTIVE T CELLS, Dianne Edgar and Maurice Zauderer, University of Rochester, Rochester, NY 14642.

The potential for T cells to recognize antigen in association with specific MHC determinants is positively selected during thymic differentiation in the absence of foreign antigens. Research in this laboratory has demonstrated the occurrence of a surprisingly large subpopulation of mature T cells that has retained or reacquired this ability to be stimulated by self-MHC alone. We have intensively investigated the origin, specificity, and function of these autoreactive T cells.

Our current research focuses on characterization of the self MHC-specific receptors expressed by cloned lines of autoreactive T cells. We are attempting to determine the relative diversity of genes for MHC-specific receptors expressed in autoreactive T cells and the relationship of these receptor genes to those expressed by antigen-specific T cells. We anticipate that because of the unique importance of MHC-recognition in T cell differentiation and function, the subset of MHC-specific autoreactive T cells will prove to be a particularly interesting "window" on the T cell population.

T 143 THE INFLUENCE OF THE THYMUS ON T CELL RECEPTOR EXPRESSION, Pamela Fink, Louis Matis, Simona Sorger, David McElligott and Stephen Hedrick, University of California at San Diego, La Jolla, California 92093

We have conducted an ongoing analysis of the T cell receptors used by murine helper T cells specific for pigeon cytochrome c. Our initial studies have indicated that the MHC congenic strains B10.A and B10.S(9R) use different V alpha and V beta gene elements to encode receptors expressed by T cells with very similar functional phenotypes. Since these two strains of mice have the same pool of T cell receptor gene elements, we sought an explanation for this differential expression. We are studying the receptors used by cytochrome c-specific T cells from F_1[B10.A x B10.S(9R)] →B10.A and F_1 →B10.S(9R) radiation chimeras to distinguish the influences of the imposition of self tolerance, positive selection for MHC restriction specificity, and antigen presentation on this differential strain distribution of T cell receptor gene expression. Preliminary studies using chimeric T cells indicate that the environment in which T cells differentiate does influence the T cell receptor genes they express, even among T cell populations of similar antigen specificity.

T 144 ARE T CELL RESPONSES IN THE FROG, XENOPUS, MHC RESTRICTED? Fiona Harding, David Watkins, and Nicholas Cohen, University of Rochester Medical Center, Rochester, NY 14642

The frog, *Xenopus*, is being used to investigate the ontogeny and phylogeny of the immune system. Like mammals, *Xenopus* has an MHC and T and B cells. Unlike mammals, *Xenopus* embryos and free-swimming larvae can be experimentally manipulated in a variety of immunologically relevant ways. For example, at 24 hours postfertilization, thymus/lymphocyte chimeras can be created such that hemopoietic stem cells of one MHC haplotype pass through the educating environment of the thymus of another MHC haplotype. We are currently investigating any effects that the thymus might exert on the repertoire of developing T cells in these chimeric animals (e.g., MHC restriction, tolerance). To investigate whether *Xenopus* T cells are MHC restricted, we are analyzing both alloreactive and anti-TNP T cell lines grown with homologous TCGF-rich supernatants. T cell lines generated against MHC disparate stimulators respond specifically to the immunizing haplotype in proliferative and cytotoxic *in vitro* assays. Preliminary results suggest that cell lines generated against minor histocompatibility antigens and TNP-modified self-antigens may not respond in an MHC restricted fashion. This apparent lack of MHC restricted T cell responses in this amphibian will be discussed in the context of both the phylogeny and ontogeny of MHC antigens. (Supported by USPHS grants HD-07901 and 1T32 AI 07285.)

T 145 RABBIT T-CELL RECEPTOR β-CHAIN GENES: ISOTYPES AND ALLOTYPES. N. Harindranath, M. Komatsu, E. Lamoyi, and R. Mage, LI, NIAID, NIH, Bethesda, MD 20892
Genomic DNA from two rabbits exhibited restriction fragment length polymorphism (RFLP) of Cβ on Southern analysis (RFLP patterns I and II) which correlated with sequence differences in Cβ1 exon 1. Various restriction enzyme digests of DNA from rabbit I gave three bands whereas DNA from rabbit II gave two when probed with exon 1 of Cβ. An ~14kb cloned genomic DNA fragment from rabbit I has two copies of Cβ exon 1 and an ~6kb fragment has a third suggesting that some rabbits have three different Cβ genes. Three different subclones in plasmids, that hybridized with a Cβ exon 1 probe were obtained and characterized (pBE20, pCT56 and pB65). Based on the DNA sequences of Cβ1 cDNA from rabbit II, CLANA8 and Cβ1 genomic DNA from rabbit I, pBE20 (Cβ1a and Cβ1b allotypes), oligonucleotide probes (oligos) were synthesized that recognize the sequence differences in these Cβ genes. Sequencing AluI fragments containing the allotypic regions of Cβ exon 1 from pCT56 and pB65 explained why the oligos differ in their hybridization patterns on Southern analyses. Clones pB65 and pCT56 do not react with an oligo specific for the Cβ region encoding a potential N-glycosylation site (AsnSerSer) found in Cβ1a and a published Cβ2 sequence (Marche and Kindt) but they react with an oligo specific for the SerGlySer sequence of Cβ1b (pBE20). However, they differ from pBE20 in a downstream allotypic sequence involving Ile-Val interchanges.

T 146 ANALYSIS OF DUAL REACTIVITY OF T CELL CLONES AND HYBRIDOMAS BY mAb TO THE T CELL RECEPTOR. Osami Kanagawa, Jean F. Nicolas and Andrew L. Glasebrook. Lilly Research Laboratories, La Jolla, CA 92037.
To address the question of whether MHC restricted antigen specific T cells which also exhibit cross reactivity to Mls antigen use the same or different receptors, mAbs were obtained directed to the Vβ8+$_b$T cell receptor (TcR) of a helper T lymphocyte (HTL) clone (OI6) specific to H-Y + I-A^b (H-Y) and to Mls^a. Abs with either clonotypic (Ti) or allotypic (Vβ8) specificity were each capable of inhibiting responses to both H-Y and Mls antigens suggesting that both reactivities were mediated by the same TcR. In contrast, the anti Mls response of a dual reactive T cell hybridoma constructed by fusing OI6 cells to the BW5147 thymoma was inhibited only by anti Vβ8 while the H-Y response of the hybridoma was inhibited by both anti Ti and anti Vβ8. These data are consistent with the probability that, in hybridomas, the two sets of α and β chain from OI6 and BW5147 associate randomly and that the MHC restricted response is mediated only by the combination of OI6 α and β chain which is recognized by the anti Ti while the Mls response can be mediated by TcR which consists of OI6 Vβ8 chain associated with either OI6 α or BW5147 α chain. This possibility is supported by the fact that hybridoma variants which respond to Mls but not to H-Y, express the Vβ8 but not the Ti determinant. These results suggest that, in dual reactive T cell clones but not in hybridomas, one receptor can mediate both MHC restricted and Mls responses and that, in the present case, MLS specificity may be determined by the β chain of the TcR.

T 147 CELL TYPE SPECIFIC REGULATION OF THE T CELL RECEPTOR β CHAIN. M. Kearns and K. Kelly, Immunology Branch, NCI NIH, Bethesda, Md. 20892
The genetic regulatory mechanisms that govern tissue specific expression of the T cell receptor β chain have been investigated utilizing an in vitro model of cell type specificity. A transient expression system has been used to assay the transcription of a genomic TCR β chain gene (including 5 kb 5' of a rearranged $V_{\beta 1}$-J-C clone) in T cells, fibroblasts, and a variety of hematopoietic tumor cells. DNA sequencing of the $V_{\beta 1}$ leader and an additional 400 bp 5', in conjunction with Sl nuclease protection assays, has identified the start site of transcription. Also, a putative regulatory hexamer, CTTTCT, that is conserved in several human and murine V_{β} genes has been identified approximately 250 bp 5' to the mRNA cap site. Transfection efficiencies were normalized by determining the mRNA levels of a truncated histone gene contained within the plasmid vector as a tissue nonspecific control. Unlike immunoglobulin genes, expression of a rearranged $C_{\beta 1}$ gene was found in nonlymphoid cells. However, it was observed that T cells show a minimum of a 3 fold preferential expression of the β chain as compared to fibroblasts and monocytic cells. Furthermore, a 1.5 kb deletion in the 5' region of the $J_{\beta 1}$-$C_{\beta 1}$ intron does not preclude β chain expression in T cells or fibroblasts. Tissue specific regulation of T cell receptor β chain expression does not appear to require gene sequences within this region of the intron, but may be influenced by a conserved region of the gene 5' to the promotor.

T 148 CHROMOSOMAL ORGANIZATION OF THE MURINE T CELL RECEPTOR BETA CHAIN LOCUS, Nadine E. Lee, Tullia Linsten, and Mark M. Davis, Stanford University, Stanford, CA 94305. A panel of AKR thymic leukemia DNAs was screened with probes for 10 of the 16 known T cell receptor beta chain variable region families. In tumors which have rearranged both chromosomes as VDJ events, there are deletions of some of the variable regions, allowing a deletion order to be established. This order is (5') E1--Vβ10--86T1--TB21 and C5 families--LB2--SJL73--2B4--constant region locus--Vβ14(3'). The members of the TB21 and C5 families are interspersed at approximately 3 kb intervals. Pulsed field gel electrophoretic analysis shows that the deletion order corresponds to the physical arrangement on the chromosome, establishes that Vβ2B4 (the most proximal of this panel of Vβ genes) is located approximately 300 kb 5' to the constant region, and indicates that other variable regions (such as E1) are as much as 500 kb 5' of Vβ2B4. The deletion of variable regions in some strains of mice (e.g. SJL) is shown to be internal to the Vβ encoding region and to involve between 100 and 200 kb. These data also indicate that Vβ14 (localized by Malissen et al.) is an exception in being 3' to the constant region and that the primary mechanism of VDJ rearrangement in the beta chain locus is consistent with looping out and excision of the intervening DNA rather than inversion.

T 149 ISOLATION OF ANTI-IDIOTYPIC ANTIBODIES TO T CELLS USING AN ANTI-FRAMEWORK DETERMINANT ANTIBODY, Holden T. Maecker, Kiyoshi Kitamura, Michael B. Brenner, and Ronald Levy, Stanford University Medical Center, Stanford, CA 94305
A novel method for isolating anti-idiotypic antibodies to T cells was developed using an enzyme-linked immunoadsorption assay. The βF1 monoclonal antibody, directed at a common determinant on the human T cell antigen receptor, was used to specifically capture receptor molecules from a cell lysate preparation. Hybridoma supernatants were then tested for their ability to bind the receptor thus captured. The supernatants could also be used to capture receptor molecules from cell lysates themselves, with detection being done using enzyme-labelled βF1. Such assays were used to identify a panel of four anti-idiotypic antibodies to the T cell line HPB-ALL, all of which were negative on all irrelevent cell lines tested and on normal human peripheral blood lymphocytes. The antibodies were shown to immunoprecipitate an identical disulfide-linked heterodimer from HPB-ALL cells as did βF1 and T40/25, a known anti-idiotype to HPB-ALL. Each of these antibodies inhibited the binding of T40/25 and variably inhibited or enhanced the binding of each other and of anti-Leu 4. This suggests that they recognize distinct but associated idiotypic determinants. Two of these antibodies define sub-populations of HPB-ALL cells and receptor molecules, which may vary in the extent of α chain glycosylation. Further study of this phenomenon is underway. Also, the isolation of such anti-idiotypic antibodies to any particular T cell line or tumor promises to be useful for biological studies of T cell malignancy in humans.

T 150 TRANSFECTION AND EXPRESSION OF THE GENES CODING FOR THE Lyt-2 POLYPEPTIDE AND THE α- AND β-CHAINS OF THE T-CELL RECEPTOR. Bernard Malissen, Jean Gabert, Anne-Marie Schmitt-Verhulst, Janes Parnes and Isabelle Hue. Centre d'Immunologie de Marseille-Luminy, Case 906, 13288 Marseille cédex 9, France and Department of Medicine, Stanford University School of Medicine, Stanford, CA 94305, USA.
The recent characterization of the genes encoding the Lyt-2 polypeptide and the α- and β-chains of the T-cell receptor offers a direct may to assess by DNA-mediated gene transfer their respective role in MHC class I T cell recognition. In order to reach such a goal, we have first isolated the complete rearranged genes coding for the α- and the β-chain of the KB5-C20 alloreactive cytolytic T cell clone. KB5-C20 recognizes the K^b molecule and is susceptible to inhibition by anti-Lyt-2 monoclonal antibodies. The α and β genes were subsequently transfected and expressed into DO11.10, an Ia-restricted and L3T4 positive T cell hybridoma kindly provided to us by P. Marrack. Expression of the KB5-C20 α/β chains at the surface of the DO11.10 transfected cells was monitored with an anti-clonotypic monoclonal antibody. Functional analysis did not show any IL-2 production when the α/β positive transfectants were stimulated with K^b positive cells. Because such failure to specifically trigger IL-2 production may results from the absence of the Lyt-2 polypeptide. We are presently transfecting the α and β chain genes together with the Lyt-2 gene. Functional data obtained with this combination of genes will be presented.

T 151 T CELL RECEPTOR BETA-CHAIN VARIABLE REGION GENES: FREQUENCY OF USE IN THYMUS AND PERIPHERAL LYMPHOID TISSUE, Craig Y. Okada, Howard Gershenfeld, Richard K. Barth*, Leroy Hood*, and Irving L. Weissman, Dept. of Pathology, Stanford Medical School, Palo Alto, CA 94304; *Division of Biology, California Institute of Technology, Pasadena, CA 91125.

One component in the diversification of the T cell antigen receptor is the use of multiple gene segments to encode the TCR alpha- and beta- chains. The beta-chain repertoire is derived from a relatively limited number of variable gene segments. Twenty different V-beta gene segments belonging to 16 different subfamilies have been reported.

To examine the usage of different V-beta gene segments during T cell differentiation, we have analyzed from several different mouse strains the relative abundance of different V-beta chain mRNAs in the thymus and peripheral lymphoid tissues. Using quantitative Northerns and a highly sensitive ribonuclease protection assay we have measured the mRNA levels for 10 different V-beta gene segments. Our results indicate that not all V-beta regions are used at equal frequencies either in thymocytes or peripheral lymphocytes. In addition, differences in the relative amounts of the V-beta chain mRNAs between thymocytes and peripheral T cells are identified. We are also investigating changes in the levels of V-beta mRNA in peripheral lymphocytes caused by antigen stimulation.

T 152 RECOMBINATION WITHIN THE HUMAN T CELL RECEPTOR ALPHA CHAIN GENE COMPLEX. Mary Ann Robinson and Thomas J. Kindt, Laboratory of Immunogenetics, NIAID, NIH, Bethesda, MD 20892

The gene complex encoding the α chain of the human T cell antigen receptor (TCR) contains numerous polymorphisms detected by Southern blot analyses with probes corresponding to C and V region gene segments. Restriction fragment length polymorphisms (RFLP) of TCR α C region genes were detected in genomic DNA samples digested with BglII, TaqI or MspI. Three polymorphisms in V region genes were detected with probes derived from the V region of human and rabbit TCRα cDNA clones. Analysis of the segregation of TCRα gene RFLP in seven families made it possible to assess linkage of V and C region genes and to characterize haplotypes by examination of the combination of allelic RFLP forms present. For the majority of markers studied one of the observed allelic forms predominated in the parents of families tested; sufficient heterozygosity was present, however, to assign TCRα haplotypes to all family members. The occurrence of 14 different patterns of the association of C and V region polymorphisms in the parental haplotypes tested suggests that recombination is frequent within this gene complex. Furthermore, one child in each of two families was identified who most likely inherited TCRα haplotypes in which crossover events had occurred between V and C region markers. The present results suggest that evaluation of disease associations with TCRα genes will require testing for multiple TCRα markers.

T 153 THE MOST PRIMITIVE VERTEBRATE GENOMES HAVE SEQUENCES RELATED TO MAMMALIAN T-CELL RECEPTOR GENES.
Maryellen Ruvolo and Allan M. Maxam. Dept. of Biological Chemistry, Harvard Medical School; Laboratory of Molecular Biology, Dana-Farber Cancer Institute, Boston, MA 02115 USA.

Because T and B lymphocytes and the lymphoid organs which produce them are seen only in vertebrates, it is likely that distinct T-cell receptor and immunoglobulin gene systems first appeared in the primitive vertebrates, 400 million years ago. Using low stringency Southern blots with cloned mouse T-cell receptor alpha, beta, and gamma chain cDNA probes, we have detected homologous sequences in different vertebrate species. Under conditions allowing detection of sequences as little as 65% homologous, we found:

1. The T-cell receptor alpha chain constant region gene cross-hybridizes with sequences in the most primitively-derived vertebrate, the Pacific hagfish (Eptatratus stoutii) and in representatives of more recently-derived vertebrate groups (fish, amphibians, birds and mammals).
2. The gamma chain constant region gene cross-hybridizes as far back phylogenetically as fish.
3. The ß chain constant region gene has homologous sequences as far back phylogenetically as amphibians (Xenopus).
4. One copy of the beta and the gamma constant region genes are present in fish and amphibians, in contrast to multiple copies in mammals.

Judging by the presence of cross-hybridizing sequences under equally low stringency conditions, the T-cell receptor alpha chain constant region gene has been most conserved over evolutionary time, beta has been least conserved, and gamma has been intermediately conserved.

The T Cell Receptor

T 154 The Relationship of T Cell Receptor Gene Polymorphisms to Systemic Rheumatic Diseases.
B. P. Sanders, M.D.*, B. Kotzin, M.D.+, and E. Palmer*, M.D., Ph.D. *National Jewish Center and +V.A. Medical Center and Univ. Col. Health Science Center, Denver, Colorado.

We have studied restriction fragment length polymorphisms (RFLPS) of T cell receptor genes in man to determine if a genetic linkage exists between T cell receptor genes and systemic lupus erythematous (SLE) and rheumatoid arthritis (RA). Thirty-one SLE, 43 RA patients and 44 healthy subjects were studied. Genomic DNA was obtained from peripheral WBCs and examined by Southern analysis using the restriction enzymes of Bgl2, Hind 3 and EcoRI and two constant region probes, one for the alpha chain and beta chain. These DNAs were screened for deletions in the B chain gene complex. No deletions were found in the 254 chromosomes studied. Next, the known Bg12 RFLPs that occur in the alpha and beta chain constant regions were studied to determine if a difference in the frequency of these alleles exists between the disease and normal populations. In the healthy population, the frequency of alleles were for alpha chain, 96% 3.2 Kb allele, 4% 2.9 Kb allele, and for B chain, 57% 10.0 Kb, 43% 9.2 Kb allele. The SLE population frequency of alleles for the alpha chain is (92% 3.2 Kb allele, 8% 2.9 Kb allele) and for the B chain is (50% 10.0 Kb allele and 50% 9.2 Kb allele). The RA population frequency of alleles for the alpha chain is (93% 3.2 Kb allele, 7% 2.9 Kb allele) and for the B chain is (58.5% 10 Kb allele and 43.5% 9.2 Kb allele). No significant difference exists in the frequency of alleles between the healthy population and SLE or RA population. We conclude that RFLPs in the constant region of the T cell receptor alpha and beta chains are not linked with SLE or RA.

Thymic Differentiation

T 200 ROLE OF THE EPITHELIUM-ASSOCIATED THYMOCYTE SUBSET - A COMPLETE PATHWAY OF T CELL DEFFERENTIATION.
Willi Born, Judi Pasternak, Andrew Farr, John Kappler and Philippa Marrack, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO

Intrathymic lymphostromal interactions are known to influence thymocyte maturation and the peripheral repertoire of T cell receptors. In an attempt to dissect further the role of the thymic epithelium in thymocyte differentiation we have studied the subset of thymocytes associated with "Thymic Nurse Cells" (TNC-T). Highly purified TNC-T (approx. 1% of thymocytes) were found to be a heterogeneous cell population. Compared to PNA^+ cortical thymocytes TNC-T were enriched in most immature subsets ($IL\text{-}2R^+$), but also in most mature stages (high surface levels of T cell receptor for antigen). Moreover, T cell receptor expression was apparently not required to initiate the lymphostromal complexes, nor for the appearance of early, TcR^{low} subsets of TNC-T, but necessary for the appearance of late TcR^{high} subsets. Our ultrastructural data and previous observations by others indicated an increased mitotic activity in TNC-T compared to most cortical and medullary thymocytes. We interpret these data to suggest that TNC-T might represent an entire pathway of thymic differentiation rather than a distinct developmental stage.

T 201 BSF1 STIMULATES IMMATURE MOUSE THYMOCYTES TO BECOME CYTOTOXIC T LYMPHOCYTES,
Priscilla A. Campbell, Janet M. Collins and Louis B. Justement, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO 80206

Immature mouse thymocytes, isolated as cells not agglutinated by the sialic acid-specific lectin, lobster agglutinin 1 (LAg1), do not become cytotoxic T lymphocytes (CTL) when stimulated polyclonally with Con A + recombinant interleukin-2 (IL-2). These cells do become CTL when stimulated with Con A + the supernatant of rat or mouse spleen cells cultured with Con A for 24 hours (SUP). Experiments showed that IL-1, IL-2, IL-3, and IFNY given alone or in combination, could not replace the maturational activity in SUP. However, supernatants of the D9C1.12.17 T cell hybridoma, which secretes high amounts of BSF1, could replace the maturational activity in SUP. Further experiments showed that a monoclonal antibody to BSF1 inhibited the activity of the D9C1.12.17 supernatant, and also inhibited the maturational activity of BSF1 purified from this hybridoma supernatant. The D9C1.12.17 supernatant and the purified material had high amounts of BSF1 activity measured as the ability to stimulate B cells to express Ia antigens. These experiments suggest that BSF1, perhaps acting with IL-2 and Con A, can stimulate immature mouse thymocytes to become CTL.

T 202 FUNCTIONAL CHARACTERIZATION OF SUBSETS OF ADULT INTRATHYMIC PRECURSOR CELLS,
I. Nicholas Crispe, Richard P. Shimonkevitz, Linda A. Husmann and Michael J. Bevan, Research Institute of Scripps Clinic, La Jolla CA 92037.
Thymocytes with the surface phenotype Lyt-2$^-$, L3T4$^-$ ("double negative," DN cells) stain bimodally with a monoclonal antibody, J11d, which also recognizes all cortical-type (Lyt-2$^+$, L3T4$^+$) thymocytes. The J11d$^+$ and J11d$^-$ subsets of DN were separated by cell sorting and characterized functionally. J11d$^+$ DN injected intrathymically could repopulate the irradiated thymus of a Thy-1 congenic host and seed the periphery with T cells. In contrast, the progeny of injected J11d$^-$ DN were undetectable. In fetal thymus organ culture, J11d$^+$ DN diversified in deoxyguanosine-depleted thymus lobes to form cells with Lyt-2$^+$, L3T4$^+$, Lyt-2$^+$, L3T4$^-$, and Lyt-2$^-$, L3T4$^+$ phenotypes. The J11d$^-$ DN cells were able to recolonize the deoxyguanosine-depleted fetal thymus, but most of the cells retained the DN phenotype. A small proportion of cells in such recolonized thymus lobes expressed either Lyt-2 or L3T4. However, Lyt-2$^+$, L3T4$^+$ cells were strikingly absent. Thus, DN cells with full proliferative and differentiative potential are concentrated in the J11d$^+$ DN subset.

T 203 ROLE OF LYT-2 AND L3T4 IN T CELL ACTIVATION AND REPERTOIRE SELECTION,
Klaus Eichmann, Jan Jönsson, Ingrid Falk, and Frank Emmrich, Max-Planck-Institut für Immunbiologie, D-7800 Freiburg, FRG.
Small resting splenic T lymphocytes can be activated to express IL-2 receptors and to proliferate in the presence of IL-2 by sub-mitogenic concentrations of anti-T-cell receptor (TCR) antibodies (F23.1, KJ16-133) in combination with anti-Lyt-2 or anti-L3T4 monoclonal antibodies. Activation is achieved by various protocols of crosslinking anti-TCR antibodies to anti-Lyt-2 or anti-L3T4, but not if only one of the two types of antibodies is crosslinked and the other is applied in soluble form. In contrast, activation by crosslinked antibodies is inhibited by either one or by both soluble antibodies. Depending on the subset-specific antibody used, either only Lyt-2$^+$ or L3T4$^+$ T cells are induced to proliferate. Lyt-2$^+$ T cells are induced to become functionally active CTL. Antibodies to other T cell surface antigens may also show some activity but their synergistic effects with anti-TCR are usually at least 10 fold less than that with anti-Lyt-2 or anti-L3T4.
We propose that, upon physiological T cell activation, similar crosslinking is achieved by simultaneous binding of the T cell receptor and Lyt-2 (L3T4) to MHC class I (class II) molecules on stimulator cells. In allo-MHC-antigen recognition, a tertiary complex is formed between TCR, Lyt-2 (L3T4) and MHC. In MHC restricted recognition, a quarternary complex is formed between TCR and Lyt-2 (L3T4) on the T lymphocyte and MHC and presented antigen on the stimulator cell. In addition, we suggest that analogous crosslinking mechanisms may play a role in the selection of the MHC class I and class II restricted repertoires of Lyt-2$^+$ and L3T4$^+$ T cells, respectively, during ontogeny.

T 204 A LOW DENSITY FRACTION OF BONE MARROW ENRICHED IN PROTHYMOCYTE ACTIVITY,
Sophie Ezine, INSERM U 25, Hôpital Necker, 75743 Paris Cedex 15, France.
Thymocytes develop from a committed haematopoietic progenitor present in the bone marrow. These prothymocytes are capable of restoring the thymus of a lethally irradiated host permanently when injected intraveinously. Purification of these precursors has been rendered difficult by the fact that these cells do not express any known marker and their low representation (less than 1%) in the bone marrow.
Using a discontinuous Ficoll gradient, we have selected a low density fraction enriched in prothymocyte activity : 2.5×10^5 cells of this layer can repopulate more than 50% of the thymus of an irradiated host, 4 weeks later, against 3% with total bone marrow. This fraction contains also the precursors for the CFU-S and GM-CFC. Using the *in vitro* limiting dilution technique we are now looking for the prothymocyte frequency in this enriched population.

T 205 EXPRESSION OF TWO DISTINCT TCR STRUCTURES ON Lyt2$^-$,L3T4$^-$ ADULT THYMOCYTES. B.J. Fowlkes, Jeffrey Bluestone, Ada Kruisbeek, John Coligan, Ronald Schwartz and Drew Pardoll. LMI, LI, LIG, NIAID; BRMP, IB, NCI; National Institutes of Health, Bethesda, MD. 20892.

We have previously described a minor subset of adult murine thymocytes (3-5%) which express no Lyt2 or L3T4 and low levels of Lyl (dLyl). This population was isolated and characterized and demonstrated to contain the precursors of the major subpopulations of thymocytes (Lyt2$^+$,L3T4$^+$; Lyt2$^-$,L3T4$^+$; Lyt2$^+$,L3T4$^-$). Here we further analyze the double negative (Lyt2$^-$,L3T4$^-$) subpopulation. Adult Lyt2$^-$,L3T4$^-$ thymocytes (4-6% of total thymocytes) can be subdivided into three subsets based on expression of T cell antigen receptor (TCR): the dLyl cells which express no surface TCR complex, those which express T3/TCRγδ, and those which express T3/TCRαβ. These cell types have been phenotypically characterized by analyses for coordinate expression of T3, Vβ8, Lyl, and IL-2R; biochemically analyzed by immunoprecipitation of T3, TCRα, TCRβ, TCRγ, and Vβ8; and examined for TCRα, β and γ specific mRNA expression by _in situ_ hybridization. Data will be presented on the _in vitro_ growth requirements and the _ability_ to stimulate proliferation and/or _differentiation_ of these cells through the T3/TCR complex.

T 206 Characterization of Thy-1+, Ly-2-, L3T4- thymocyte subpopulations. W.C. Gause, A.D. Steinberg, F.D. Finkelman, and J.D. Mountz. National Institutes of Health. Bethesda, MD 20892.

Ly-2-, L3T4- thymocytes include progenitors for more mature T cell populations and probably recent prothymocyte immigrants from the bone marrow. We have analyzed this population by flow microfluorometry and _in situ_ hybridization, after killing single and double positive cells with anti-L3T4 and anti-Ly-2 + C. By not killing with anti-ly-1, we have included Ly-1 bright double negative cells in our study. We first examined Ly-24 and 6B2 (anti-B220) as possible prothymocyte markers, since both of these antigens are expressed at high levels in bone marrow and low levels in unseparated thymocytes. 30% of double negative thymocytes were Ly-24+ and 5% 6B2+. Most of the dull Thy-1+ cells were F23.1-, Ly-24+, a phenotype consistent with a bone marrow T cell precursor. In contrast, all of the F23.1+ cells were bright Thy-1+ and bright Ly-1+. Double labelling studies suggest a pathway of differentiation of dull Ly-1, Ly-24- thymocytes (52% of thymocytes) distinct from an Ly-24+ pathway. _In situ_ hybridization revealed that 8% of thymocytes expressed high c-_myb_, whereas 43% of double negative cells expressed c-_myb_ mRNA, an oncogene primarily in normal bone marrow and thymus. Examination of T cell receptor expression revealed that 5% of thymocytes but 54% of double negative cells expressed T cell gamma message, while 36% of thymocytes and 57% of double negative cells expressed T cell receptor beta message. These studies thus define subsets of double negative thymocytes and provide a basis for studies of differentiation of these cells as well as sequential gene expression.

T 207 THE DIFFERENTIATION POTENTIAL OF L3T4$^+$, LYT-2$^+$ THYMOCYTES, Linda A. Husmann, I. Nicholas Crispe, Richard P. Shimonkevitz and Michael J. Bevan, Research Institute of Scripps Clinic, La Jolla, CA 92037.

Four major categories of murine thymocytes have been identified based on Lyt-2 and L3T4 surface markers. The most frequent thymocytes, constituting 85-90% of the adult mouse thymus, are positive for both of these markers (double positive or DP cells). These cells accumulate in the thymic cortex, and some express a T cell antigen receptor complex. Unlike thymocytes negative for both markers, DP cells are unable to recolonize the irradiated thymus after intravenous cell transfer. One hypothesis is that they are intermediates in the thymocyte differentiation pathway. However, most DP cells die within a few days, both _in vivo_ and in culture. To try to identify a subpopulation of DP thymocytes that is a differentiation intermediate, we have made DP-enriched populations (either by sequential panning on antibody-coated plates or by fluorescence-activated cell sorting) and injected them directly into the thymuses of Thy-1 congenic hosts. At various time points, host thymus, lymph nodes, and spleen have been examined for cells of donor origin. Preliminary evidence indicates that DP thymocytes contain no precursors of mature T cells.

T 208 A NOVEL ANTIGEN EXPRESSED ON MURINE THYMOCYTES AND CLONED NK CELLS. *Kyogo Itoh, *Constantin G. Ioannides, †Ryuji Suzuki, *Chris D. Platsoucas, and *Charles M. Balch, *M.D. Anderson Hospital, Houston, TX 77030 and †Tohoku Univ. Sendai, Japan.
To identify lymphocyte surface molecules expressed on interleukin 2 (IL2)-activated cells, we utilized an IL2-dependent murine cloned natural killer (NK) cell line ($ASGM_1^+$, Ly-5.1$^+$, Thy-1.2^{dim+}, Lyt-1.1$^-$ and Lyt-2.1$^-$) as immunogen for monoclonal antibody (mAb) development. A rat IgG mAb named B4813-7 (B48) was developed by fusing X63.653.Ag8 cells and lymph node cells from rats immunized with the cloned NK cells. The B48 mAb immunoprecipitated from ^{125}I-labeled cloned NK cells a 110 kd molecule under non-reducing conditions and 47 kd and 38 kd polypeptide chains under reducing conditions. The B48 mAb also immunoprecipitated from ^{125}I-labeled thymocytes from 4 wk old mice a 106 kd molecule under non-reducing conditions, and a 36 kd polypeptide chain under reducing conditions. Immunofluorescence analysis using $F(ab)'_2$ fragments of the B48 mAb and FITC-conjugated $F(ab)'_2$ fragments of goat anti-rat IgG revealed that both murine thymocytes and ConA-activated splenic T lymphocytes expressed the antigen recognized by the B48 mAb. Among adult thymocytes, large size cells (10-20% of total thymocytes) were stained brightly by the B48 mAb. Thymocytes of smaller size (80-90% of the total) were dimly stained by the B48 mAb. In contrast, the antigen recognized by the B48 mAb was not detected on the surface of freshly prepared NK cells or T lymphocytes from spleen, lymph nodes or peripheral blood.

T 209 PHENOTYPIC ANALYSIS OF HUMAN LYMPHOKINE-ACTIVATED KILLER CELL CLONES. Eric O. Long, Roberto Biassoni, Alessandro Moretta and Silvano Ferrini, LIG, NIAID, NIH, Bethesda, MD 20892; Ludwig Institute for Cancer Research, Lausanne, Switzerland and IST, Genova, Italy.
Human lymphokine-activated killer (LAK) cells were derived from normal peripheral blood lymphocytes. LAK activity, as defined by lysis of fresh tumor cells, was present in both T11$^+$ and T11$^-$ cells separated by E-rosette formation. T11$^+$ cells were cloned under limiting dilution conditions with 1% PHA, 10^5 irradiated feeder cells and 50 units/ml of recombinant IL-2 added at weekly intervals. Most of the clones exhibiting LAK activity were of T3$^-$T4$^-$T8$^-$ phenotype whereas a few clones exhibited the T3$^+$T4$^-$T8$^+$ phenotype (Ferrini et al., Int. J. Cancer, in press). Unlike MHC-restricted effector cells most LAK cells belong to the double-negative T4$^-$T8$^-$ subset and may thus be derived from immature thymic precursors. To test what stage of differentiation these cells might represent and what genes might be expressed in these cells we are currently analyzing RNA transcripts with DNA probes for the various chains of the T cell receptor, for components of the T3 complex and for other T cell differentiation antigens.

T 210 INDUCIBILITY OF INTERLEUKIN-2 RNA EXPRESSION IN INDIVIDUAL T LYMPHOCYTES FROM THYMUS AND SPLEEN, Kathleen L. McGuire and Ellen V. Rothenberg, Division of Biology, California Institute of Technology, Pasadena, CA 91125.
Expression of the gene for the T cell growth hormone, interleukin-2 (IL2), is subject to at least two kinds of control. It is not only tissue specific, i.e., restricted to T lymphocytes, but also strictly dependent upon activation of the producing T cell. In mature cells, IL2 production is usually triggered via the cell surface receptor for antigen. To study the regulation of the murine IL2 gene in T-cell populations differing in their state of maturation, we have used a calcium ionophore in conjunction with the phorbol ester, TPA, to stimulate IL2 gene transcription while bypassing the requirement for triggering through a mature cell surface receptor. RNA probe protection and *in situ* hybridization analyses have allowed us to quantitate accumulated cytoplasmic IL2 RNA as well as to enumerate the cells capable of inducing the IL2 gene in mature, immature, and precursor (i.e., double negative thymocyte) T-cell populations. We will report evidence for a molecular distinction between the IL2 induction responses of different T cells, according to their degree of maturity and to their functional subclass. Mature splenic T cells of the L3T4$^+$ phenotype are characterized by accumulation of IL2 transcripts to over 100 copies per producing cell. However, we find that many T-lineage cells, especially in immature populations, show induction-dependent IL2 gene expression but only accumulate low levels of IL2 mRNA per cell. Additionally, we will report evidence that many of the thymocytes capable of accumulating low levels of IL2 RNA are of the L3T4$^+$ Lyt-2$^+$, or double positive, phenotype despite the previously reported inability of these cells to secrete IL2 in response to the appropriate stimuli.

T 211 REARRANGEMENT AND EXPRESSION OF T CELL ANTIGEN RECEPTOR GENES IN SUBSETS OF EARLY MURINE THYMOCYTES. Martin Pearse, Ken Shortman & Roland Scollay, The Walter & Eliza Hall Institute of Medical Research, Melbourne, Victoria 3050, Australia.

A group of Ly 2^- L3T4$^-$ thymocytes which represent 4% of lymphoid cells in the adult murine thymus has been identified as early precursor cells. This group, however, has been shown to be heterogeneous by a series of six other surface markers and seems to form two groups, those of medullary phenotype tendency Ly 1↑ Thy 1↓ B2A2↓) and those of a cortical phenotype tendency (Ly 1↓ Thy 1↑ B2A2↑). Many molecular studies to date have omitted the former group, absent from embryonic thymus and depleted in selections for Ly 1-dull cells. Since many of these are surface positive for the β-chain specific monoclonal F23.1, their receptor gene status is of particular interest. The degree of T cell receptor gene rearrangements within this group and subsets of this group is currently being studied. In situ hybridization on cell smears is being used to access the level of T cell receptor gene expression in early subsets. To date α-chain expression can readily be detected in EL-4, a thymoma cell line and in adult thymocytes using a ^{35}S-RNA probe prepared from a cDNA clone (Pα∂) which contains VJC. The other chains are being tested. The aim of current studies is to access which, if any, subgroups of Ly 2^-, L3T4$^-$ thymocytes express an effective T cell receptor. This should help determine the exact point at which selection of the specificity repertoire would commence and help access whether Ly 2 and L3T4 need be co-expressed for this to occur.

T 212 PHORBOL ESTER INDUCED MODULATION OF Lyt-2 and L3T4 ANTIGENS ON MURINE LYMPHOMA CELL LINES, E.R. Richie, B.B. McEntire, S. Helfritz and J. Phillips, Univ. of Texas Sys. Cancer Ctr., Science Park-Res. Div., Smithville, TX 78957.

The precise lineage relationships among thymocyte subsets defined on the basis of Lyt-2 and L3T4 differentiation antigen expression are not completely resolved. Cells that do not express either antigen are precursors of cells with a single positive phenotype. However, it is not clear if the major population of Lyt-2^+, L3T4$^+$ cortical cells (double positive) is an intermediary in this process, or a separate, and perhaps, nonfunctional lineage. To study alterations in differentiation antigen expression induced by protein kinase C activation on clonal T-cell populations of Lyt-2^+, L3T4$^+$ cells, we established tissue culture lines from AKR/J thymic lymphomas. Incubation of two double positive cell lines in the presence of 0.01 μM phorbol ester for 72 hr, resulted in loss of both Lyt-2 and L3T4 cell surface antigen expression as detected using an indirect immunofluorescence assay and flow cytometry. Other antigens such as Thy, H-2 and Lyt-1 showed unchanged or enhanced expression indicating that the loss of Lyt-2 and L3T4 antigens was a selective event. L3T4 antigen expression was diminished as early as 1 hr after incubation with phorbol ester, whereas Lyt-2 antigen expression was not significantly altered until 24-48 hr after exposure to phorbol ester. Cells exposed to phorbol ester for 72 hr, washed and recultured, demonstrated a double positive phenotype, similar to untreated controls after an additional 72 hr of culture. The selective modulation of Lyt-2 and L3T4 antigens by activation signals presented during intrathymic residence may be a physiologically significant event during differentiation of the double positive subset.

T 213 ONTOGENY OF INTERLEUKIN-2 RECEPTOR INDUCIBILITY IN THE MURINE THYMUS, Ellen V. Rothenberg, Paul D. Boyer, James P. Lugo, and Rochelle A. Diamond, Division of Biology, California Institute of Technology, Pasadena, CA 91125.

T-cell precursors differentiating in the thymus not only acquire antigen recognition structures but also become programmed for inducible expression of specific "response" genes. We have examined the ontogeny of the interleukin-2 (IL2) receptor expression response in thymic lymphocytes, focussing on two questions. The first is to what extent IL2 receptor expression is correlated with mitogenic activation in different populations of immature T cells. We have previously reported that most dividing cells in the adolescent mouse thymus fail to express IL2 receptors. The cells that do express IL2 receptors fall mainly within the immature Lyt-2^- L3T4$^-$ and Lyt-2^{low} L3T4$^-$ populations. Even in these populations, receptor expression is not correlated with proliferation; acridine orange staining shows that it is only randomly associated with cycling. Thus, intrathymic mitogenic signals do not necessarily activate IL2 receptor expression, and are not required to maintain existing IL2 receptor expression. Secondly, we have asked when in development IL2 receptor expression becomes inducible, as in mature T cells. Most or all thymocytes of mature phenotype, and most immature double-negative thymocytes that are not already expressing IL2 receptors, can be induced to express these receptors *in vitro* within 24 hr of stimulation with calcium ionophores and phorbol esters. Thus, IL2 receptors may become inducible early in the differentiation of all T-cell precursors. Unlike both immature double-negative cells and mature T cells, however, the "common" (double positive) cortical thymocytes seem uniquely unable to express IL2 receptors in response to induction. We are currently investigating the basis of this lesion.

T 214 KINETIC ANALYSIS OF THYMOCYTE SUBPOPULATIONS, INCLUDING SUBSETS OF DOUBLE NEGATIVE EARLY PRECURSORS. Roland Scollay, Mark Egerton & Ken Shortman. The Walter & Eliza Hall Institute of Medical Research, Melbourne, Victoria 3050, Australia.

Studies on T cell development which go beyond static analysis to real precursor/product experiments are relatively rare, but are essential if we are to understand the relative role of thymocyte subsets and the cortex and medulla. We have used a cell kinetic approach and have looked at the cell cycle status and turnover rates of the major thymocyte subpopulations (Ly 2^- $L3T4^-$, Ly 2^+ $L3T4^+$, Ly 2^- $L3T4^+$ and Ly 2^+ $L3T4^-$) and of a number of putatively very early subsets of the Ly 2^- $L3T4^-$ thymocytes. Under semi-continuous ^{3}HTdR labeling conditions, the Ly 2^+ $L3T4^+$ cells, which make up the majority (95%) of cortical cells, very rapidly (2-3 days) reach saturation labeling of almost 90%, while the single positive "medullary" populations accumulate label very slowly, reaching only 25% after 4 days. Amongst the double negatives, several subgroups are turning over slowly, while cells in the largest group (the dull Ly 1, high Thy 1, high B2A2 subset) are dividing rapidly, with the majority disappearing immediately, presumably becoming double positive. The cells leaving the thymus (emigrants) accumulate label very slowly, showing that they cannot be the progeny of the typical rapidly labeling cortical subpopulation. The implications of these data for intrathymic development will be discussed.

T 215 PHENOTYPIC ANALYSIS OF EXPRESSION OF CELL SURFACE ANTIGENS AND T CELL RECEPTOR COMPLEX IN MURINE FETAL THYMUS ORGAN CULTURE, S. O. Sharrow, D. A. Stephany, J. A. Bluestone, D. H. Sachs and D. DeLuca, NIH, Bethesda MD 20892 and Univ. of S. C. 29425.

The development of T cell subpopulations was analyzed in murine fetal thymus organ culture by immunofluorescence and multi-color flow cytometry. Thymic lobes from 14 or 15 day fetuses cultured in vitro for 5 days were relatively enriched for $Lyt2^+$,$L3T4^+$ cells (65%) and cells bearing low amounts of T cell receptor (T3 dull = 40%), but also contained $Lyt2^+$ and $L3T4^+$ single positives (20%), as well as 10-15% T3 bright cells. In contrast, by 12 days of culture, fetal thymuses were: a) enriched for $Lyt2^+$ and $L3T4^+$ single positives (50%); b) contained cells bearing cell-surface V β 8 (KJ16=10%); c) displayed strikingly high frequencies of T3 bright cells (75-85%); and d) were enriched for an unusual $Lyt2^-$, $L3T4^-$ cell which expressed high levels of Ly1 and T3. Also evaluated was the expression of a Class I MHC antigen, Qa-2, which is present on all peripheral T cells and on functional thymocytes. It was found that while negative on cells from 5 day cultures, Qa-2 was expressed on 50% of cells from 12 day cultures. It was also determined that both in normal thymocytes and in fetal thymus organ culture, Qa-2 expression defines two subpopulations ($Qa\text{-}2^+$ and $Qa\text{-}2^-$) in single positive ($Lyt2^+$ or $L3T4^+$) thymocytes as well as in double negative ($Lyt2^-$,$L3T4^-$) thymocytes. These studies show that fetal thymus organ cultures not only allow development of mature T cells, as defined by surface phenotype and receptor expression, but also demonstrate the enrichment in these cultures of a novel cell which bears surface receptor as defined by T3 (T3 high, $Lyt2^-$,$L3T4^-$, Ly1 bright, $Qa\text{-}2^+$). This cell may either be terminally differentiated or a precursor expressing surface receptor.

T 216 MAJOR HISTOCOMPATIBILITY COMPLEX TOLERANCE INDUCTION BY MURINE THYMOCYTES, Richard P. Shimonkevitz and Michael J. Bevan, Research Institute of Scripps Clinic, La Jolla CA 92037.

T lymphocytes acquire the ability to discriminate self versus non-self in the thymus. While major histocompatibility complex (MHC) class II self-tolerance and restriction of antigen reactivity appear influenced by thymic bone marrow-derived cells and stromal cells, class I tolerance and antigen reactivity remain less well characterized. Since class I MHC antigen-bearing T cells can "veto" specific recognition by allogeneic cytolytic T lymphocyte precursors, one possibility is that thymocytes impose class I MHC tolerance upon themselves. The ability of immature thymocytes to induce in vivo tolerance to allogeneic MHC antigens was tested by using direct intrathymic adoptive transfers. Preliminary experiments indicated that L3T4/Lyt-2 (double)-negative donor thymocytes could repopulate Thy-1-congenic host thymuses, remaining in situ for up to 21 days before migrating to peripheral lymphatic organs. $(AxB)F_1$ double-negative thymocytes injected intrathymically into lethally irradiated and syngeneic bone marrow-reconstituted parent A hosts could induce and maintain a lasting tolerance among reconstituting host T cells to parent B class I and II MHC antigens as determined by in vitro proliferation and cytotoxicity assays. Administration in vivo of anti-Thy-1 monoclonal antibodies directed specifically against donor F_1 T cells abrogated the tolerance both intrathymically and peripherally. The tolerance to parent B MHC antigens could be induced using Thy-1-positive-FACS-sorted L3T4/Lyt-2 double-negative $(AxB)F_1$ thymocytes as donors for intrathymic transfer into parent A hosts.

T 217 PHENOTYPE, PROLIFERATIVE CAPACITY AND RECEPTOR STATUS OF EARLY THYMOCYTES: TWO DISTINCT LINEAGES? Ken Shortman, Anne Wilson, Tania Ewing & Roland Scollay, The Walter & Eliza Hall Institute, Melbourne, Victoria 3050, Australia.
The early precursor thymocytes of adult murine thymus, defined as Ly 2^- $L3T4^-$, are clearly heterogeneous. We have subdivided them, cross-correlating by two-color flow cytometry the expression of six other markers. Two populations are dominant, namely Ly 1^- Thy 1^+ $B2A2^+$-$M169^+$ (also IL-2R^{+and-} MEL-14^{+and-} Pgp-1^{+and-}) and Ly 1^+ Thy 1^- $B2A2^-$-$M169^-$ (also IL-2R$^-$ MEL-14$^+$ Pgp-1^{+and-}). The latter population is absent from embryonic thymus. The Ly 1^- Thy 1^+ $B2A2^+$ cells are rapidly dividing in vivo but proliferate poorly in single-cell cultures. Conversely the Ly 1^+ Thy 1^- $B2A2^-$ cells are slowly or non-dividing in vivo but proliferate well on stimulation in culture. Surprisingly, even the putative earliest members of the $B2A2^-$ group (Ly 2^- $L3T4^-$ Ly 1^- Thy 1^- $B2A2^-$ Pgp-1^+) are 25% strongly positive for the T cell β chain V-region monoclonal F23.1; the receptor status of all early subsets is under study. We are attempting to determine by reconstitution, culture and ^{3}H-TdR uptake kinetic studies if these early subsets are stages in a single lineage, or whether there are two distinct developmental streams from the earliest stages detected, perhaps with different prothymocyte precursors. Present data suggests the Ly 1^+ Thy 1^- $B2A2^-$ group has a medullary orientation, whereas the Ly 1^- Thy 1^+ $B2A2^+$ group has a cortical orientation. As well as the Ly 2^- $L3T4^-$ cells, a small group of thymocytes which are Ly 2^+ $L3T4^-$ and Ly 2^- $L3T4^+$, but $B2A2^-$, have been isolated. We are testing whether these are an intermediate developmental stage, or are activated peripheral T cells which have reseeded in the thymus.

T 218 TPA INDUCES GROWTH FACTOR DEPENDENCE IN A PRE-T CELL LINE AND LOSS OF TERMINAL TRANSFERASE RNA. Allen E. Silverstone and Janice L. Beland, SUNY-Health Sciences Center , Syracuse, NY 13210.
When treated with nmolar amounts of the phorbol ester TPA,a clone of the 8402 acute lymphoblastic leukemia derived cell line undergoes growth arrest, and, within 48 hours, loses its characteristic nuclear enzyme marker, terminal deoxynucleotidyl transferase(TdT), as indicated by loss of immunologically reactive material. Pulse labelling experiments with radioactive methionine indicate a significant loss of TdT biosynthetic capacity within 4 hours of exposure to TPA. This loss is explained by an extraordinarily rapid disappearance of TdT specific RNA. Thus, cells that are still considered to be TdT positive by immunoflourescence assays, have ceased to produce TdT and TdT RNA.

Increasing the amount of TPA allows the cells to resume growth and proliferation, although at a slower rate. From 30-90% of the entire population of "normal" 8402 cells can be shifted to a TdT negative, TPA dependent phenotype within 24 hours. The dependence on TPA is irreversible, and, in conjunction with the phenotypic loss of TdT as well as the gain and loss of other T-cell markers, suggests a linked step in early T-cell differentiation.

T 219 CLONAL DYNAMICS IN THE HAEMATOPOIETIC SYSTEM: EMPHASIS ON THE THYMUS Ralph Snodgrass and Gordon Keller, Institute for Immunology, Basel.
We have studied the variation, with time, in the clonal makeup of the haematopoietic system of mice that were irradiated and reconstituted with bone marrow cells. To study the repopulation of the thymus, the recipients were grafted under each kidney capsule with embryonic thymuses. The bone marrow cells used for reconstitution were infected with a defective retrovirus, and the proviral intergration sites were utilized as clonal markers. DNA was prepared from cells of various lineages isolated from the same animal at two time points following reconstitution. The clonal composition was determined by Southern blots utilizing a probe specific for the retrovirus used in the experiment. The following conclusions can be drawn. New clones appeared and/or expanded in all of the lineages studied. The grafted thymuses were often repopulated with multiple identical clones which were derived from stem cells whose expression was more limited in the various linages, whereas the endogenous thymus contained a smaller number of clones derived from pluripotent stem cells. Most of the clones found in the thymuses were also seen in the periphery, suggesting that there are not major clones which never leave the thymus. At any point in time, there were many more clones in the peripheral T cells than in the thymuses, which we interpret as a result of clonal waves passing through the thymus into the periphery. These and other data suggests that all the lineages of the haematopoietic system following reconstitution are in constant clonal flux, and the thymus at a point in time is populated by a very limited number of major clones, which change with time.

T 220 HETEROGENEITY OF THYMIC PRE-T CELLS, Fumio Takei, University of British Columbia and Terry Fox Laboratory, B.C. Cancer Research Centre, Vancouver, B.C., V5Z 1L3 Canada.

Murine thymocytes lacking Lyt-2 and L3T4 (pre-T cells) were isolated from young adult mice by an indirect panning method. Flow cytometric analysis showed the isolated pre-T cells to be heterogeneous with respect to the expression of Thy-1 and IL-2 receptor. Southern blot analysis using the T cell receptor β (TcRβ) gene probe also showed that some pre-T cells have the non-rearranged gene whereas others have rearranged β gene. Therefore, the pre-T cell population was further separated into two populations based on the densities of Thy-1 antigen. The population with low Thy-1 was found to have non-rearranged TcRβ gene while the gene in the cells with high Thy-1 is already rearranged. These results suggests that Lyt-2^- L3T4^- thymocyte population consists of at least two subpopulations, one with low Thy-1 and germ line TcRβ gene and the other with high Thy-1 and rearranged β gene. The isolated pre-T cells were stimulated with ionomycin and TPA and maintained in cultures over a month in the presence of Con A stimulated spleen cell conditioned media. Recombinant IL-2 failed to maintain the long term cultures. After three weeks of cultures, most of the cells retained pre-T cell-like phenotype (Lyt-2^- L3T4^- IL-$2R^+$ Thy-1 low). Therefore, no apparent differentiation of pre-T cells into more mature T cells were induced under this condition.

T 221 T-CELL RECEPTOR EXPRESSION EARLY IN THYMOCYTE DIFFERENTIATION. M.L. Toribio, A. de la Hera, J.R. Regueiro, C. Marquez, M.A.R. Marcos, R. Bragado, A. Arnaiz-Villena and C. Martínez-A. Dept. Immunology. Clínica Puerta de Hierro. San Martín de Porres, 4. 28035 Madrid. SPAIN.

"Double negative" ($T11^+3^+4^-6^-8^-$) thymocytes constitute a functional intermediate human ontogenic stage arising from early prothymocytes ($T11^+3^-4^-6^-8^-$) and able to generate $T11^+3^+4^+6^-8^-$ and $T11^+3^+4^-6^-8^+$ mature T cells. Developmental regulation of T-cell receptor expression was analysed in these thymic subpopulations at the DNA, RNA and protein levels, demonstrating that multiple Tiβ gene rearrangements and β RNA transcripts are already evident at the early prothymocyte stage. In addition, similar levels of both Tiα and Tiβ RNAs to those observed in mature thymocytes, were also present in transitional "double negative" thymocytes, but not in prothymocytes, correlating with the surface expression of Tiα and Tiβ molecules.

Thus, T-cell receptor expression is a very early event in T-lineage development, being initially expressed in ontogeny on $T3^+4^-8^-$ "transitional" thymocytes in assotiation with the T3 complex and prior to the expression of T4 and T8 glycoproteins.

T 222 SCANNING ELECTRON MICROSCOPY OF THYMIC NURSE CELLS IN SITU, W. van Ewijk* and E. Wisse**, *Dept. Cell Biology & Genetics, Erasmus University, Rotterdam, The Netherlands, **Dept. Cell Biology, Free University, Brussels, Belgium.

The three dimensional network of thymic tissue was analyzed with scanning electron microscopy. To this purpose mice were fixed using a whole body perfusion method. Lymphoid tissues were excised, post-fixed, critical point dried and sputter coated with gold. Electron microscopic analysis revealed clear cut architectural differences between the thymic stroma in cortex and medulla. In addition, large spherical lympho epithelial structures were observed in the subcapsular cortex. These structures are similar to thymic nurse cells in terms of localization, cell size and content of lymphoid cells.

The T Cell Receptor

T 223 T CELL RECEPTOR-NEGATIVE VARIANTS OF BW5147 AS NEW TOOLS FOR THE GENERATION OF T CELL HYBRIDOMAS. Janice White, Willi Born, Philippa Marrack and John Kappler. National Jewish Center for Immunology and Respiratory Medicine, Denver, CO 80206.

The AKR thymoma and most frequently used T cell fusion line BW5147.G1.4.Ou/r.1 produces functional transcripts for both alpha and beta chains of the T cell receptor for antigen, but does not express the heterodimeric receptor complex on the cell surface. However, upon fusion to normal T cell receptor-positive T lymphocytes, BW's receptor is coexpressed with the normal cell's receptor, as well as mixed receptor molecules generated by random combinations ofthe four receptor chains. We are attempting to generate radiation - induced variants of BW5147 which are negative for the production of mRNA for alpha and beta chain or both. These variants would prove useful in studying the receptor repertoire and antigen specificities of normal T cell populations without interference the BW-derived receptor.

T 224 EFFECTS OF INTERLEUKIN 4 (B CELL STIMULATORY FACTOR 1) ON PROLIFERATION OF MURINE FETAL AND ADULT THYMOCYTES. Albert Zlotnik, Gregory Frank, Melissa Fischer, John Ransom, Hajime Hagiwara and Maureen Howard. DNAX Research Institute, Palo Alto, CA 94304.
The supernatant from a Concanavalin-A (Con A)-induced T cell hybridoma induced proliferation of Peanut agglutinin (PNA)(-) but not PNA(+) thymocytes. Using recombinant interleukin (IL)-4 and IL-2 as well as monoclonal antibodies against these lymphokines we have shown that the effect of this supernatant is due to a synergistic effect of Con A and IL-4. In adult thymocytes, IL-4 and either Con A or phorbol ester (PMA) stimulated the proliferation of PNA (-) but not PNA (+) thymocytes. The nonresponsive population was identified as double positive (L3T4+,LyT2+) thymocytes. In contrast, purified double negative (L3T4-,LyT2-) thymocytes proliferated to IL-4 and PMA. Fetal thymocytes (day 14-19 of gestation) were also induced to proliferate with IL-4 and PMA. Fetal thymocytes (day 14-19 of gestation) were also induced to proliferate with IL-4 and PMA. Both day 14 fetal thymocytes as well as double negative adult thymocytes proliferate in vitro for at least 5 days in medium supplemented with IL-4 and PMA. Neither IL-4 nor PMA alone induced significant proliferation. Interestingly, none of these responses were inhibited with neutralizing anti-IL-2 monoclonal antibodies, indicating that these responses are IL-2-independent. Analysis using an S1-nuclease protection assay indicates that IL-4-specific mRN is induced in adult PNA(-)-enriched thymocytes following stimulation with the calcium ionophore A23187 and PMA. These observations suggest a role for IL-4 in T cell ontogeny.

Class I MHC Structure/function

T 225 PEPTIDES FROM THE α_1 and α_2 DOMAINS OF HLA-A2 SPECIFICALLY INHIBIT ALLOREACTIVE CYTOTOXIC T LYMPHOCYTES, Carol Clayberger, Peter Parham, Sherri Zorn, David Ludwig, Gary Schoolnik, and Alan M.Krensky, Stanford University, Stanford, CA 94305.
Class I major histocompatibility complex (MHC) molecules are central for the recognition of target cell antigens by cytotoxic T lymphocytes (CTL). However, despite a great deal of information about the structure and polymorphic variation of Class I MHC molecules, relatively little is known about the regions of these molecules which are involved in functional interactions with molecules on the T cell surface. In this study we show that peptides derived from the α_1 and the α_2 domains of HLA-A2 specifically inhibit lysis of target cells by HLA-A2 specific CTL. HLA-A2 and the related HLA-Aw68 molecule are identical from residues 98-113 except at position 107, where HLA-A2 contains tryptophan, and HLA-Aw68 contains glycine. The peptide with tryptophan at position 107 inhibits lysis by HLA-A2 specific CTL, whereas the peptide with glycine does not. We have recently identified a peptide from the α_1 domain of the HLA-A2 which can specifically inhibit CTL specific for HLA-A2 and HLA-B17. These results identify regions of the HLA-A2 molecule that are probably involved in binding to the T cell receptor and also raise the possibility that alloreactive CTL recognize processed fragments of Class I MHC antigens.

T 226 SITE-DIRECTED MUTAGENESIS OF HLA-A3 IDENTIFIES AMINO ACIDS CRUCIAL TO THE FORMATION OF DETERMINANTS RECOGNIZED BY CTL. Elliot P. Cowan, Mary Lou Jelachich, John E. Coligan, and William E. Biddison, Laboratory of Immunogenetics, NIAID and Neuroimmunology Branch, NINCDS, NIH, Bethesda, MD 20892.

Amino acids involved in the formation of class I determinants recognized by CTL have been identified by examining primary structural differences between CTL-defined class I subtypes. Two such subtypes of HLA-A3, A3.1 and A3.2, differ at only positions 152 (A3.1=glutamic acid, A3.2=valine) and 156 (A3.1=leucine, A3.2=glutamine). To determine if both of these differences are necessary to alter the class I determinants recognized by CTL, we have used site-directed mutagenesis to generate A3 molecules that differ at either position 152 or 156 (152:A3.1-156:A3.2 and 152:A3.2-156:A3.1). HLA-A3.1-restricted influenza-specific CTL efficiently lysed influenza-infected mouse cell transfectants expressing the A3.1 or 152:A3.1-156:A3.2 molecules, whereas targets expressing the A3.2 or 152:A3.2-156:A3.1 molecules were not significantly lysed. In contrast, an alloreactive CTL line specific for HLA-A3.2 lysed targets expressing A3.2 and 152:A3.2-156:A3.1 molecules but not those expressing A3.1 or 152:A3.1-156:A3.2 molecules. We conclude from these studies that it is the change from glutamic acid in HLA-A3.1 to valine in HLA-A3.2 at position 152, independent of the change from leucine to glutamine at position 156, that results in the differential CTL recognition of these two class I molecules. These data suggest that charge differences on class I molecules may form epitopes that are selectively recognized by T cell receptors.

T 227 Functional sites of human class I MHC Molecules P. Parham, R. Salter, P. Ennis, D. Ludwig, D. Ludwig, G. Schoolnik, S. Zorn, C. Clayberger and A. Krensky.

A combination of approaches has been used to localize regions of human class I molecules that form the determinants recognized by antibodies and alloreactive cytotoxic T cells (CTL). Particular emphasis has been placed on HLA-A2 and a related group of molecules: the HLA-A2/A28 family. Comparison of primary sequences has identified localized regions of variation that correlate with immunological specificity. In vitro mutagenesis within these regions and the use of synthetic peptides derived from these sequences has confirmed these initial assignments. We have been able to specifically inhibit alloreactive CTL with peptides derived from appropriate regions of the class I molecule. Our results suggest that in some cases these CTL may specifically recognize peptide fragments of class I seen in association with intact class I molecules.

T 228 T-CELL SPECIFIC EXPRESSION OF A RABBIT CLASS I MHC GENE. Marie-Christine Rebiere, Patrice N. Marche and Thomas J. Kindt, Laboratory of Immunogenetics, NIAID, NIH, Bethesda, Maryland 20892

Four rabbit class I cDNA clones, corresponding to 4 different transcripts have been isolated from a T cell cDNA library. Expression studies using Northern analyses and S1 mapping experiments revealed that one of these, pR27, is transcribed only in thymus, spleen, appendix and in the T-cell line RL-5. This tissue-specific expression is particular to pR27, the two other class I cDNA are ubiquitously expressed. We are currently searching for factors controlling the expression of class I genes. Constructs including the different regions of pR27 or of other class I genes are being prepared. These will be used in assays to detect DNA binding proteins and to assign specific regulatory functions to different sequences of the class I genes. The same type of studies can be conducted on class II genes. In further experiments, genes encoding T cell antigen receptor and those class II genes that are expressed in T cells will be analyzed for correlative elements that regulate their tissue expression.

The T Cell Receptor

T 229 STRUCTURAL AND FUNCTIONAL ANALYSIS OF THE HLA-A2 MOLECULE, J. Santos-Aguado, S.J. Mentzer, M. Crimmins, J.A. Barbosa, S.J. Burakoff and J.L. Strominger, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA 02115.

The localization and characterization of the regions and, more precisely, the particular amino acid residues responsible for the serological specificity and CTL recognition of the HLA-A2 molecule has been investigated using a combination of oligonucleotide site-directed mutagenesis, DNA-mediated gene transfer and immunological techniques. The approach involves the replacement of specific amino acids (aa) in the HLA-A2 molecule with residues present at the same position in the HLA-B7 molecule. Individual and combined substitutions have been introduced in both α-1 (aa 9, 43 and the highly polymorphic cluster of residues 62-83) and α-2 domains (aa152, 156) in order to investigate the effects of the mutations on the recognition of the molecule by a panel of HLA specific mono- and polymorphic monoclonal antibodies and by HLA-A2 and -B7 allospecific CTLs. The effect of these mutations on the association of the heavy chain with mouse or human β2-microglobulin will be also discussed.

T 230 CHARACTERIZATION OF DUAL REACTIVE $H\text{-}2K^b$-RESTRICTED ANTI-VESICULAR STOMATITIS VIRUS AND ALLOREACTIVE CYTOTOXIC T CELLS, James M. Sheil, Leo Lefrancois and Michael J. Bevan, Research Institute of Scripps Clinic, La Jolla CA 92037.
Alloreactive and self H-2-restricted antigen recognition by cytotoxic T lymphocytes (CTL) represent two means of interaction between the CTL receptor and its target determinant on the cell surface. To examine the functional characteristics underlying both forms of antigen recognition, we isolated dual reactive CTL effectors specific for: (1) vesicular stomatitis virus in the context of $H\text{-}2K^b$, and (2) an $H\text{-}2K^{bm8}$ mutant allodeterminant(s). While the response of normal B6 mice to bm8 showed no measurable cross-reactivity on VSV-infected targets, the response of VSV-primed B6 mice to bm8 stimulation was almost entirely cross-reactive. Reactivity on EL4 ($H\text{-}2^b$) tumor lines transfected with various VSV genes revealed that the internal N protein is the major VSV target antigen. Analysis by cold target competition and antibody blocking indicated that the bulk of dual reactive CTL have a higher avidity for VSV-B6 targets than for bm8 targets. The extreme case of this is illustrated by a fraction of CTL clones (isolated and maintained on bm8 stimulations) which lysed VSV-B6 targets but did not lyse bm8 targets. One such CTL clone was shown to be specific for the bm8 mutant in proliferation assays. These results demonstrate: (1) The specificity of an alloreactive CTL response may be dramatically altered by previous antigenic encounters; (2) Dual reactive CTL display a significant difference in affinity of the CTL receptor-determinant interaction, depending upon the target which is recognized; and (3) Some CTL may exhibit a lower affinity requirement for triggering a proliferative response than for inducing lytic activity.

Lymphocyte Activation

T 231 EVIDENCE FOR RESTRICTED AG RECOGNITION IN THE T CELL RESPONSE TO DNP, Beverly E. Barton and Leslie C. James; Allergan Pharmaceuticals, Inc. and Herbert Laboratories, 2525 Dupont Drive, Irvine, CA 92715

Contact sensitivity is an acute cutaneous inflammatory response initiated by sensitized T cells. The response is often directed against small haptens, such as DNP. In order to study the initiating events of contact sensitivity, T cell lines specific for DNP were raised and cloned from C3H/HeJ and DBA/2J mice. In 72 hr proliferation assays, the T cells (both cloned and uncloned) responded to DNP-albumin, but not to albumin, ovalbumin, DNP-gamma globulin, or DNP-hemocyanin. Thus, all of the T cells raised recognized the DNP moiety as it is bound to albumin, and not to DNP on other proteins, nor to unhaptenated albumin. Furthermore, DNP-lysine, and to a lesser extent, DNP-tyrosine, inhibited the proliferative response to DNP-albumin, presumably by competition for the Ag-binding region of the T cell receptors, because DNP-glycine and ABA-tyrosine were not found to compete under the same experimental conditions. More characterization of the lines and clones revealed that they responded to IL-2 but not to IL-3, synthesized both IL-2 and IL-3, and were Ia-restricted in their response to DNP. Since all the T cells raised to DNP-albumin were specific for the DNP-moiety, even in the uncloned populations, we feel this restricted recognition of Ag may be a reflection of the T cell repertoire, and may play a role in the etiology of the phenomenon of contact sensitivity.

T 232 MODULATION OF HUMAN T CELL ACTIVATION AND CELL CYCLE PROGRESSION BY ANTIBODIES TO CD3 AND ACTIVATION OF PROTEIN KINASE C. L. Davis, M.C. Wacholtz and P.E. Lipsky. UTHSCD, Southwestern Medical School, Dallas, TX. 75235.

Human T cell proliferation can be induced by immobilized antibodies to CD3 (anti-CD3) or the combination of soluble anti-CD3 and the phorbol ester, phorbol dibutyrate (PDB), but not by soluble anti-CD3 alone. Cross-linking CD3 molecules by staining cells with anti-CD3 (OKT3) followed by goat anti-mouse Ig (GaMIg) induced an immediate increase in intracellular calcium concentration measured after fura-2 labeling, but the cells were not stimulated to proliferate or respond to IL2. Moreover, they were inhibited in their capacity to respond to PHA but could proliferate in response to immobilized anti-CD3. In addition, they responded when cultured with PDB indicating a requirement for stimulation of protein kinase C (PKC) to induce proliferation. The following experiments were undertaken to determine the length of PDB mediated PKC stimulation necessary to activate the cells. OKT3+GaMIg-stained cells that had been incubated with PDB for 30 minutes at 37°C and then washed, responded like cells that had not been exposed to PDB. After incubation with PDB for 18 hours at 37°C, however, anti-CD3 and GaMIg pulsed cells proliferated when cultured in fresh medium without PDB and produced IL2. After a 4 hour incubation with PDB, anti-CD3 and GaMIg pulsed cells acquired IL2 responsiveness and were able to respond to PHA but did not proliferate spontaneously. These studies indicate that incubation with PDB progressively induces IL2 responsiveness, loss of inhibition of PHA-responsiveness, and finally IL2 production. Thus, maximal anti-CD3-induced T cell activation and proliferation require prolonged stimulation of protein kinase C.

T 233 GL RESPONSIVE T CELL CLONES, R.H.DeKruyff, S-T.Ju, J.Laning, D.T.Umetsu, M.E.Dorf, Children's Hosp. at Stanford, Stanford Univ, Stanford, CA, Harvard Med. Sch., Boston, MA.

The synthetic random copolymer of L-glutamic acid and L-lysine is nonimmunogenic in all inbred strains of mice. Theories proposed to account for nonresponsiveness to GL include a deficient T cell repertoire, failure of antigen presenting cells to present the antigen and the presence of suppressor cells. We examined mechanisms for nonresponsiveness to GL at the T cell clonal level. We demonstrate the existence of GL reactive T cell clones in nonresponder mice which can be isolated with relatively high frequency. Analysis of the antigen and alloreactivity patterns of these clones indicates that they express distinct antigen receptors, representing at least 5 different clonotypes. These clones are also heterogeneous with regard to their interaction with B cells. Clone E10 is very effective in inducing antibody production by purified B cells, and responds to antigen presented by B cells. In contrast, clone F3 does not respond to antigen presented by B cells, and does not induce antibody production even at high concentrations of antigen in the presence of macrophages. These studies imply that the T cell repertoire of "non-responder" $H\text{-}2^d$ mice includes multiple GL reactive T cell clones which can function effectively as helper cells in induction of antibody synthesis, and that the APC of these mice are effective in processing and presenting GL. Although GL specific suppressor cells have not previously been identified, lack of response to GL may indeed be due to suppressor cells, since we have identified GL specific suppressor T cells in the lymph nodes of GL primed mice.

T 234 NOVEL T CELL ACTIVATION ANTIGEN PRESENT ON ALLOSPECIFIC SECONDARY LYMPHOCYTES AND DEFINED BY VG01 MONOCLONAL ANTIBODY. Denegri JF, Gilks CB, Altin M, Peterson J, Lafleur L. Immunology laboratory, Div. of Hematopathology, Dep. of Pathology, Vancouver General Hospital and University of British Columbia. Canada.

This study describes VG01, a monoclonal antibody to a novel T cell activation antigen. The cells reacting with the antibody were characterized by indirect immunofluorescence and FACS analysis. The antigen defined by VG01 was expressed on circulating T lymphocytes (23.8 +/-9.0) and on the majority of large granular lymphocytes and monocytes (>90%), while granulocytes stained weakly. All other cells tested so far, thymocytes, hematological malignancies and several cell lines were negative. Upon activation with mitogens and allogeneic cells more than 90 % of the transformed lymphocytes became VG01 positive. The kinetic studies demonstrated that the antigen defined by VG01 lags the expression of IL-2 receptor by 48 hr and remained present for up to 18 days in culture. Thus this antigen does not seem to be involved in the initial steps of T cell activation and its expression continues after cell division has stopped. Assays with purified sorted populations showed that lymphocyte proliferation to mitogens and in mixed lymphocyte culture was primarily found in the VG01⁻ population. The responding cells became VG01+ during activation and when alloantigen primed lymphocytes were restimulated with the original stimulators after sorting all the memory response came from the VG01+ population. Thus, VG01 is efficient in selecting for alloreactive T lymphocytes and has potential for selective modulation of immune responses. Its reactivity with activated T cells and NK cells may also help define a common functional program for these two cell types, adding to the understanding of their mechanism of action and/or their origin.

T 235 **A 275 BP FRAGMENT AT THE 5' END OF THE INTERLEUKIN-2 (IL-2) GENE ENHANCES EXPRESSION FROM A HETEROLOGOUS PROMOTER IN RESPONSE TO SIGNALS FROM THE T CELL ANTIGEN RECEPTOR** David B. Durand, Mark R. Bush, John G. Morgan, Arthur Weiss*, and Gerald R. Crabtree. Department of Pathology, Stanford University Medical School and *Department of Medicine, the University of California, San Francisco.

Using a transient transfection assay we have defined the sequences required for the activation of the IL-2 gene in response to signals from the T cell antigen receptor. A 275 bp fragment between 52 and 326 bp upstream of the IL-2 gene transcription initiation site is required for expression of a linked indicator gene in the Jurkat T cell line. The fragment functions in both orientations and will enhance the expression from the promoter of an unrelated gene upon stimulation with phytohemagglutinin (PHA) plus phorbol 12-myristate 13-acetate (PMA). This inducible enhancer function can be reconstituted by duplication of either the upstream 162 bp or the downstream 113 bp of the 275 bp fragment suggesting that a region of homology shared by the two subfragments may be involved in enhancer function. The 275 bp fragment activated the expression of a linked gene following binding of a monoclonal antibody to the Jurkat T cell antigen receptor in the presence of PMA. In addition, calcium ionophore induced the expression of the linked gene through this 275 bp sequence in the presence of PMA. Finally, in a mutant Jurkat cell line lacking T3-antigen receptor complexes at the cell surface, no expression due to the IL-2 5'-flanking region was seen after exposure to antibody to the T cell antigen receptor plus PMA or to PHA plus PMA. In contrast, calcium ionophore plus PMA did induce the expression of a linked gene through the IL-2 5'-flanking region in the mutant line. We have further shown that 1 ng/ml cyclosporin A completely inhibits the induction of an indicator gene through the IL-2 5'-flanking region.

T 236 ACTIVATION OF Ca^{2+} MOBILIZATION IN MATURE AND IMMATURE T CELLS BY CROSSLINKING OF ANTI-RECEPTOR ANTIBODY. T.H. Finkel, J.A. Pasternak, M. McDuffie, J. Kappler, P. Marrack and J. Cambier. Natl. Jewish Ctr. for Immunol. & Resp. Med., Denver, CO.

T cells develop sequentially from immature cells with no detectable antigen receptor to immature thymocytes with few receptors, eventually becoming mature thymocytes with 20,000-40,000 receptors per cell. Recent studies in our lab have shown that incubation of the fetal mouse thymus in vivo or in vitro with the anti-T cell receptor antibody KJ16-133 (KJ16) (an antibody directed against the products of the Vβ8 gene family), prevents the development of mature, $KJ16^+$ thymocytes. Immature, $KJ16^+$ cells, on the other hand, are apparently unaffected by treatment with the antibody. We are interested in identifying the signal transduction mechanisms responsible for this interruption of development.

Using the Ca^{2+}-sensitive fluorescent dye, indo-1, we have demonstrated specific anti-receptor antibody mediated induction of calcium mobilization in epitope positive peripheral T cells and thymocytes of murine strains which express determinants recognized by the anti-receptor antibodies, F23.1 (F23) and KJ23-588 (KJ23), used in conjunction with rabbit anti-mouse Ig antibodies (RAMIG). KJ23 also induced Ca^{2+} mobilization in thymocytes from strain C57/Br, a strain which produces only immature $KJ23^+$ thymocytes. Thus, at least some immature thymocytes appear to be capable of mobilizing Ca^{2+} in response to binding of the T cell antigen receptor.

T 237 ACCESSORY CELL INDEPENDENT T CELL PROLIFERATION INDUCED BY IMMOBILIZED ANTI-CD3 MONOCLONAL ANTIBODIES (Mab). TD Geppert, and PE Lipsky. UTHSCD, Dallas TX 75235
The capacity of soluble and immobilized anti-CD3 Mab to induce accessory cell (AC) independent T cell proliferation was examined. Highly purified peripheral blood T4 or T8 cells were cultured with immobilized or soluble anti-CD3 Mab, 64.1 or OKT3, with or without AC (glass adherent cells), IL-2, or Mab directed at CD5 (10.2) or TP44 (9.3). Mab were immobilized by allowing them to adhere to plastic microtiter wells. Immobilized but not soluble anti-CD3 Mab induced AC independent T4 and T8 cell proliferation. Moreover, there was a direct relationship between the density of the immobilized anti-CD3 Mab and the number of T cells entering the cell cycle. High density 64.1 induced nearly all T4 cells to enter the cell cycle and induced T cell proliferation that was not enhanced by AC, IL-2, 9.3 or 10.2. Lower densities of 64.1 induced responses that were enhanced by AC, IL-2, 9.3 or 10.2, whereas responses induced by still lower densities were dependent on these additional signals. Immobilized OKT3 stimulated only a minority of the T4 or T8 cells to enter the cell cycle. However, when immobilized but not soluble Mab directed at non-CD3 T cell surface proteins including class I MHC antigens, LFA-1 or CD2, were also present, immobilized OKT3 induced responses comparable to those induced by 64.1. Immobilized anti-CD4 and anti-CD8 Mab enhanced immobilized OKT3 induced T4 and T8 cell proliferation, respectively, whereas soluble anti-CD4 Mab inhibited T4 cell responses. The data indicate that maximum anti-CD3 induced cell activation is dependent on multiple anti-CD3-CD3 interactions without internalization. When the number of interactions is suboptimal, responses are dependent on additional signals or the establishment of interactions via non-CD3 molecules.

T 238 MUTATIONAL ANALYSIS OF SIGNAL TRANSDUCTION BY THE T CELL ANTIGEN RECEPTOR,
Mark A. Goldsmith and Arthur Weiss, Department of Medicine and Howard Hughes Medical Institute, University of California, San Francisco, CA 94143.
The molecular interactions mediating transmembrane signalling by the human CD3/antigen receptor (Ti) complex were explored by the derivation and analysis of T cell mutants that are unable to respond to conventional activation stimuli. Using fluorescence-activated cell sorting with the Ca^{++} indicator Indo-I, we isolated mutants of the T cell line Jurkat that fail to generate phosphoinositide (PI) second messengers or subsequent increases in cytoplasmic free Ca^{++} in response to lectins or monoclonal antibodies against Ti (C305) or CD3 (OKT3). Interestingly, cross-linking of such antibodies by two methods partially restores their agonist properties, indicating that at least part of the signal transduction pathway is intact. AlF_4^-, an activator of G proteins, elicits PI metabolism and a concomitant Ca^{++} increase in these cells, further suggesting that the mutation affects a proximal part of the pathway. As anticipated, production of interleukin-2 in response to anti-receptor antibodies in the presence of PMA is abolished, further implicating the PI metabolites in activating this cellular response in wildtype cells. However, modulation of CD3/Ti from the surface of the mutants in response to anti-Ti antibody is unaffected, thereby dissociating this process from receptor-activated PI turnover. While the CD3 and Ti structures appear grossly normal in the mutants, experiments are in progress to elucidate the site of the mutation and to identify structural requirements for competence in signal transduction.

T 239 EFFECTS OF IN VIVO ADMINISTRATION OF A MONOCLONAL ANTI-MURINE T3 ANTIBODY ON T LYMPHOCYTE FUNCTION. R. Hirsch, D.H. Sachs, J.A. Bluestone, NIH, Bethesda, MD 20892.
Anti-human T3 monoclonal antibodies (mAb) have been used in vivo as an immunosuppressive therapy to prevent kidney graft rejection. In vitro, anti-T3 mAbs have been shown to both activate and inhibit T cell function. Thus, the precise mechanism of the in vivo effect of the anti-T3 mAb remains unclear. We have recently developed an anti-murine T3 mAb that, like OKT3, induces resting spleen cells to proliferate, produce IL-2 and become cytolytic, but inhibits in vitro allogeneic CTL responses. The in vivo effects of this anti-T3 mAb were therefore examined. Animals demonstrated an adverse reaction to the initial injection, as was also observed in humans given OKT3 mAb. One hour after intravenous administration of the mAb all peripheral blood T cells disappeared from circulation. However, T cells were evident in the spleen and lymph node throughout the 10 day treatment period. Most of these T cells had either modulated T3 or had anti-T3 bound to the cell surface. Functional analysis of these cells showed that the mAb both activated and suppressed T cell function. Unlike control spleen cells, spleen cells from mice treated 1 hr previously with anti-T3 proliferated in the presence of IL-2 alone. By 3 days after injection, splenic T cells did not proliferate to IL-2, despite the presence of anti-T3 mAb bound to a significant number of T cells. In addition, the immune response to Concanavalin A and to allogeneic cells was markedly decreased. These results suggest that the presence of anti-T3 on the surface of T cells may deliver a tolerogenic signal to T cells in vivo. Thus, in vivo injection of anti-T3 mAb leads to an initial activation (which may account for the early toxicity observed in humans and mice), followed by suppression of T cell responses.

T 240 LY-1 NEGATIVE AND THY-1 NEGATIVE T CELL VARIANTS PRODUCE IL2 IN RESPONSE TO MITOGENS. Nurit Hollander, Tel-Aviv University, Israel.
The T cell tumor line LBRM331A5 responds synergistically to IL1 and PHA by releasing IL2. It was demonstrated that anti Ly-1 monoclonal antibodies and PHA co-stimulated LBRM331A5 cells, as did IL 1 plus PHA. Thus, anti Ly-1 antibodies mimic the effect of IL1, suggesting a role for Ly-1 in T cell activation, perhaps by serving as an IL1 receptor. To further study the functional role of Ly-1 and its relation to IL1 receptor, Ly-1 negative variants of the LBRM331A5 cell line were selected and analyzed for IL2 production in response to PHA plus IL1. It was demonstrated that Ly-1 negative clones were capable of IL2 production as efficiently as Ly-1 positive clones. These results indicate that the Ly-1 and IL1 receptor are distinct molecules, which are involved in different activation pathways. Crosslinking of the Thy-1 molecule results in T cell activation, suggesting that Thy-1 may play a role in T lymphocyte triggering. To further study its functional role, Thy-1 negative variants were selected and analyzed for IL2 production in response to phorbol-12-myristate-13-acetate or to concanavalin A. It was demonstrated that Thy-1-negative clones were capable of IL2 production as efficiently as Thy-1 positive clones. These results indicated that although Thy-1 crosslinking triggers cell activation, a signal provided by Thy-1 is not indispensable for T cell activation.

T 241 SELECTION OF TCR-SPECIFIC MONOCLONAL ANTIBODIES FOR CLINICAL APPLICATION.
R. Kurrle and F.R. Seiler, Research Labs. of Behringwerke AG, 3550 Marburg, FRG
We have generated three monoclonal antibodies BMA031, BMA032 and BW239/347 (all of IgG2b isotype), which previously have been charaterized to recognize epitopes within the framework of the human T-cell receptor (TCR). In different test systems (proliferation assay, induction of in vitro IgM synthesis and release of immunomediators, triggering/blocking of cytotoxicity), the functional capacities of these anti-TCR mabs are compared to the well-known T-cell functions triggered by two CD3-specific mabs (BMA030, IgG2a; BMA033, IgG3). Although, in contrast to the CD3 mabs, none of the TCR-specific mabs is mitogenic for T-cells in the majority of blood donors (IgG2b low-responders) or is able to trigger Ca^{2+}-influx, TCR-specific mabs are highly effective in the suppression of SAC and/or PWM-induced IgM synthesis. When the capacity of all TCR/CD3 mabs to induce the release of immunomediators is analyzed, it turns out that only one of the TCR-specific mabs is able to induce synthesis of high γ-IFN titers. This finding indicates that besides specificity and isotype other characteristics of the antibody are essential as well. Up to now it is unclear which of all these (and other) antibody functions are really essential to suppress T-cell functions in vivo without causing severe adverse reactions simultaneously. To answer these questions, the prophylactic and therapeutical use of BMA031 (TCR-mab) in organ transplantation is now under clinical investigation.

T 242 NOVEL GLYCOPROTEIN SUBUNIT OF THE HIGH-AFFINITY INTERLEUKIN-2 RECEPTOR.
Warren J. Leonard, Richard D. Klausner, Bryan R. Cullen*, Richard Chizzonite*, and Michael Sharon. Cell Biology and Metabolism Branch, NICHD, NIH, Bethesda, MD 20892, and Dept. of Molecular Genetics, Hoffman La-Roche, Inc., Nutley, NJ 07110.
We have used DSS and DSP to covalently cross-link ^{125}I-IL-2 to activated human T cells which express both high and low affinity IL-2 receptors in order to analyze the biochemical differences between these receptors. Only the high affinity receptors are known to be involved in receptor mediated endocytosis and signal transduction. Under high affinity conditions cross-linked bands were identified at 68-72 kD and 85-92 kD. Both species could be precipitated with anti-IL2 MoAb 17A1, but only the 68-72 kD band could be precipitated with Abs to the Tac antigen (p55). Thus the 68-72 kD moiety represents IL-2 cross-linked to p55, and the 85-92 kD moiety represents IL-2 cross-linked to a different species. Cross-linking over a range of IL2 concentrations indicated that both moieties are detected under high affinity conditions, but only the smaller moiety was detected under low affinity conditions. These data indicate the existence of a novel subunit which when associated with p55 forms a high affinity IL-2 receptor. To identify this subunit we cross-linked unlabeled IL2 to ^{3}H-mannose labeled HUT-102B2 cells with the cleavable cross-linker DSP, followed by precipitation with 17A1 and analysis of a 2-D non-reducing/reducing gel. The subunit was identified as a 65-77 kD doublet (p70) below the diagonal, consistent with the cleavage of one IL2 molecule. We have identified p70 on several human T cell lines which lack p55, indicating that these proteins are not necessarily coordinately expressed. We propose that p55 and p70 be referred to as the α and β subunits, respectively of the high affinity IL-2 receptor.

T 243 AlF_4^- INDUCES PHOSPHORYLATION OF THE T CELL ANTIGEN RECEPTOR COMPLEX: EVIDENCE FOR INVOLVEMENT OF A G PROTEIN IN T CELL ACTIVATION VIA THE ANTIGEN RECEPTOR, J. J. O'Shea, K. B. Urdahl, H. Luong, L. E. Samelson and R. D. Klausner, National Institutes of Health, Bethesda, MD 20892.
Guanine nucleotide binding (G) proteins have been shown to be involved in coupling receptors to activation of adenylate cyclase. More recently G proteins have been implicated in phosphatidylinositol (PI) metabolism. Antigen activation of T lymphocytes results in PI turnover and phosphorylation of three of the subunits of the antigen receptor complex (Samelson et al., Cell 46: 1083, 1986). We therefore asked whether a G protein mediates signal transduction via the T cell antigen receptor. Because fluoride ions (F^-) have been shown to activate G proteins we used F^- to probe for G proteins in T cells. We found that F^- induced PI turnover and phosphorylation of 2 subunits of the antigen receptor complex, gp21 (γ) and p25 (ϵ), in a dose dependent manner. Addition of $AlCl_3$ markedly potentiated the F^- effect. Elevation of intracellular cAMP levels blocked antigen and anti-receptor antibody induced phosphorylations but was unable to block the F^- induced phosphorylations of the receptor subunits. We interpret these data to suggest that a G protein couples the antigen receptor to kinases which upon activation phosphorylate the antigen receptor. cAMP uncouples the signal transducing mechanism at or proximal to the level of a G protein.

The T Cell Receptor

T 244 TRANSMEMBRANE SIGNALLING VIA ANTIGEN-DEPENDENT AND ANTIGEN-INDEPENDENT PATHWAYS OF HUMAN T CELL ACTIVATION. G.Pantaleo, D.Olive, A.Poggi, C.Bottino, L.Moretta and A.Moretta. Ludwig Institute for Cancer Research, Lausanne Branch, Epalinges, Switzerland. In this study, we investigate the mechanism by which stimulation via T11 or T44 molecules induces activation in Jurkat cells. We show that one of the early events during cell activation mediated via either T11 or T44 molecules is represented by an increase in the concentration of a free cytoplasmic Ca^{2+} ($[Ca^{2+}]i$). The initial T11-mediated increment in $[Ca^{2+}]i$ was detectable also when extracellular Ca^{2+} was depleted by EGTA, indicating that Ca^{2+} from intracellular stores is mobilized. In contrast, anti-T44 increase in $[Ca^{2+}]i$ occured only in presence of extracellular Ca^{2+}, suggesting that perturbation of T44 molecules fails to mobilize Ca^{2+} from intracellular stores. We next measured the levels of phosphatidilinositol bisphosphate (PIP2) which is hydrolysed to generate inositol triphosphates (IP_3) (the putative mobilizer of Ca^{2+} from internal stores) and 1,2 diacylglycerol (DAG) (the physiological activator of protein Kinase C). Similarly to mAbs directed to the TcR complex stimulatory combination of anti-T11 mAbs provoke a rapid breakdown of PIP2, the parental product from which IP3 and DAG derive. In addition antibodies to either the T11 or TcR complex induce marked elevations in IP3, other inositol phosphate compounds and DAG. In contrast, stimulation via T44 molecules does not induce increments of inositol phosphates. Therefore, the process of transmembrane signalling via T11 molecules induced hydrolysis of PIP2 in order to generate IP3 and DAG, whereas stimulation mediated by the T44 molecules proceeds via a mechanism independent from the typical inositol lipid metabolism.

T 245 CONTACT IS IMPORTANT FOR T-B COLLABORATION, Robert Rasmussen and Kim Bottomly, Yale University School of Medicine and the Howard Hughes Medical Institute, New Haven, CT. 06510. We have examined the nature of the interaction between helper T cells and B cells leading to the formation of plaque forming cells (PFC) and have devised a system to assess the need for contact between the two cell types. In this system cloned helper T cells (Th) were cultured together with unimmunized splenic B cells in a 2 chamber culture system. Cloned Th cells and B cells in the top chamber were separated by a nucleopore membrane from an equal number of B cells in the bottom chamber. The activation of B cells on both sides of the membrane was determined. It was found that for the generation of TNP-specific PFC, B cells in contact with (or in very close proximity to) Th cells gave a response 10-20x greater than separated B cells indicating that 90% of the response is contact mediated. Increasing the number of cloned T cells or antigen concentration did not alter the PFC response of the separated B cells. Lymphokine concentrations, as measured by both HT-2 proliferation and Ia induction assays were of equal magnitude on both sides of the membrane. A preferential requirement for contact was also observed for the generation of polyclonal PFC. However, only 50% of the total polyclonal response required contact. Using B cells fractionated by Percoll gradient centrifugation preliminary data suggest that unfractionated, small and large B cells showed a similar need for contact for both TNP-specific and polyclonal PFC responses. The results are consistant with the idea that T cell help is not mediated solely by lymphokines and that contact (or close proximity) is required for the optimal generation of PFC. The nature of the contact mediated signal sent to the B cell is currently being investigated.

T 246 EPITOPE RECOGNITION AND ACTIVATION OF PROLIFERATION BY ANTIBODIES AGAINST THE ANTIGEN RECEPTOR OF A HELPER T CELL LINE, Jose M. Rojo and Charles A. Janeway, Jr. Section of Immunobiology, Howard Hughes Medical Institute at Yale University School of Medicine, New Haven, CT 06510 USA.

The mouse helper T cell line D10.G4.1 (D10) can be activated to proliferation and lymphokine secretion by anti-T cell receptor (Ti) antibodies in soluble form, and immunization of mice with D10 induces the generation of anti-D10 Ti antibodies with high frequency. We have studied a series of monoclonal anti-D10 Ti antibodies termed, in order of their relative avidity for D10: 3D3, C233.20B, C193.5A: C193.6D, C193.10B, F23.1, C193.16A, C251.4B, KJ-16 and C193.13A. All of them are mouse IgG anticlonotypic antibodies raised by immunization with D10, except F23.1 (a mouse IgG) and KJ-16 (a rat IgG), which recognize an allotypic determinant present in the Ti of a subpopulation of T lymphocytes in most strains of mice.

We have determined the ability of these antibodies to compete with each other for the binding of Ti, and the results indicate the existence of at least five different epitopes, defined by 3D3, C193.6D, C193.5A, F23.1 and C193.16A. The ability of soluble anti-L3T4 antibodies to enhance or suppress the proliferative response induced by the anti-Ti antibodies correlates with two of the epitopes, those recognized by C193.16A and C193.5A, respectively.

When activation of D10 was considered, and the concentration of antibody required for 50% maximal activation taken as a measure of the activating efficiency of each antibody, the most efficient was 3D3 followed by C193.10B, C233.20B, C193.6D, C193.5A, C251.4B, F23.1, C193.16A, C193.13A and KJ-16. Comparison of the relative avidity of these antibodies to their efficiency as D10 activators revealed some striking differences between these two parameters among the antibodies considered, and suggest that optimal stimulation of D10 by soluble antibodies is not a direct function of the avidity of the antibody considered, and there are other factors, namely the particular epitope recognized by the antibody, which influence the outcome of the response.

T 247 Pleiotropic loss in activation pathways in a T cell receptor α chain deletion variant of a cytolytic T cell clone, Anne-Marie Schmitt-Verhulst[1], Annick Guimezanes[1], Pierre Kaldy[1], Martin Poenie[2], Roger Tsien[2], Michel Buferne[1], Claude Boyer[1], and Lee Leserman[1]. [1]Centre d'Immunologie INSERM-CNRS de Marseille-Luminy, Case 906, 13288 Marseille, FRANCE. [2]Department of Physiology, University of California, Berkeley, CA, USA.
Use of the Ca^{++} ionophore ionomycin (iono) and the phorbol ester phorbol myristic acetate (PMA) as substitutes for Ti-mediated activation, combined with immunoselection using the anti-clonotypic (anti-Ti) mAb Désiré-1 permitted isolation from the H-$2K^b$-specific cytolytic T (CTL) clone KB5-C20 of a variant lacking surface expression of the Ti. The variant was selected with methotrexate-containing liposomes targeted to the T cell receptor via coupled anti-Ti mAb. Northern analysis indicated that the variant lacked mRNA for the α chain of the Ti. Other cell surface markers such as Lyt2.2, Thy-1 and H-$2K^k$ were expressed at similar levels on clone KB5-C20 and the Ti^- variant. Functional analyses indicated that the Ti^- variant was defective in lectin-mediated (Concanavalin A or Leucoagglutinin) activation whether measured by increase in intracytoplasmic Ca^{++}, CTL effector function or gamma-interferon (g-IFN) synthesis. The variant expressed normal levels of Thy-1, but was unresponsive to anti-Thy-1 mAb activation as measured by g-IFN secretion, whereas it responded to iono plus PMA. These results indicate that in a non-transformed, functional mature T cell, Thy-1 mediated signalling is not an alternative to, but might depend on elements associated with the Ti/CD3-mediated T cell activation pathway.

T 248 BIOCHEMICAL PATHWAYS IN THE ANTIGEN RECEPTOR REGULATED EXOCYTOSIS OF GRANULES IN CYTOTOXIC T LYMPHOCYTES (CTL). Michail Sitkovsky, Guido Trenn and Hajime Takayama, LI, NIAID, NIH, Bethesda, Maryland 20892
Using a novel CTL activation assay, based on the quantitative measurements of exocytosis of granules we demonstrate the existence of two counteracting biochemical pathways. One of them is triggered by the crosslinking of T cell receptor (TcR), involves transmembrane protein kinase C activation, translocation of extracellular Ca^{++} through plasma membrane Ca^{++} channels, calmodulin binding proteins and results in the exocytosis of granules. Another inhibitory pathway is mediated by cAMP dependent protein kinase and counteracts the TcR-triggered processes in CTL. It is suggested that responsiveness of CTL to low surface density of antigen is determined by basal levels of cAMP. The model of "ON" and "OFF" signalling, which is based on the interplay of these two pathways and secretion of granule-associated enzyme(s) will be discussed.

T 249 STIMULATION OF MURINE T CELL HYBRIDOMAS WITH ANTI-THY-1 MONOCLONAL ANTIBODIES RESULTS IN IL-2 PRODUCTION AND INHIBTION OF GROWTH, J.J. Sussman, M. Mercep and J.D. Ashwell. BRMP, NCI, NIH, Bethesda, MD 20892.
Activation of antigen-specific murine T cell hybridomas with the appropiate antigen/MHC combination results in their production of interleukin-2 (IL-2) and inhibtion of their growth. We have extended these observations to G7, a rat monoclonal antibody that binds to a nonpolymorphic determinant on Thy-1, and which stimulates normal T cells to proliferate and produce IL-2. Incubation with G7 inhibited the growth of 2B4.11 and C10.9, antigen-specific murine T cell hybridomas, and EL4, a murine T cell lymphoma, in a dose-dependent fashion. Concentrations of G7 that caused growth inhibtion in 2B4.11 also increased intracellular free Ca^{2+}, phosphatidyl inositol metabolism, and IL-2 production. A subclone of 2B4.11, derived by repetitive cloning at limiting dilution, was found to lack mRNA for three of the four Ti chains carried by the 2B4.11 parent, and did not express the T3 complex on its surface. Although this subclone bears as much Thy-1 on its surface as the parent 2B4.11 hybridoma, incubation with optimal concentrations of G7 did not cause an increase in phosphatidyl inositol metabolism or in intracellular free Ca^{2+}, and did not lead to IL-2 production or growth inhibtion. The T3-negative subclone, like the parent hybridoma, could be induced to make small amounts of IL-2 after incubation with the phorbol ester PMA. These data support the hypothesis that the Ti/T3 complex plays a role in transmembrane signalling via the Thy-1 molecule.

T 250 CYCLIC AMP DEPENDENT PROTEIN KINASE MEDIATED INHIBITORY PATHWAY IN THE ANTIGEN RECEPTOR REGULATED EXOCYTOSIS OF CYTOTOXIC T-LYMPHOCYTES. Hajime Takayama, Guido Trenn and Michail Sitkovsky. LI, NIAID, NIH, Bethesda, Maryland 20892
Sequential interactions of cytotoxic T-lymphocytes (CTL) with several target cells (TC) imply the existence of an "on" and "off" molecular switching mechanisms in T-lymphocyte activation. We suggest here that cAMP dependent protein kinase (PK-A) is involved in an inhibitory biochemical pathway, which counteracts protein kinase C and Ca^{++}-mediated stimulatory, T-cell antigen receptor (TcR) triggered pathway. Pretreatment of CTL with cholera toxin, but not with pertussis toxin, dramatically inhibits TcR-triggered "lethal hit" delivery to the target cell and blocks TcR-triggered exocytosis of granules from CTL. Other agents that raise intracellular level of cAMP, including forskolin (but not di-deoxy-forskolin), inhibitor of cAMP phosphodiesterase also inhibited TcR-triggered CTL activation and induced increases in cAMP levels in CTL cloned lines. Involvement of cAMP-dependent protein kinase in an inhibitory pathway is suggested by the combined effects of cyclic nucleotide analogs 8Br-cAMP and N^6 benzoyl cAMP in inhibition of TcR-triggered exyocytosis.

T 251 ANTIGEN-RECEPTOR REQUIREMENT FOR CYTOTOXIC T-LYMPHOCYTE (CTL) ACTIVATION CAN BE BYPASSED BY A NOVEL PROTEIN KINASE C ACTIVATORS OF A BRYOSTATIN FAMILY. Guido Trenn, Hajaime Takayama, George Pettit* and Michail Sitkovsky. LI, NIAID, NIH, Bethesda, Maryland 20892 and *Arizona State University, Tempe, Arizona 85287
The effect of two novel protein kinase C activators bryostatin 1 and bryostatin 2 on the activation and differentiation of murine cytolytic cells was studied. Both reagents were able to trigger Ag-nonspecific and to inhibit Ag-specific cytotoxicity of cloned CTL. Bryostatin 1 and 2 synergize with Ca^{++} ionophores in triggering the exocytosis of granules from CTL at very low concentrations. Bryostatin 1 completely inhibited the development of mature CTL from both in vivo primed spleen cells and unprimed small resting lymph node T-cells. Bryostatin 2 in combination with IL-2 and/or BSF-1 induced activation and differentiation of both resting and primed T cells. The possible implications of these results for the strategy in the development of immunomodulating agents are discussed.

T 252 STRUCTURAL AND FUNCTIONAL CHARACTERISTICS OF THE CD3 (T3) MOLECULAR COMPLEX ON HUMAN THYMOCYTES. C.D. Tsoukas, B. Landgraff, D.A. Carson, J.H. Vaughan. Scripps Clinic and Research Foundation, La Jolla, CA 92037.
The mitogenic properties of anti-CD3 (T3) antibodies have suggested that the CD3 complex may be the intracellular transducer of the antigenic signal. In the present invesitgation, we have studied some of the characteristics of CD3 on human thymocytes. Comparison of anti-CD3 immunoprecipitates between thymocytes and peripheral T cells by two-dimensional gel electrophoresis revealed several differences. The most notable difference is the presence of an intense, relatively acidic (pI 5.5-6.0) spot contained in the 20-22 kD polypeptides of CD3 from thymocytes. Anti-CD3 antibodies are not by themselves mitogenic for thymocytes, indicating that functionally active monocytes are absent from the thymocyte population. Even addition of peripheral monocytes does not allow a response of thymocytes to anti-CD3 antibodies. However, when the anti-CD3 antibody 64.1 is added in the presence of exogenous rIL2, a strong antibody and lymphokine dose-dependent response ensues. Over half of the thymocytes coexpress CD3 and CD1 (T6) on their surfaces. However, there exist distinct subpopulations displaying only CD3 or CD1. Anti-CD3 plus rIL2 stimulated only $CD1^-$ $CD3^+$ cells. Thus, the mere expression of CD3 on the $CD1^+$ $CD3^+$ subpopulation apparently is not sufficient to render the cells responsive. In conclusion, CD3 molecules of thymocytes and peripheral T cells have several structural and functional differences.

The T Cell Receptor

T 253 STIMULATION OF HUMAN T LYMPHOCYTES BY CD3 McAb ISOTYPE SWITCH VARIANTS, R. van Lier, J. Boot, G. v. Seventer, A. Verhoeven, E. de Groot, M. Brouwer, L. Aarden, Central Lab. Neth. Red Cross Blood Transf. Service, Amsterdam, The Netherlands.

From a CD3 monoclonal antibody (McAb) of the γ1 subclass we isolated 4 switch variants, i.e. γ2b, γ2a, ε and α successively. All five antibodies recognize the CD3 antigen as determined by immunoprecipitation. In proliferation experiments with the γ1, γ2b and γ2a variants we could confirm the reported Fc-receptor polymorfism for mouse Ig on human monocytes. In addition all donors responded to the ε-CD3 antibody, while no α responders were detected. We were able to confirm this polymorfism in CD3 antibody induced non-specific lysis of monocytes and cell lines by CTL-clones. In purified T cells none of the switch variants proved to be mitogenic by itself. However, in marked contrast to the other antibodies, the ε-CD3 antibody induced a strong proliferation in combination with IL-2. This led us to assume that the binding of the ε-CD3 McAb and thus the activation signals delivered might differ from the other switch variants. Indeed while the γ1, γ2b, γ2a and α variants blocked effectively the binding of labeled γ2a variant, the ε variant was much less effective on a molar basis. Furthermore, the ε variant was found to be less effective in the induction of T3 modulation and Ca^{2+} mobilization. Because we were able to exclude possible dose effects we assume that the ε antibody, in contrast to the other variants, binds monovalently to the CD3 antigen. This monovalent binding results in a different T cell triggering leading to differences in proliferation requirements for purified T cells. We feel that this series of isotype switch variants might be very helpful not only to study Fc-receptors but also to get insight in the changes in the T cell membrane that lead to T cell activation.

T 254 PHENOTYPIC AND FUNCTIONAL ANALYSIS OF NOVEL T CELL SUBSETS IN AUTOIMMUNE MICE. Scott Wadsworth, Katsuyuki Yui, Yasuhiro Hashimoto and Mark I. Greene. Univ. Pennsylvania Philadelphia PA 19104.

Mutant mice of C3H/HeJ, C3H-gld/gld develop age-related massive lymph node enlargement with autoimmunity. We defined two novel T cell subsets in gld lymph nodes, namely, a Thy-1^+ Lyt-2^- L3T4^- T cell receptor $(TcR)^+$ subset (Double-negative cells) and a Thy-1^- Lyt-2^- L3T4^- TcR^+ subset (Null cells). Messenger RNA expression of these T cell subsets paralelled the expression of cell surface proteins.

Functional analysis of these T cell subsets revealed that both Double-negative and Null cells responded to stimulation by T cell factors including IL-2. However, Null cells and most Double-negative cells were not induced to express detectable levels of IL-2 receptor and did not proliferate after stimulation with Concanavalin A even in the presence of an exogenous source of IL-2. Double-negative cells but not Null cells responded to allogeneic stimulator cell in the presence of rat Con A supernatant. Lyt-2^- L3T4^- class I specific CTL clones were established from Double-negative cells. These studies provide novel insights into the growth and function of phenotypically immature T cells and indicate a distinct relationship between expression and function of T cell receptor and T cell differentiation molecules in C3H-gld/gld mice.

T 255 ROLE OF Ly-5 AND Lyb-2 IN LPS-INDUCED B CELL ACTIVATION, H. Yakura, G. Tate, H. Sakata, I. Kawabata, T. Ashida and M. Katagiri, Department of Pathology, Asahikawa Medical College, Asahikawa 078, Japan.

Lymphocyte activity is regulated by cell surface molecules through which outside information is transformed into biochemical signals that affect a cell's machinery. We have demonstrated that two mouse B cell surface molecules, Lyb-2 and Ly-5, are involved in the regulation of antigen- and mitogen-driven B cell differentiation. In this study, we have further examined the role of these two molecules in LPS-induced B cell activation. The results showed that monoclonal Ly-5 antibody reduced the generation of IgG-secreting cells without affecting IgM or proliferative responses whereas Lyb-2 antibody did not have any effect on these responses. Analysis on the selective inhibition of IgG responses by Ly-5 antibody revealed that the Ly-5 antibody acted upon both Lyb-5^+ and Lyb-5^- B cells and reduced primarily the precursor frequency but only partially the clone size of IgG-secreting cells and that the inhibition occurred at post-transcriptional levels. It has been shown that LPS-induced IgG secretion is regulated by B cell stimulatory factor (BSF)-1. BSF-1 induces IgG1 production with concomitant reduction in IgG2b and IgG3. Ly-5 antibody could not compete with the action of BSF-1. Interestingly enough, Lyb-2 antibody which might detect a molecule closely associated with BSF-1 receptor, interfered with BSF-1-induced IgG1 production but not IgG2b/IgG3 reduction. These results suggest that BSF-1-induced IgG1 production and IgG2b/IgG3 reduction might be regulated by a distinct mechanism and that Lyb-2 molecule is somehow involved in the signaling events initiated by the interaction of BSF-1 and its receptor.

The T Cell Receptor

T 256 STRUCTURE AND FUNCTION OF THE MURINE TAP MOLECULE AND RELATED PROTEINS, Kenneth L. Rock, Hans Reiser, John Coligan* and Edward T.H. Yeh, Harvard Medical School, Department of Pathology, Boston, MA 02115, *NIAID, Bethesda, MD 20892.
We have recently described the TAP/TAPa membrane proteins that displayed the unusual ability of causing antigen independent activation of T cells upon crosslinking. TAP/TAPa are physically independent and biochemically distinct from the TCR/T3 complex. However, their signal(s) synergise with TCR/T3 stimulation. To determine the role of TAP in T cell activation, we have derived a panel of T-T hybridomas that are selectively deficient in surface expression of TAP/TAPa. These expression mutants show a concordant impairment in activation. Analysis of this defect suggests that TAP may be involved in receptor signal transduction. Given these findings, it is of interest that, TAP expression correlates developmentally with the acquisition of immunocompetence. TAP is anchored to the membrane by a rare phosphatidyl inositol lipid linkage. The only other known T lymphocyte membrane protein with similar anchorage is Thy-1, which is also activating. The gene encoding TAP maps to the Ly-6 locus. This locus contains two families of structurally similar proteins, all of which may have activating potential. Given the distribution of these antigens, the role of TAP-like proteins may extend to other cell types and tissues. Putative TAP cDNA clones have been isolated.

T Cell Effector Mechanisms

T 300 PHOSPHORYLATION OF THE T8 ANTIGEN ON CYTOTOXIC HUMAN T CELLS IN RESPONSE TO PHORBOL MYRISTATE ACETATE OR ANTIGEN PRESENTING B CELLS. R.B. Acres, P.J. Conlon, D.Y. Mochizuki, and B. Gallis. Immunex Corporation, 51 University Street, Seattle, Wa., 98101.
We have used the monoclonal antibody OKT8 to demonstrate that the T8 antigen on cytotoxic human T cells undergoes a previously unreported protein modification. Immunoprecipitation of T8 with OKT8 from a $T8^+T4^+$ cell line prelabeled with $[^{32}P]PO_4$ demonstrates that T8 can be constitutively labeled with phosphate. T8 undergoes rapid and intense phosphorylation in serine upon addition of phorbol myristate acetate (PMA) to the cells. T8 phosphorylation is induced upon addition of heterologous, Epstein-Barr virus (EBV)-transformed B cells, which cause proliferation and are target cells for a cytotoxic $T8^+T4^+$ line and $T8^+T4^-$. Phosphorylation induced by targets is dose-dependent, rapid, and followed by a fast dephosphorylation. EBV-transformed B cells which do not induce proliferation of and are not targets for the $T8^+T4^+$ line and the $T8^+T4^-$ clone do not induce T8 phosphorylation. A $T8^+T4^-$ clone which proliferations in response to and kills targets only in the presence of lectin does not undergo B cell induced T8 phosphorylation. These data suggest that induction of T8 phosphorylation is antigen specific and is correlated with the cytotoxic response. Finally, preincubation of effector and target cells with an antibody to a monomorphic determinant of major histocompatibility complex (MHC) class I antigens reduces target-induced T8 phosphorylation to a greater extent than antibody to an MHC class II subregion (DR) monomorphic determinant, reinforcing the notion that MHC class I antigens interact with $T8^+$ cells.

T 301 Phosphorylation of T cell antigen receptor-associated proteins: correlation with activation for killing and/or for gamma-interferon production by a cytolytic T cell clone. Claude BOYER, Claire LANGLET, Annick GUIMEZANES, Michel BUFERNE, Christine HUA and Anne-Marie SCHMITT-VERHULST. Centre d'Immunologie INSERM-CNRS de Marseille-Luminy, Case 906, 13288 Marseille, CEDEX 9, FRANCE.
The activation-induced phosphorylation of T cell receptor (Ti) associated proteins was investigated in order to analyse possible signal-transduction mechanisms leading to distinct effector functions of a mouse cytolytic T cell clone (KB5.C20), which are target cell killing (not dependent on protein synthesis) and *de novo* production of gamma-interferon (g-IFN) (dependent on g-IFN gene expression). Ti-associated T3-like proteins were first identified by immunoprecipitation of ^{125}I-labeled cell surface proteins from 1% digitonin lysates of clone KB5.C20 by one and two (non reduced (NR)/reduced (R)) dimension gel electrophoresis. In addition to the α and β chains of the Ti (NR: 80 kD; R: 43 and 40 kD), two doublets of 35-37 kD (NR) and 32-34 kD (NR) leading to bands of 25, 16 and 14 kD (R) were identified, as well as three bands (25, 23, 22 kD (NR)) leading to 27, 25, 21 kD bands (R). Activation of clone KB5.C20 (prelabeled with ^{32}P-orthophosphate) with either anti-Ti mAb or exposure to both ionomycin (iono) and phorbol myristic acetate (PMA) induced the phosphorylation of 21 and 25-27 kD (R) Ti associated proteins, whereas exposure to either iono or PMA alone induced only weak phosphorylation of 21 kD (R) components. Functional studies suggested that activation for g-IFN production was observed only when both the 21 and 25-27 kD proteins were phosphorylated, whereas activation for killing (when measured by PMA induced non specific killing) may occur in conditions where no phosphorylation of the 25-27 kD protein was detected.

T 302 ROLE OF T CELLS IN B CELL ACTIVATION AND PROLIFERATION, Eiro Kubota, Jeffrey Wall, Richard W. Dutton and Susan L. Swain. University of California, San Diego, La Jolla, CA 92093.
Non-antigen specific helper T cell hybridomas (Th) were developed which in the presence of Concanavalin A (Con A) could activate the majority of normal resting B cells and stimulate considerable proliferation. These Th made BSF_1 and IL2 and were able to stimulate B cells of any haplotype to respond. The Th-dependent response was blocked by monoclonal antibodies (mAb) to B cell class II MHC antigen and to L3T4 on the T cells. Anti-L3T4 did not block lymphokine production by Th. By glutaraldehyde fixing the Th cells we were able to dissociate the stimulation into two events. Fixed Th cells would stimulate B cell activation and proliferation but only when exogeneous BSF_1 was added. Thus, we could identify two effects of Th: a direct T-B cell interaction, dependent on Con A which could be mediated by fixed Th and a lymphokine-mediated event requiring BSF_1. We suggest that the direct Th-B interaction may provide a signal transduced through class II molecules that BSF_1 acts both during activation and proliferation events.

T 303 EXPRESSION OF T CELL RECEPTOR IN CTL HYBRIDOMAS AND THEIR LOSS VARIANTS. Zelig Eshhar and Tova Waks, Department of Chemical Immunology, The Weizmann Institute of Science Israel,
Allorestricted CTL hybridomas that constitutively interact with, and lyse EL4 are reminiscent of memory cells in a sense that upon antigenic (or mitogenic) stimulation, a potentiation of the lytic activity and secretion of IL-2, IL-3 and GM-CSF could be obtained. Variants defective in various stages of target cell recognition, lytic process and inductive production of interleukines have been isolated from subclones of aged cultures. To better understand the mode of target cell recognition and induction of effector processes mediated by CTL, with the help of T. Yokota, K. Arai (DNAX) and M. Davis (Stanford), we cloned the α, β, and γ T-cell receptor genes, from cDNA library prepared form two independent hybridomas. Sequence analysis revealed that although the hybridomas had similar antigenic and allorestricted specificity, they differ in the variable region of both the α and β chains of the TcR. Upon stimulation of the CTL hybridomas with either EL4 cells or Con A, increased transcription of α and β TcR RNA was observed, followed by surface expression of the antigen specific receptor. Using specific $V\alpha$ and $V\beta$ probes we identified defective variants that do not transcribe the α or β chains. Some functional subclones lost their γ chains without impairing their response to the target cells. Such defined loss-mutants can serve as an ideal tool in complementation experiments in which the missing gene(s) can be transfected and regaining of specific function can be followed. Initial experiments along this line will be described.

T 304 PROPERTIES OF CLASS II MHC-SPECIFIC CYTOTOXIC T CELL CLONES; Paul V. Lehmann and Zoltan A. Nagy, Preclinical Research, Sandoz Ltd., 4056 Basel, Switzerland
Cytotoxic T lymphocytes (CTL) generated in the class II Mhc-disparate strain combinations B1o.AQR anti-B1o.T(6R) and vice versa were cloned and characterized for surface phenotype, specificity, and function. All clones recognized one of the inducing class II molecules, A^k, E^k, or A^q. No requirement for the recognition of additional cell-surface molecules (e.g., class I Mhc or non-Mhc) was noted, in order for the lysis to occur. The clones were either L3T4+Lyt2-, or L3T4-Lyt2+. Both phenotypes of CTL were induced equally well by all three stimulator molecules tested. Some L3T4+ clones, but not Lyt2+ clones, exhibited bystander killing of tumor targets in the presence of stimulator cells. However, bystander lysis was not demonstrable with lymphoblast targets. The data suggest that bystander killing is due to a lymphokine (perhaps tumor necrosis factor), which is not involved in the lysis of relevant targets by helper-type CTL.

T 305 PRELIMINARY CHARACTERIZATION OF MURINE PERFORIN 1 cDNA CLONES. D.M. Lowrey, F. Rupp, T. Aebischer, P. Grey, H. Hengartner and E.R. Podack. New York Medical College, Valhalla, NY 10595, University of Zurich, Switzerland and Genentech, Inc., San Francisco, CA.

In order to study the structure and function of Perforin 1, a Ca-dependent pore-forming protein contained in the cytolytic granules of murine CTL and NK, we have obtained a P1 cDNA clone by screening a recombinant λgt11 cDNA library using mono-specific polyclonal anti-mouse perforin 1 antisera. The clone directed the synthesis of a 150-160 kD fusion protein reactive with both anti-P1 and anti-β-galactosidase antisera on Western blot analysis. The deduced amino acid sequence of this clone could be aligned with the C-terminal human C9 protein sequence and possessed 27% homology. The homologous region includes the complete identity of the six cysteine residues of the EGFP-type cysteine rich domain contained in C9. The cloned CTL cell line HY3-AG3 expressed an RNA species of approximately 2.1 kb which hybridized under stringent conditions with radiolabeled insert DNA in Northern blot analysis. Several B and T cell lines examined by Northern blot analysis which do not kill target cells were negative. These results are consistent with the properties expected for a perforin 1 cDNA clone.

T 306 SELF REACTIVE DELAYED TYPE HYPERSENSITIVITY, David Naor and Itzhak Klein, The Lautenberg Center for General and Tumor Immunology, The Hebrew University-Hadassah Medical School, Jerusalem 91010, Israel.

X-irradiated or normal A mice injected with syngeneic concanavalin A- induced lymphoblasts (syn-Con A-blasts) developed inflammatory response in their footpads 24 h to 72 h after injection of syngeneic lipopolysaccharide-induced lymphoblasts into these tissues. This immunological activity was designated syngeneic delayed type hypersensitivity (syn-DTH), because Lyt-1^+ cells transferred the response to naive recipients. Analysis on Ultrogel or Sephadex G-50 columns revealed that a Con A blast extract contains two syn-DTH-stimulating antigens: a small antigen (6000-7000 dalton) and a large antigen (apparent M.W. of 175,000 dalton). This conclusion held true even when protease inhibitors were included in the fractionation procedure. The approximate molecular weights of these antigens estimated by the gel filtrations were confirmed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The large lymphoblast syn-DTH-stimulating antigen contains carbohydrate residues but not products of the H-2 genetic region. The small antigen does not contain glucosyl or mannosyl residues, but it expresses affinity to anti-H-$2D^d$ monoclonal antibody. The small antigen does not cross-react with the large antigen. Since A mice immunized with the small antigen generated syn-DTH after challenge with lymphoblasts of B10.T (6R) mice which share the H-$2D^d$ subregion with A mice but not the H-2K or the H-2I subregions, we suggest that genes located at the H-$2D^d$ subregion control the Syn-DTH.

T 307 **A NOVEL GLYCOPROTEIN ASSOCIATED WITH ISOLATED CAPS OF THE LYMPHOCYTE SIALOGLYCOPROTEIN (LSGP) AND THE LEUCOCYTE COMMON ANTIGEN (LC) ON THE THYMOCYTE SURFACE.** **Mary R. Newton, Christopher E. Turner and David M. Shotton, Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, England.**

We have identified a novel 205Kd Con A binding glycoprotein on the surface of rat thymocytes, associated with the isolated caps of 2 major thymocyte surface glycoproteins, LSGP and LC, induced by monoclonal antibodies W3/13 and OX 1. When either of these glycoproteins are capped, cytoskeletal proteins, including fodrin and actin, co-cap, and the capped cell surface glycoprotein becomes attached to the detergent-insoluble cytoskeleton. Isolated caps sheared from the residual cytoskeleton of detergent extracted capped cells contain the capped cell surface glycoprotein (either LSGP or LC), together with gp205 and actin. We are currently investigating the possibility that gp205 may function as a "protein X", postulated to mediate the interaction of other cell surface glycoproteins with the cytoskeleton.

T 308 LYT1$^+$, L3T4$^+$ CYTOTOXIC EFFECTOR T CELLS IN TUMOUR SURVEILLANCE, Atsuo Ochi and Greg Woods, Mount Sinai Hospital Research Institute, Departments of Immunology and Medical Genetics, 600 University Ave., Toronto, Ontario, Canada M5G 1X5

The effector mechanisms of Lyt 1$^+$ T lymphocytes in tumour immunity are not well defined. To study the function of Lyt 1$^+$ T cells in tumour specific immunity BALB/c mice (H-2^d) were immunized with a B cell hybridoma (A20-2JxB6; H-2$^{b/d}$) established from the fusion between an A20-2J cell line (H-2^d, Ia$^+$) and C57BL/6 spleen B cells (H-2^b). After 10 days the spleen cells were restimulated and cultured in vitro with mitomycin C treated A20-2J. T cell hybridomas derived from these in vitro cultured cells were screened for direct cytotoxicity against A20-2J and a particularly cytotoxic cell line against A20-2J, but not normal C57BL/6 spleen cells, was detected. This hybridoma (103L2) appeared to recognize an unique determinant on A20-2J and the A20-2JxB6 hybridoma; there was no evidence for cytotoxicity against a variety of tumour cell lines including pre-B, monocyte, T cell lines and LPS induced blast cells. In addition to this strong cytotoxicity this cell line released abundant amounts of interleukin 2 when stimulated by A20-2J target cells. Both the cytotoxicity and IL2 production were inhibited by antibodies to I-E^d and L3T4 and compatible with this finding the cell line was of the "helper" phenotype as it was Lyt1$^+$, L3T4$^+$, Lyt 2$^-$. In contrast to classical cytotoxicity this cell line was cytotoxic against a variety of non-participating bystander cells suggesting the production of soluble cytotoxic factors. We propose that this cell line represents a class of tumour surveillance and that this bystander killing of various cell types may prevent the escape of tumour antigen negative mutant cells from tumour surveillance.

T 309 CLONED T CELLS AS TARGETS FAIL TO INDUCE Ca^{++} MOBILIZATION AND KILLING IN EFFECTOR CTLs. Hanne L. Ostergaard and William R. Clark, University of California, Los Angeles, CA 90024.

Previous studies in our laboratory have shown that most cloned cytotoxic T lymphocytes (cCTL) and T helper (T_H) hybridomas are relatively refractory to lysis by other cCTLs even though these cells express class I MHC molecules and are able to form specific conjugates. One possible explanation for this relative resistance to lysis is that long-term T cell clones lack a recognition structure that is required to activate the lytic program in the cCTL effector. To determine if these cloned T cell targets are activating the cCTL effector cells, we measured the changes in intracellular free Ca^{++} in these latter cells after binding to cloned (resistant) or normal (lysable) target cells. We found that when a cCTL binds to its specific lysable target, there is an increase in intracellular free Ca^{++}. As expected there is no Ca^{++} influx when a nonspecific target is used. If a specific cloned T cell target is used, there is no Ca^{++} influx even though the appropriate class I molecule is present and a firm CTL-target conjugate is formed. An increase in effector CTL intracellular Ca^{++} is also seen when a nonspecifc but lysable target cell is pulsed with ConA, but not when a cloned T cells is pulsed with ConA. These results indicate that cCTL targets are unable to activate Ca^{++} mobilization in effector cells and may therfore be lacking an early activation signal.

T 310 CHARACTERIZATION OF NONLYTIC VARIANTS DERIVED FROM A MURINE CTL CLONE
Sueihua Pan, Department of Pathology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854

The mechanisms by which cytotoxic lymphocytes mediate the lysis of target cells involve complex biochemical and physiological processes. We have isolated several nonlytic variants from a murine CTL line, B6.cl 4. B6.cl 4 lyses H-2 K^b-bearing cells that are infected or transformed by SV40. One of the nonlytic variants, B6.4.127, is of particular interest. This variant clone expresses H-2 K/D, Lyt.2 and T cell receptor molecules on its surface at levels comparable to those of the lytic B6.cl 4 cell lines in FACS analysis. T cell receptor was studied utilizing four anti-idiotypic monoclonal antibodies. Immunoprecipitation of ^{35}S methionine labelled cell extracts with anti-granule antibodies demonstrates that B6.4.127 also contains high amounts of cytolysin in its' granules. Furthermore, this lack of killing can not be overcome by adding Con A in ^{51}Cr release assays.
Interestingly, the B6.4.127 clone is morphologically different from the parental, lytic B6.cl 4 clones. B6.4.127 appears flatter and looks more like a macrophage than a T-lymphocyte in culture.

T 311 LOSS OF ADHESION: A DISTINCT AND NOVEL EVENT DURING CTL-TARGET INTERACTION, John H. Russell, Deborah E. McCulley and Patricia Meleedy-Rey, Dept. of Pharmacology, Washington University Medical School, St. Louis, MO 63110.

The interaction between the CTL and its target provides a unique opportunity to investigate cell-cell interactions. In addition to the standard type of membrane lesion induced by soluble immune mediators the CTL initiates several events in which the target appears to play an active role. In this report we demonstrate a previously unrecognized consequence of this interaction--the loss of adhesiveness by adherent targets. Before obvious damage is done to the integrity of such a target cell, it loses its ability to adhere to a substrate. Such a phenomenon does not occur during target cell attack by soluble agents like Ab + C'. The CTL induced loss of adhesiveness is a unique event which can be separated from the actual lytic process by adding a calcium selective chelating agent at various times after initiating CTL-target interaction. A variety of third-party mixing experiments have demonstrated that loss of adhesion is the direct result of CTL-target interaction and is not mediated by soluble products produced during that interaction. The phenomenon has been observed with both cloned CTL and CTL harvested from an in vivo allograft rejection site (PEL) as effectors and with tumor and primary fibroblast as well as macrophage targets. This previously unrecognized T-cell function could play an important role in the initiation of both beneficial and pathological inflammatory processes.

T 312 MHC-RESTRICTED, ANTIGEN SPECIFIC, GRANULE EXOCYTOSIS FROM HELPER T CELLS: ANOTHER INDICATOR OF RECEPTOR MEDIATED T CELL ACTIVATION, Michael Taplits, Pierre Henkart, and Richard Hodes National Cancer Institute, Bethesda MD, 20892

Recently, it has been observed that some T cells contain trypsin-like serine esterases. Helper T cell clones derived in our lab contain high levels of these enzymes (BLT esterase), which are associated with high and low density cytoplasmic granules. Using supernatant release of these enzymes as a marker, we examined granule exocytosis as a consequence of T cell activation via the T cell receptor. Clones 8-5(A^b,KLH), 10-2(A^b,KLH), and A13(A^k,KLH) all released enzymatic activity over background(constitutive release) when cultured with antigen plus syngeneic antigen presenting cells (APC)(37%, 50%, 27% of total activity, respectively). APC alone contained negligible enzyme activity. Incubation of clones with syngeneic APC or with antigen plus non-syngeneic APC gave only background release. Other stimuli which induced strong granule exocytosis were conconavalin A and an antibody against the mouse T3 complex. Using antigen pulsed APC, exocytosis was detected as early as one hour after the initiation of T cell stimulation. In contrast, when activation was induced by incubation of cloned T cells with interleukin 2 (IL-2), no secretion above background was observed despite the fact that substantial DNA synthesis occurred. Thus, it appears that granule exocytosis may be another means of analyzing T cell activation pathways initiated by T cell receptor-ligand interactions. Furthermore, these activation sequences may be distinquished from those occurring after IL-2 interaction with its receptor, since this event does not induce granule exocytosis.

T 313 FUNCTIONAL HETEROGENEITY IN THE RESPONSE OF CLONED HUMAN INDUCER T CELLS TO ANTIGEN PRESENTED BY B CELLS, Dale T. Umetsu, Haifa H. Jabara, Peter Hauschka, and Raif S. Geha, Stanford Univ, Stanford, CA, Harvard Medical School, Boston, MA.

We examined the ability of a large panel of human inducer T cell clones (n=23) to respond to antigen presented by B cells or by monocytes. With one exception, all T cell clones examined responded to antigen presented both by monocytes and by lightly but not heavily irradiated resting B cells. One alloreactive clone, Clone A1, which recognized an HLA-DP associated antigen, proliferated in response to allogeneic monocytes, but not in response to allogeneic B cells expressing the stimulatory alloantigen. Clone A1 was not cytotoxic for the allogeneic B cells, and B cells did not exert inhibitory effects on the proliferative response of A1 to allogeneic monocytes. Pretreatment of B cells with neuraminidase did not reverse the inability of B cells to stimulate Clone A1. Allogeneic B cells failed to cause a rise in intracellular Ca2+ ion concentration and failed to induce IL-2 receptor expression in Clone A1. Neither recombinant IL1 (rIL-1) nor phorbol myristate acetate reversed the inability of Clone A1 to proliferate to allogeneic B cells. However B cells were capable of supporting the proliferation of Clone A1 to Sepharose bound OKT3, indicating that once cross-linking of the T cell receptor complex occurred, B cells could provide late secondary signals, which could not be provided for by rIL-1, for induction of proliferation of Clone A1. These results indicate that the failure of B cells to stimulate this clone was due to the inability of B cells to efficiently cross link the antigen receptor on this T cell clone, and suggest that Clone A1 recognizes an epitope of HLA-DP which is either absent, greatly diminished, or altered on B cells.

The T Cell Receptor

T 314 PROGRAMMED TARGET CELL SUICIDE IN IMMUNITY, David S. Ucker,
Medical Biology Institute, 11077 N. Torrey Pines Rd., La Jolla, CA 92037

The mechanism by which cytotoxic T lymphocytes (CTL) kill their targets is unresolved. CTL induce a cytotoxic process in target cells which, like the glucocorticoid-mediated cytolysis of immature thymocytes, effects a characteristic degradation of chromosomal DNA well before any cytoplasmic or membrane distintegration can be detected. To explore whether these two lethal processes act via common pathways, I have examined glucocorticoid resistant mutants for their susceptibility to CTL mediated killing. When selection schemes are designed to demand retention of hormone responses other than the cytotoxic effect, a class of so called "deathless" thymoma mutants can be obtained. I have found that such mutants are resistant to killing not only by glucocorticoids but by CTL as well! Complement-mediated killing, however, is unaffected in these mutants. Reversion studies demonstrate that resistance to both glucocorticoids and CTL can result from mutational events at a single genetic locus. These results provide evidence that cells possess an endogenous suicide pathway which can be triggered by different effectors. This is different from the mechanism of complement-mediated lysis: in CTL-mediated cytolysis, target cells play an active role in their own death!

The Receptor in Disease

T 315 T-CELL RECEPTOR GENE ARRANGEMENTS AND EXPRESSION IN NORMAL HUMAN LARGE GRANULAR LYMPHOCYTES (LGL) AND THEIR PATHOLOGICAL EXPANSIONS, Paola Allavena, Pier G.Pelicci*, Alessandro Rambaldi, Paola Pirovano, Fedro Peccatori, Riccardo Dalla Favera*, and Alberto Mantovani. Lab. of Human Immunology, Ist. "Mario Negri", Milano Italy. • Dpt. of Pathol. New York Univ. NY. USA.

The lineage to which normal LGL/NK cells belong is controversial; in fact they share some surface markers and functional activities with monocytes, but also with T lymphocytes. We have investigated the relationship of LGL to the T cell lineage with the analysis of T cell receptor (T-rec) genes. Pure preparations of human LGL and their $CD11^+$ $CD8^-$ and $CD11^-$, $CD8^+$ subsets had Tβ gene in its unrearranged germline configuration. Expression of Tα and Tβ genes was also not detectable. The organization of Tγ gene, which is of particular importance because it occurs early in T cell ontogeny was also found in its germline configuration.
A rare type of lymphoproliferative disorder termed Tγ-LPD is characterized by expansion of cells whith LGL morphology. These LGL have ADCC and some of them NK cytotoxicity, they express NK-associated markers (CD16 , HNK-1 and variably CD11 and CD19), produce IL-1 and IFN γ and have in vitro chemotaxis. Of 15 patients with Tγ-LPD studied for T-rec rearrangement, 12 displayed clonal (1 polyclonal) rearrangement of Tβ and Tγ loci and were CD3 +, while 2 cases were in germline configuration and were CD3 - . Similar to the very small subset of CD3+ LGL recently described, most Tγ-LPD cases are CD3 + and have T-rec genes rearranged. These data suggest that either a subset of LGL or a particular step of differentiation may be related to the T cell lineage; they also demonstrate that,in contrast to previous views, most TγLPD are monoclonal, presumably neoplastic, lymphoproliferative disorders.

T 316 EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS IN THE RAT: T-CELL RECEPTOR VARIABLE REGION GENE EXPRESSION.
Bernhard Arden, Hermann J. Schlüsener+, Hartmut Wekerle+, and Lee Hood, California Institute of Technology, Pasadena, CA 91125 and + Max-Planck-Society, Clinical Research Group for Multiple Sclerosis, Würzburg, West Germany.
Experimental allergic encephalomyelitis (EAE), an animal model for autoimmune disease, can be induced in Lewis rats by injection of myelin basic protein (MBP). T-cell lines specific for MBP have been isolated from Lewis rats. The MBP-specific T-cell lines can transfer EAE to naive syngeneic recipients. They recognize the encephalitogenic peptide sequence from amino acid residues 68-88 of MBP. These cells carry the $W3/25^+$, $OX8^-$ surface marker, a phenotype defining rat T helper/inducer cells. These T-cell lines recognize MBP with class II-MHC antigens on astrocytes and subsequently kill the antigen presenting cells. The aim of this study is to determine whether certain Variable gene segments are predominantly used in this T-cell response. Construction of cDNA libraries from messenger RNA of these T-cell lines is underway. The expressed V_α and V_β genes are isolated using mouse constant region probes, and their nucleotide sequences will be determined. The clonal heterogeneity in DNA genomic rearrangements of these T-cell lines is investigated using mouse β-chain probes.

T 317 PREDISPOSITION TO VIRUS-INDUCED AUTOIMMUNE MYOCARDITIS MAPS TO CHROMOSOME 14 OF THE MOUSE. Kirk W. Beisel, Emory Univ., Atlanta, GA 30322; Monica D. Traystman, Ahvie Herskowitz, Johns Hopkins Med. Inst., Baltimore, MD 21205; Muriel N. Nesbitt, Univ. California, San Diego, La Jolla, CA 92093

Previous studies have demonstrated that a non-Mhc gene(s) controls the predisposition of certain mouse strains to the development of Coxsackievirus B3 (CVB3)-induced autoimmune myocarditis. Recombinant inbred strains (RIS), AXB and BXA, were studied to determine the chromosomal location(s) of this susceptibility gene(s). The progenitor inbred strains of the AXB/BXA RIS lines are A/J and C57BL/6J, which are respectively susceptible and resistant to CVB3-induced autoimmune heart disease. Two-week-old animals with 10-20 animals per line were intraperitoneally infected with 10^5 TCID $_{50}$ of CVB3. Three weeks after infection, the animals were bled and the hearts removed for pathologic examination. Each individual serum was typed for the presence of heart-specific autoantibodies using an indirect immunofluorescence assay. The heart tissue sections were examined for the presence of active focal lesions and a diffuse interstitial mononuclear cell infiltrate. About 50% of the 27 AXB/BXA RIS lines developed autoimmune myocarditis as determined by the presence of heart-specific autoantibodies and myocardial inflammation. Analysis of the data demonstrated a linkage of both autoantibody and pathology with the nucleoside phosphorylase-2 (Np-2) locus on chromosome 14 with a map distance of ~7 cM. Recently, the AXB/BXA lines have been characterized for the Tcrα gene (Schwab, H, V Garr, MM Nesbitt and E Palmer, personal communication). Preliminary data indicates that the gene(s) controlling predisposition to autoimmune myocarditis is associated with or closely linked to the Tcrα. These data suggest that a single autosomal or set of linked genes on chromosome 14 controls the development of CVB-3 induced autoimmune myocarditis. Both the production of heart-specific autoantibody and heart inflammation appear to be under similar genetic regulation by the Tcrα linked gene(s). (This work was funded by a grant from the American Heart Association, Maryland Affiliate and by USPHS grants HL-30144 and HL-38276).

T 318 ANTIBODIES WHICH REACT WITH HUMAN T CELL ANTIGEN RECEPTOR BETA CHAIN V REGION FAMILIES IDENTIFY MALIGNANT T CELL PROLIFERATION AW Boylston, DM Clark, P Hall, and S Carrel. Departments of Pathology, St. Mary's Hospital and St. Bartholomew's Hospital, London, England and Ludwig Institute for Cancer Research, Lausanne, Switzerland.

Monoclonal Abs that react with normal human PBLs which use the VB5 or VB8 variable region gene families to form the Beta chain of their TCR have been used to study a panel of normal and malignat human lymphoid tissues. In reactive lymphoid tissues these Mabs stain individual cells and rarely if ever react with more than three contiguous cells. In the thymus cells in both the cortex and medulla react. The frequency of reactive cells suggests that most/all the T3 positive cells in the thymus (60-70% with UCHT1) also express mature TCR heterodimers.

In a panel of malignant T cell tumors about 10 % react with one of these Mabs, a frequency similar to the frequency of positive cells in PBLs. Unlike reative lymphoid tissue the staining is homogeneous and readily identifies small foci of positive cells. Thus such Mabs are tumor markers identifying the monoclonal proliferation. VB families can be subdivided by other Mabs which recognize a subset of the cells which react with the broader reagent. These Mabs allow a more precise definition of the malignant population.Anti VB Mabs have both diagnostic and therapuetic potential.

T 319 T CELL RECEPTOR β CHAIN REARRANGEMENT IN HYBRIDOMAS DERIVED FROM THE EXPANDED POPULATION OF DOUBLE NEGATIVE T CELLS IN MRL/Mp-lpr/lpr (lpr) MICE. Thomas W. Croghan, Rebecca Rapoport, and Philip L. Cohen, University of North Carolina, Chapel Hill, North Carolina 27514.

MRL mice spontaneously develop an autoimmune syndrome which closely resembles human Systemic Lupus Erythematosus. Expression of the recessive lymphoproliferation (lpr) gene on the MRL background results in acceleration of the disease and expansion of an unusual population of T cells, which fail to express L3T4 and Ly2, in the spleen and lymph nodes. Previous work from ths laboratory has shown diminished surface expression of the T cell receptor (TCR) on the aberrant cell population, although there appears to be polyclonal rearrangement of the β chain and normal, or even increased, amounts of full length transcript for the α, β, and T3δ subunits. In order to better appreciate the nature of the aberrant cells, we have derived a series of hybridomas from double negative lpr cells, using BW5147 as the fusion partner. Out of 19 lines from a single fusion, 13 had the Thy 1.2^+, $L3T4^-$, $Ly2^-$ phenotype. The remaining lines failed to express Thy 1.2 and were not further characterized. Lines with the lpr phenotype were cloned by limiting dilution. No clone expressed the TCR on the surface, as detected by FACS analysis with KJ16-133 and F23.1 monoclonal antibodies which are directed against epitopes encoded by $V_{\beta}8$ variable region family. Initial analysis of β chain rearrangement revealed only two patterns of D-J joining events in the 5 clones tested. These results suggest the presence an important subpopulation within the lpr lymph node which may contribute to the disease process.

T 320 RESISTANCE OF SWR/J MICE (H-2^q) TO THE INDUCTION OF ARTHRITIS WITH TYPE II COLLAGEN: INVESTIGATION OF POSSIBLE ROLE OF V_β T CELL RECEPTOR DELETION. Chella S. David, Subhashis Banerjee, Harvinder S. Luthra and John M. Stuart*. Mayo Clinic, Rochester, MN 55905; and *VA Medical Center, Memphis, TN 38104.
The susceptibility to the induction of arthritis with type II collagen in Freund's adjuvant in mice is linked to the H-2 complex, with mice of H-2^q and H-2^r strains being susceptible and other strains being resistant. However, SWR mice (H-2^q) are completely resistant to the development of collagen induced arthritis (CIA). This could potentially be due to the genomic deletion of close to 50% of their V_β TCR genes. To investigate this further, F_1 and F_2 crosses of SWR with B10 (H-2^b, a strain resistant to CIA and having a normal complement of TCR genes) were made and were injected with 100 μg of native bovine type II collagen (BII) in complete Freund's adjuvant intradermally. (SWR x B10)F_1 mice were backcrossed to both parents and mice heterozygous and homozygous for H-2^q were injected similarly with BII. Five of the 25 (20%) (SWR x B10)F_1 mice developed low grade arthritis when observed over 14 weeks following BII immunization. One out of 4 (25%) SWR backcrosses developed moderate grade arthritis when followed up over an 8 week period and 3 out of 20 (15%) B10 backcrosses developed moderate to severe arthritis 4 weeks after BII immunization. The F_2 and backcross mice are being monitored further for development of arthritis and their TCR V_β genes are being analyzed.
Supported in part by the Minnesota Chapter of the Arthritis Foundation.

T 321 T CELL RECEPTOR BETA CHAIN GENE POLYMORPHISMS ARE ASSOCIATED WITH CERTAIN AUTOIMMUNE DISEASES. A.G. Demaine, B.A. Millward, R.W. Vaughan and K.I. Welsh, Department of Molecular Immunogenetics, Guy's Hospital, London SE1 9RT, England.
Autoimmunity is thought to be a defect in the ability of the immune system to differentiate between self and non-self antigens. Autoimmune diseases are associated with such phenomena as elevated levels of activated T cells, autoantibodies and infiltration of the affected tissues. Many have associations with immunoregulatory genes,. particularly with major histocompatibility complex (MHC) class II antigens and immunogobulin allotypes. The recent cloning of the genes which encode the alpha and beta chains of the T cell antigene receptor (TCR) allows further investigation of the immunogenetic background. Using restriction fragment length polymorphism (RFLP) analysis and TCR probes we have studied patients with insulin dependent diabetes mellitus (IDDM), membranous nephropathy (MN) and Graves' disease (GD). Using the restriction endonuclease Bgl II, significant associations between a TCR constant beta chain RFLP phenotype (10.0;9.2 kilobase) and these particular diseases was found (P = 0.003; 0.003; 0.007 respectively). All these diseases are significantly associated with MHC class II antigen DR3. In GD 16/19 patients had both the 10.0;9.2 kb TCR beta phenotype and DR3, compared to 7/22 DR3 normal controls. These results suggest TCR genes contribute to the immunogenetic background of autoimmune diseases. In some diseases this association may be particularly significant in those individuals possessing DR3.

T 322 OLIGOCLONAL T LYMPHOCYTES IN THE CEREBROSPINAL FLUID OF PATIENTS WITH CHRONIC PROGRESSIVE MULTIPLE SCLEROSIS, David A. Hafler, Allan D. Duby, Soon Jin Lee, Deborah Benjamin, J.G. Seidman, and Howard L. Weiner, Harvard Medical School, Boston, MA 02115. We have investigated the T cell populations in the cerebrospinal fluid (CSF) of chronic progressive multiple sclerosis (MS) patients. Individual T cells from the CSF and blood were cloned prior to expansion and their clonotypes defined by analysis of rearranged T-cell receptor β-chain and γ-chain genes. Eighty seven T cell clones from blood and CSF of two patients with chronic progressive MS were examined for common T-cell receptor gene rearrangement patterns. In one patient, 18 of 28 CSF derived T-cell clones demonstrated common T-cell receptor gene rearrangements indicating oligoclonal T cell populations; in the blood, two patterns were found twice among 26 T cell clones. In another patient, five of 27 CSF derived clones had common T-cell receptor gene rearrangement patterns. In contrast, no common β-chain rearrangement pattern was found among 67 T cell clones derived from the blood or CSF of a patient with subacute sclerosing panencephalitis or among 20 clones from the CSF of a patient with herpes zoster meningoencephalitis. A subject with atypical, fatal MS of eight month's duration was also studied and did not have oligoclonal T cells in the CSF or blood. These results demonstrate that clonal expansion of T cells can occur in an immune compartment such as CSF during the course of a non-malignant inflammatory response.

T 323 THE T CELL RESPONSE IN EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS: CLONALITY AT THE LEVEL OF ANTIGEN SPECIFICITY AND T CELL RECEPTOR GENE REARRANGEMENTS. Mary Pat Happ, Aniko S. Kiraly, Halina Offner*, Arthur Vandenbark*, and Ellen Heber-Katz. The Wistar Institute, Phila., PA 19104 and *The Veterans Administration Medical Center, Portland OR 97207.

Experimental Allergic Encephalomyelitis (EAE), induced by immunization with myelin basic protein (MBP) in CFA, serves as a model for the study of CNS autoimmune diseases. Using T cell hybridomas to study the repertoire of the Lewis rat T cell response to guinea pig (GP) MBP, we found that fusion of a primary in vitro culture generated a population in which 60-70% of the hybrids responded to the major encephalitogenic determinant, residues 68-88, of GP MBP. When the primary in vitro culture was subjected to two further rounds of in vitro stimulation with GP MBP before fusion, approximately 90% of the hybrids were specific for 68-88. Southern blot analysis of rearrangements at the T cell receptor B chain locus revealed that all of the 68-88-specific hybrids share one of two rearranged bands that is not shared by those clones responding to non-encephalitogenic determinants. The use of one band versus the other seems to be dictated by use of $C_{\beta}1$ versus $C_{\beta}2$. In following the generation of GP MBP-specific long-term T cell lines we consistently observed the appearance of these two bands as the only detectable rearranged bands in the line, coinciding with the selection of 68-88-responding cells previously shown to occur in such lines. This suggests that these rearrangements observed in the hybrids are productive and relevant to the disease. That all of the 68-88-specific T cells relevant to the induction of EAE may share the usage of one particular T cell V_{β} gene suggests a basis for the anti-idiotypic nature of the protection against EAE induced by immunization with encephalitogenic T cell clones.

T 324 FAMILIAL T-CELL RECEPTOR COMPLEX DEFICIENCY, José R. Regueiro*, Miguel-López Botet+, Manuel Ortiz de Landazuri+, José Alcamí*, Alfredo Corell*, José M. Martin-Villa*, José L. Vicario* and Antonio Arnaiz-Villena*, Inmunologia *Hospital Primero de Octubre 28041 MADRID, and +Hospital de La Princesa, MADRID. (SPAIN)

A T-cell receptor complex expression defect has been found in two children belonging to an otherwise healthy Spanish family. One of the sibs (who had been vaccinated with attenuated poliomyelitis virus) showed immunodeficiency clinics with an autoimmune syndrome, but the other (older) sib (vaccinated with attenuated rubella, measles, mumps and poliomyelitis viruses)has been symptomless throughout life. In contrast to both sibs' normal expression of other peripheral leukocyte markers by flow cytometry (including CD1,CD2,CD4,CD8 and CD16) only about 6% of $CD2^+$ T cells expressed surface antigen-specific T-cell receptor (Ti), and only about 45% weakly expressed surface CD3 determinants; on the respectively remaining $CD2^+$ T cells the expression of Ti and CD3 was undetectable. Natural Killer (NK) activity was not increased in any of the sibs, ruling out a high content of NK cells among their $CD2^+$ lymphocytes. In vitro lymphocyte proliferative response to anti-CD3 was markedly impaired in both sibs, and the response was not restored by addition of rIL-2 to the cultures. PMA exerted a slight but significant synergistic effect with anti-CD3. In contrast, lymphocyte activation via CD2 in the presence of PMA was found to be normal. In addition, low lymphoproliferative responses to alloantigens, tetanus toxoid and PHA associated to lo IL-2 production were observed. Genes encoding for Ti alpha, beta and gamma chains did not show major alterations by Southern blot analysis, and beta chain genes rearrangements were detected in both children T cell blasts. Family clustering suggests a genetic pathogenesis, but linkage to HLA or other blood group markers has not been found.

T 325 ANALYSIS OF T-CELL RECEPTOR GENE REARRANGEMENTS IN T LYMPHOCYTES FROM CEREBROSPINAL FLUID, Francien T.M. Rotteveel, Ingrid Kokkelink and Cornelis J. Lucas, Central Lab. Netherl. Red Cross Blood Transfusion Service, incorporating the Lab. of Exp. and Clin. Immunology of the Univ. of Amsterdam, Amsterdam, The Netherlands.

Characterization of T cells, present in the cerebrospinal fluid (CSF) of patients with multiple sclerosis (MS), may contribute to the understanding of the putative immunopathologic role of these cells. To analyze the diversity of T cells in the CSF of MS patients, 30 cloned T-cell lines from each of two MS patients were surveyed for their patterns of T-cell receptor (TcR) β-chain gene rearrangements. Rearrangements of the gene segments of the T-cell receptor β-chain are required for functional β-chain gene expression. The availability of a T-cell receptor β-chain specific cDNA probe allows analysis of the gene rearrangement pattern with a number of restriction endonucleases. Southern blot analysis of the DNA of these T-cell clones indicated that all clones have rearrangements in the TcR β-chain genes. So far, no indications were obtained for identical rearrangement patterns in multiple clones from a single patient. An additional 30 clones of one patient are analyzed at present. The results suggest that if there is an oligoclonal population of T cells, it represents a relatively minor fraction of the total number of T cells in the CSF of MS patients. At present CSF T cells from patients with other neurological diseases (including 1 Guillain-Barré syndrome (GBS) patient and 1 post-infectious encephalomyelitis patient) are studied in a similar fashion. In a model study, the clonality of T-cell responses in vitro was studied. Repeated stimulation with tetanus toxoid was surveyed by analysis of TcR β-chain rearrangements.

The T Cell Receptor

T Cell Receptor Structure/function

T 326 ANALYSIS OF THE T CELL RECEPTOR α and β CHAINS OF KJ-23^{+}, I-E BINDING, T cell HYBRIDOMAS. Marcia Blackman, Terri Wade, Jerry Bill, John Kappler and Philippa Marrack. National Jewish Center For Immunology and Respiratory Medicine, Denver, CO 80206

The relative contribution of α and β chains to the specificity of a T cell receptor is not known. An examination of V gene usage in T cell receptors specific for closely related antigen and restricting molecules has not revealed a simple correlation between T cell specificity and particular Vα or Vβ genes. However, using a monoclonal antibody, KJ-23, we have identified an unusual Vβ (KJ-23) that often recognizes I-E. Southern blot analysis of KJ-23^{+} hybridomas reveals that all Jβ1 and Jβ2 gene segments can contribute to an I-E binding, KJ-23^{+} chain, indicating that the J component of the β chain is not critical for I-E recognition. We have examined the diversity of α chains that can be used in association with the KJ-23^{+} Vβ for I-E recognition. RNA isolated from hybrids was screened with a panel of Vα-specific probes and Jα-specific oligomers. The contribution of these data to our understanding of T cell recognition of MHC will be discussed.

T 327 **INDUCTION OF INOSITOL PHOSPHOLIPID HYDROLYSIS BY ANTI-T-CELL RECEPTOR ANTIBODY OR ANTIGEN STIMULATION IN A MURINE, ANTIGEN-SPECIFIC, HELPER T-CELL LINE.** **Ezio Bonvini, William L. Farrar[1], Karen DeBell, Thomas Hoffman, Richard Hodes[2] and Michael Taplits[2]; Lab. of Cell Biology, DBBP, CDB, US-FDA, Bethesda, MD 20892, [1]Lab. of Molecular Immunoregulation, BRMP, NCI-FCRF, Frederick, MD 21701 and [2]Immumology Branch, NCI, Bethesda, MD 20892.** **Several pieces of evidence indicate that a rise in cytoplasmic calcium, possibly mobilized from intracellular stores, may be part of the mechanism whereby the T-cell antigen receptor signal is transduced. Activation of inositol phospholipid turnover, which lead to the formation of the calcium-mobilizing molecule, inositol (1,4,5)trisphosphate (Ins(1,4,5)P_3), may represent an early event in transmembrane signalling by the antigen receptor. Clone 10-5-17 (a B10 I-A^b mouse spleen-derived L3T4$^+$, Lyt2$^-$, Keyhole-Limpet Hemocyanin (KLH)-specific, IL-2-dependent, helper T-cell line), which is positive for the T-cell receptor V_{Beta8} determimant recognized by the F23.1 antibody (F23.1Ab), was used in this study. 10-5-17 cells proliferate when treated with F23.1Ab conjugated to sepharose beads, or in response to KLH presented by I-A^b-positive antigen presenting cells (APC). Treatment of [^{3}H]-inositol-prelabeled 10-5-17 cells with sepharose-F23.1Ab resulted in rapid intracellular accumlation of [^{3}H]-inositol phosphates ([^{3}H]-InsP, [^{3}H]-InsP_2 and [^{3}H]-InsP_3), concomitant with a decrease in the proportion and of [^{3}H]-inositol-labeled phosphatidylinositol (4)phosphate and phosphatidylinositol (4,5)bisphosphate. Accumulation of [^{3}H]-inositol phosphates was also observed after treatment of 10-5-17 cells with KLH-pulsed, T-cells-depleted, spleen cells from B10 mice, although a delayed response (30 min.) was observed, probably due to a requirement for cell-to-cell contact. KLH-pulsed, B10.A APC failed to elicit a similar response.**

T 328 CHARACTERIZATION OF T CELL RECEPTOR GENES DISTINCT FROM THE MATURE α/β HETERODIMER. David Cohen[+], Jean-Pierre deVillartay[+], Ellen Nielsen[+], Frits Koning*, David Lewis°, and John Coligan*. [+]LCB, NIDDK and *LIG, NIAID, NIH, Bethesda, MD and °Dept. of Pediatrics, University of Washington, Seattle, WA.
The isolation of additional T cell receptor (TCR) genes may help to clarify the pathways of T cell differentiation, the process by which a T lymphocyte learns to recognize self major histocompatibility antigen, and the diverse function of helper and suppressor T lymphocytes, which have been postulated to reside in distinct TCR's. As the first step in understanding the function of these additional TCR's, we are seeking to define the scope of the TCR-γ gene family, and of the genes encoding proteins which pair with γ to form a TCR. We will present sequence data concerning TCR cDNA clones which we have isolated and characterized from immature T cell tumors, and data concerning their expression in immature human and mouse thymic tissue. Two such human γ-related clones can be differentiated from each other by mRNA size (1.9 vs. 2.0Kb) and by their pattern of hybridization. One clone is expressed in the T cell lines PEER, HPB-ALL, and K7, while the other is expressed in K7, but not in PEER or HPB-ALL. Studies will be presented to distinguish whether these clones represent 2 related members of a gene family, similar to Cγ1 and Cγ4 in the mouse, or whether they correspond to some other unexpected transcripts.

T 329 ANTIGEN SPECIFIC MHC UNRESTRICTED T CELL HELP: EVIDENCE FOR DUAL RECOGNITION. Mary K. Crow, Juan A. Jover, Elliot K. Chartash and Steven M. Friedman, The Hospital for Special Surgery, New York, N.Y. 10021.
The mechanism by which T cells are constrained to recognize antigen only in association with self MHC determinants remains unresolved. We recently reported that direct interaction between cloned allospecific human helper T (T_h) cells and allogeneic B cells bearing the relevant DR antigen leads to the expression of a B cell specific activation antigen, BLAST-2. Presumably, BLAST-2 expression is triggered by binding of the T_h cell antigen receptor to DR antigen on the B cell surface. If so, T_h-induced BLAST-2 expression may provide a sensitive assay for investigating the antigenic structure recognized by the T cell receptor. To this end, we have examined the induction of BLAST-2 by a cloned, trinitrolphenyl (TNP) altered-self reactive human T_h cell, E-11. After 16 hours of coculture with E-11 cells, > 30% of all TNP-modified allogeneic B cells, regardless of their DR haplotype, are induced to express BLAST-2. While E-11 does not trigger BLAST-2 expression on unmodified B cells, even if they express the "restricting" DR antigen, we have determined that E-11 cells incubated with TNP (but not DNP) conjugated soluble proteins or TNP-conjugated Ia^- cells induce BLAST-2 on unmodified B cells. These results suggest that the T cell receptor for antigen consists of two distinct binding sites which appear to function sequentially. One is a high affinity (or easily accessible) site, which recognizes foreign antigen alone. The other is a low affinity (or normally inaccessible) MHC binding site which is either induced, increases its affinity for, or is exposed to bind polymorphic class II determinants only after the binding site for foreign antigen has been engaged.

T 330 DIVERSITY OF T-LYMPHOCYTE ANTIGEN RECEPTORS IN RESPONSES TO HAPTENS AND TO AUTOANTIGENS, Jörg T. Epplen, Johanna Chluba, Ari Hinkkanen, Ute Hochgeschwender, Bettina Kempkes, Victor Steimle and Hubertus Stockinger, Junior Research Unit, Max-Planck-Institute for Immunobiology, PO Box 1169, D7800 Freiburg, FRG.
Vertebrate T lymphocytes are able to recognize specifically a multitude of antigens in the context of MHC-encoded glycoproteins by their T cell receptor (TCRs). The diversity in antigen/MHC specificities results from the combination of multiple germline elements, junctional and so-called N-region diversity as well as combinatorial association of the TCR α and ß chains . We investigated the actual repertoire in a "primary" response of C57/BL6 (B6) mice to a hapten: Almost 40% of the T cell clones in a panel H-$2K^b$/TNP-specific killers expressed an identically composed α/ß chain combination as their TCR (Hochgeschwender et al. 1986a,b). These results are currently compared with a panel of T cell clones from hyperimmune mice. In addition the TCRs have been analyzed in several autoimmune situations: i) Autoreactive T cell clones and hybridomas from B6 mice that secreted Interleukin-2 after stimulation with MHC class II antigens (i.e. IA^b) alone; ii) MHC class II-restricted autoreactive T cell clones from patients with reactive arthritis; iii) Autoaggressive rat T cell clones specific for myelin basic protein that elicit experimental autoimmune encephalomyelitis. Implications of the TCR structure on the pathogenesis of autoimmune disease are discussed.

Supported by the Deutsche Forschungsgemeinschaft.

Hochgeschwender, U. et al. Nature 322, 376 (1986a).
Hochgeschwender, U. et al. (1986b submitted).

T 331 ANALYSIS OF T-CELL RECEPTOR STRUCTURE AND FUNCTION USING CHIMERIC T-CELL RECEPTOR/IMMUNOGLOBULIN MOLECULES. Pila Estess, Mark Davis and Vernon T. Oi. Becton-Dickinson Immunocytometry systems, Mt. View, CA. and Stanford University Medical School, Stanford, CA.
The similarity between T-cell receptor alpha and beta chains and immunologlobulin heavy and light chains has been the basis for a series of molecular constructions designed to test structure/function relatedness between the two kinds of effector molecules. Chimeric DNA molecules having murine dansyl specific immunoglobulin heavy and light chain variable region DNA spliced 5' to T-cell receptor alpha and beta constant region DNA have been constructed and transfected into murine T and B cell lines. Preliminary northern blots indicate expression of TcR mRNA in Sp2/0 cells, although no dansyl binding molecules are detectable on the cell surface. Analysis of intracellular and cell surface chimeric proteins is in progress. Additional constructs having TcR variable regions spliced to immunoglobulin heavy and light chain constant regions have also been constructed and will be similarly transfected. It is our expectation that such molecules will provide useful information about the tertiary nature of T-cell receptors and be valuable reagents in the study of intermolecular interactions in the Ti complex.

T 332 ANALYSIS OF THE BALB T-CELL REPERTOIRE TO ANTIGENS OF DEFINED SEQUENCE AND CONFORMATION,
A. Fotedar, M. van Hoof, P. Kilgannon, J. Ratanavongsiri, M. Boyer, A. Fu, T.G. Wegmann and B. Singh, Dept. of Immunology, University of Alberta, Edmonton, Alberta CANADA T6G 4R7.

The T cell receptor genes encoding the α and β chains of the T cell receptor are being analyzed from our panel of T cell hybridomas reactive to beef insulin and poly 18 (a synthetic polypeptide antigen of defined sequence and conformation). Beef insulin reactive IA^d restricted T cell hybridomas obtained from BALB mice can be classified into two groups on the basis of antigen specificity. The first group of hybrids are reactive to beef insulin, but not pork insulin; A20.2.15 and L38 are members of this group. The second group of hybridomas are reactive to both beef and pork insulin; B8C3X is a prototype member of this group. The α and β T cell receptor genes expressed in A20.2.15 a prototype Group I hybrid were analyzed by sequencing cDNA clones. The β gene expressed by this cell uses $V_{\beta1}$, $D_{\beta1.1}$, $J_{\beta1.1}$. The α gene expressed uses novel $V\alpha$ and $J\alpha$ genes not previously described. L38, a beef insulin hybrid with an antigen specificity very similar to A20, does not use $V_{\beta1}$, but expresses $J_{\beta1.1}$. This is confirmed by the finding that antisera raised against synthetic $J_{\beta1.1}$ peptides precipitate heterodimers suspiciously like TCR molecules from both A20 and L38 but not other T cells. On the other hand B8C3X (a Group II hybrid) has deleted $C_{\beta1}$ and does not use $J_{\beta1.1}$. This data seems to imply that antigen specificity in this particular case seems to follow more $J\beta$ than $V\beta$. We are currently studying the BALB anti-poly 18 T cell response, to assess the generality of these findings.

T 333 SECRETION OF CHIMERIC T CELL RECEPTOR-IMMUNOGLOBULIN FUSION PROTEINS,
Nicholas R.J. Gascoigne*, Christopher Goodnow, Karla Dudzik and Mark M. Davis, Scripps Clinic and Research Foundation, La Jolla CA 92037(*), Stanford University, Stanford CA 94305.

To enable us to produce large quantites of soluble T cell receptor protein for detailed biochemical and biophysical studies, we have investigated the use of immunoglobulin-T cell receptor gene fusions. We have constructed chimeric genes containing T cell receptor variable regions and immunoglobulin constant regions. When such genes are transfected into plasma cells, chimeric proteins are produced that express both immunoglobulin and T cell receptor antigenic determinants. These include a T cell receptor idiotypic determinant and the protein A binding sites of IgG. A chimeric heavy-chain protein containing the T cell receptor alpha-chain variable domain and the constant domains of immunoglobulin- 2a associates with a normal immunoglobulin -light chain to form a tetrameric (H_2L_2) immunoglobulin molecule which is secreted. Substantial amounts of the protein are easily purified by passage over protein A columns.

T 334 CHIMERIC T-CELL RECEPTOR GENES AS TOOLS IN ANALYZING T-CELL/TARGET-CELL INTERACTIONS.
Joan Goverman and Leroy Hood, California Institute of Technology, Pasadena, CA 91125.

We have recently proposed a model of T-cell antigen recognition in which the T-cell receptor and immunoglobulin (Ig) molecules have structural and functional similarities (Goverman, J., Hunkapiller, T. and Hood, L., Cell 45, 475, 1986). In order to test the premise of this model, we have constructed hybrid T-cell receptor genes in which the variable regions of the T-cell receptor are replaced with the variable regions of an Ig molecule specific for the antigen phosphorylcholine. We are introducing chimeric genes into T cells to generate T cells specific for a soluble antigen rather than cellularly presented, MHC-associated antigen. Using these transfected T cells and the ability to conjugate the antigen to different types of target cells, we will examine independently the interactions of T-cell cell-surface molecules and target-cell molecules and the influence of these interactions on T-cell activation and effector function.

T 335 MOUSE ANTIBODY TO THE MURINE HOMOLOGUE OF T3, Kathryn Haskins, Barbara Davis Center for Childhood Diabetes, U. Colo. Health Sciences Center, Denver, CO 80262.

We have immunized NZB mice with the T3 complex isolated from a human T cell line, HPB-MLT (HPB), to see if antibodies could be raised that crossreact with the murine T3 equivalent. Three animals were immunized with an affinity-purified preparation of the T3 complex and after 3-5 immunizations, all of the mice were producing antisera that precipitated the T3 proteins from mouse T cell hybridomas, as well as from the human line. The antisera was characterized by immunoprecipitation of surface-labeled whole cell lysates and subsequent analysis by SDS-PAGE. Autoradiograms of gels showed two low molecular weight (MW) bands, one at about 20,000 daltons and another somewhat lower, in lanes containing samples from immune mice, but not in a control which was run with serum from an unimmunized mouse. If samples from immune mice were reduced and run in the 2nd dimension, in addition to one or two spots lying on the diagonal in the 20-25 kd region, a third spot with an apparent MW of 20-21 kd could be observed slightly above the diagonal. Spleen cells from two of these animals were fused to the mouse myeloma P3-X63Ag8.653 to obtain monoclonal antibodies to murine T3 molecules. At the time of writing, one monoclonal antibody has been obtained which binds to two mouse T cell hybrids in addition to HPB.

T 336 Molecular Analysis of the Antigen Receptor of Myoglobin-specific T Cell Clones. Ishihara, Toshiki, Alexandra Livingstone, Tullia Linsten, Mark Davis and C. Garrison Fathman, Medicine/Immunology, Stanford University, Stanford, CA 94305

Despite all the information which has been generated concerning the T cell receptor for antigen, little is known about the structure/function relationship of the ternary complex of the T cell antigen receptor, antigen receptor, antigen and MHC. In order to investigate this, we have established four sperm whale myoglobin reactive T cell clones (8.2, 9.4, 11.3 and 12.2) which are derived from different individual mice, but have similar recognition patterns to a panel of synthetic peptides defining the epitope 112-118, and are restricted by the I-E^d molecule. These T cell clones were examined for rearrangement of their T cell receptor alpha and beta chain genes. Southern and Northern blot analyses of beta chain genes showed that these T cell clones all utilized V_βC5.2, J_β2.7 and C_β2. Rearrangement of the alpha chain genes for these four clones was also examined. Using the pulsed field gel electrophoesis technique, it was shown that all four T cell clones had different patterns of alpha gene rearrangement. To investigate alpha gene usage, six probes from different V alpha gene families were used to hybridize with Northern blot filters. Data suggested that similar V alpha genes might be used by these four clones. However at least two different $\mathcal{J}$ alpha genes were used by Northern blot analysis with several $\mathcal{J}$ alpha probes. Sequence studies are underway to confirm the expected identity of V beta usage and to examine the question of possible homology among V alpha usage.

T 337 CORRELATION OF T CELL RECEPTOR STRUCTURE AND FUNCTION. N. A. Johnson, F.M. Carland, J. Heilig*, S. Tonegawa*, P.M. Allen+, and L. H. Glimcher, Harvard Sch. of Public Health, Boston, MA, *Mass. Inst. of Tech., Cambridge, MA and +Washington Univ. Sch. of Med., St. Louis, MO, USA

Correlating T cell receptor gene structure with patterns of functional T cell activation requires a very well- defined nominal antigen, a panel of cloned T_H cells specific for that nominal antigen, and a way to define the recognition site(s) in the Class II molecule. We have established such a system in our laboratory by producing a panel of cloned T cell hybridomas specific for the well-defined protein antigen hen egg lysozyme (HEL), identifying the precise location of the HEL amino acids recognized by such T cells and utilizing a panel of cloned Class II mutant antigen-presenting cell lines to define sites on the Class II molecule that interact with the T cell receptor and the HEL moiety. This analysis has revealed multiple distinct specificities recognized by the panel of HEL-specific T hybridomas, consistent with the enormous diversity of the T cell repertoire. We have now extended these observations to the molecular level by sequencing the T cell receptor alpha and beta chain genes isolated from cDNA libraries of eight of these hybridomas. The combinatorial relationship of the variable, diversity and joining segments of these hybridomas' α and β chain genes to antigen/Ia recognition will be discussed.

The T Cell Receptor

T 338 BIOSYNTHESIS OF THE HUMAN TCR. EVIDENCE FOR FREE INTRACELLULAR β CHAIN PROTEIN AND ASSOCIATION OF β CHAIN WITH CD3 IN ABSENCE OF α CHAIN. Frits Koning, Andrew M. Lew, W. Lee Maloy, Robert Valas and John E. Coligan, LIG, NIAID, NIH, Bethesda, MD 20892.

Studies on the biosynthesis and assembly of the TCR-CD3 complex have been seriously hampered by the lack of reagents specific for the individual components of the complex. Using recently developed anti-peptide antisera specific for the TCR α and β chains, and the CD3 δ chain, we have studied the biosynthesis and assembly of CD3-TCR in a $CD3^+$ cell line, a $CD3^+$ T cell clone and in several $CD3^-$ T cell lines (as judged by FACS-analysis). In all cell lines tested, a 30-35 kD molecule could be precipitated with the anti-β chain antiserum after metabolic labeling. N-terminal amino acid analysis of this protein from two cell lines confirmed that this protein is the TCR β chain. Moreover, part of this β chain protein is intracellularly associated with the CD3 complex, even in $CD3^-$ cell lines which have no α-chain mRNA. Although both the intracellular β-chain and CD3 proteins contain N-linked glycosylation unit(s), extensive charge heterogeneity due to sialic acid residues is lacking, indicating that the majority of these proteins have not undergone final processing associated with cell surface expression. The results show that both TCR expressing and non-expressing cell lines can produce β chain which associates intracellularly with the CD3 complex. This strongly supports the notion that the α chain protein is the limiting factor in TCR expression.

T 339 CHARACTERIZATION OF MURINE T3 AND T3-ASSOCIATED MOLECULES. R.T. Kubo, M.L. Pigeon, W. Born, P. Marrack, J. Kappler. National Jewish Center for Immunology and Respiratory Medicine, Denver, CO.

Serologic identification of the murine T3 has been achieved using antisera prepared from Armenian hamsters immunized with affinity purified human T3. These antisera are capable of immunoprecipitating the human T3/T cell antigen receptor (TcR) complex, and also they detect similar molecular complexes by immunoprecipitation of surface labeled murine T cells indicating the presence of a cross-reactive epitope(s) on human and murine T3. Several hamster-mouse hybridomas secreting anti-human T3 monoclonal antibodies (Mab) which cross react with murine T3 as assessed by immunoprecipitation have been produced. These Mab do not detect surface expressed T3 epitopes as they did not react with live T cells by immunofluorescent staining nor did they activate T cells. Using the mild detergent,digitonin, to prepare lysates of surface labeled thymocytes from day 16 and 17 fetal thymuses, the anti-T3 Mab immunoprecipitated, in addition to the T3 molecules, a heterodimeric molecule (approximate molecular weight of 80,000) composed to two subunits of 45 kD and 34 kD. This heterodimer was not detected by a xenogeneic pan-reactive rabbit anti-mouse TcR antiserum and may represent an new class of TcR molecule.

T 340 POSSIBLE ROLE OF NONRANDOM DNA SEQUENCE VARIATION IN THE EVOLUTION OF IMMUNE GENE STRUCTURE AND EXPRESSION, Gene Levinson and George A. Gutman, University of California, Irvine CA 92717.

Repetitive DNA sequences provide raw material from which a variety of important controlling elements may arise, including (but not restricted to) a variety of immune system functions. Many controlling elements contain simple tandem repeats; examples include sites that function in generation of T-cell receptor and antibody diversity, immunoglobulin class-switching, and transcriptional enhancement of mouse MHC genes. Computer analysis of a broad range of published and unpublished sequence data suggest that such simple tandem repeats arise by accumulation of tandem insertions, apparently generated by a slipped-strand mispairing mechanism.

Modulated methylation of CG dinucleotides is a mechanism involved in control of gene expression. Computer analysis of sequence data suggests that elimination of CG dinucleotides by deamination, at sites where they are not needed, may have contributed to dramatic patterns of nonrandom sequence composition evident in both peptide coding and noncoding regions of a broad range of genes, including genes involved in immune responsiveness.

T 341 HETEROGENEITY OF THE T CELL RESPONSE TO SPERM WHALE MYOGLOBIN, Alexandra M. Livingstone, Jonathan B. Rothbard and C. Garrison Fathman, Stanford University, Stanford CA 94305.
Antigen-specific T cell proliferative lines were established from individual DBA/2 mice immunised with sperm whale myoglobin. We had previously used synthetic peptides to identify two epitopes (spanning residues 70-78 and 112-118 respectively) within the middle cyanogen bromide fragment (residues 56-131) of sperm whale myoglobin. The lines were therefore selected for reactivity to fragment 56-131, then cloned by limiting dilution. An initial screen with synthetic peptides divided the clones into three groups: 1) specific for epitopes involving residue 109; 2) specific for epitopes within the sequence 110-121; and 3) specific for unidentified epitopes within residues 56-131. Analysis of these clones on the fluorescence-activated cell sorter, using monoclonal antibodies specific for T cell receptor V_β gene products, suggested that I-E^d -restricted clones showed a disproportionately high usage of the TcR $V_\beta C5$ ($V_\beta 8$) gene family (see the abstract by P. Morel et al.). Further analysis of these clones showed that the T cell response to a defined antigenic region could be extremely heterogeneous. Three distinct reactivity patterns could be observed for I-E^d -restricted clones specific for epitopes within the eight residue sequence 111-118. Similarly, clones specific for epitopes involving residue 109 had three completely different reactivity patterns on synthetic peptides spanning this residue. To determine the lower limits of this heterogeneity, we have asked whether clones with indistinguishable antigen specificity and MHC restriction, from different mice, use the same T cell receptor genes. The results are discussed in the accompanying abstract by T. Ishihara et al.

T 342 NEW INTERACTIONS OF HUMAN T CELL RECEPTORS IN RESPONSE TO ANTIBODY BINDING, Nadia Marano, David Holowka and Barbara Baird, Cornell University, Ithaca NY 14853.
Interactions and redistribution of the T3 complex on T-cell line Jurkat were examined using the anti-T3 monoclonal antibody OKT3. Binding ^{125}I-OKT3 to Jurkat cells is complete in <10min. at 4°C. When the cells are warmed to room temperature or 37°C in the presence of ^{125}I-OKT3 there is an energy-dependent 33-100% increase in binding sites. This increase may be due to expression of latent receptors from a cytoplasmic pool or limited receptor recycling. No evidence was found for "shedding" of the T3 antigen at room temperature or 37°C. When cells with bound ^{125}I-OKT3 are incubated without any additional antibody some radioactivity is released but the T3 antigen remains cell associated as it is able to rebind antibody when fresh ^{125}I-OKT3 is added. After overnight incubation of cells at 37°C with unlabeled OKT3 very little ^{125}I-OKT3 can bind compared to cells that have not been exposed to antibody, but after incubation with ^{125}I-OKT3 overnight all initially bound radioactivity remains cell associated. Evidence that internalization is occuring comes from the observation that less than 30% of the bound ^{125}I-OKT3 is acid dissociable after 1-2 hours at room temperature compared to almost 90% when the cells are at 4°C. Cytoskeletal association of the T3 antigen in response to ^{125}I-OKT3 was examined. The divalent antibody which crosslinks T3 renders 25-40% of the T3 resistant to solubilization by Triton X-100 compared to only 10-15% with monovalent Fab' fragments. Thus crosslinking of the T3 complex appears to facilitate new interactions with cytoskeleton which may be similar to those cytoskeletal associations shown to correlate with cell triggering in the IgE receptor system.

T 343 ANALYSIS OF HUMAN T CELL AND ANTIGEN RECEPTOR REPERTOIRE IN KIDNEY ALLOGRAFT REJECTION. C. Miceli, T. Barry, O. J. Finn., Div. Immunology, Duke Univ. Med. Center.
Infiltrating T cells present in needle biopsies of kidneys undergoing rejection have been established as long term lines. Over 45 biopsies have been cultured. Every cell line analyzed exhibits specific cytotoxic killing directed against one or more of the donor HLA specificities. While most cell lines contain a mixture of T4+ and T8+ subsets, a predominance of T4+ cells correlates with class II antigen directed killing. A T4+, HLA-DR-3 specific clone has been isolated from one parental line confirming a role for T4+ class II specific cytotoxic T cells. In a number of cell lines a subpopulation of cells co-expressing T4 and T8 antigens has been observed. Coexistence of three subpopulations in bulk cultures (T4, T8, T4T8) has allowed us to analyze different T cell subsets from the same rejection and to correlate their function and phenotype with their fine specificity for donor antigens. MH3 line is a mixture of T4, T8 and T4T8 cells cytotoxic against donor antigens HLA-A11, -A29 and -DR7. All three cell subsets have been isolated and their function and specificities analyzed. Northern blots have shown these T cells to express Ti α and β but not γ messages. We are currently analyzing the T cell receptor gene repertoire used by these T cells. Predominant Tiβ gene rearrangements have been seen on Southern blots from EH1 and J2L bulk cell lines. EH1 is a predominately T4+, donor specific, cytotoxic T cell line that has been cultured for only 6 weeks prior to DNA extraction, while J2L is a T4+ DR-3 specific cytotoxic line that has been carried for 2 years. Each of these lines exhibits only one rearranged Tiβ band. This preliminary data suggests the expansion of a limited number of T cell clones within an allograft.

T 344 MECHANISMS OF IMMUNE RESPONSE GENE CONTROL, Kazumasa Ogasawara, W. Lee Maloy and Ronald H. Schwartz, Laboratory of Immunolgy, NIAID, MD 20892. Immune response (Ir) gene regulated nonresponsiveness is thought to arise by one of two distinct mechanisms. Either the major histocompatibility complex (MHC)-encoded molecules fail to physically interact adequately with the antigen, preventing activation of T cells with appropriate receptors, or they limit the expressed repertoire of T cell clones such that no T cells are available to be activated by existing complexes of MHC-encoded molecules and antigens. However, the relative importance of each of these mechanisms has not been clearly established. The antigenic determinant consisting of residues 43 to 58 of the pigeon cytochrome c sequence has been mapped to identify residues that may interact with the T cell receptor and those that may interact with the Ia molecule. By using single amino acid substitutions at these positions, it was possible to change the peptide without altering its ability to interact with the MHC-encoded molecule, thus providing a method for selectively probing for holes in the T cell repertoire. In B10 mice 18 different analogs were tested and all were immunogenic, eliciting at least some T cell clones specific for the immunogen. These experiments demonstrate the vast potential of the T cell population to recognize many different analogs, each in a unique way, and suggest that constraints on the diversity of the T cell repertoire may not be a major explanation for Ir gene defects.

T 345 EXPRESSION AND REACTIVITY OF A HYBRID T CELL RECEPTOR, Jennifer Payne and Brigitte T. Huber, Tufts University School of Medicine, Boston, MA 02111.
During T cell antigen recognition, three molecules: the T cell antigen receptor (Tcr), a major histocompatibility antigen, and foreign antigen, associate to trigger T cell activation. Precisely how the trimolecular complex aggregates is unclear. Several insulin-specific T hybridomas of helper phenotype have been isolated and characterized in this laboratory. Three, differing in Ia restriction and antigen fine specificity were selected for these studies. Fusion of two of the T hybridomas, which use different but closely related Tcr α and β genes, generates a novel reactivity in the resulting double hybridomas. We believe the new reactivity is due to the expression of a hybrid Tcr. The expression of the hybrid Tcr is being verified by several methods. Tcr α- and β-loss variants have been identified and are being isolated to: 1) transfect with non-parental α- or β-cDNA in expression vector constructs; 2) fuse with reciprocal loss variants. Idiotype expression and further antigen binding characteristics will be examined in the hybrid as compared to the parental Tcr. This information may reveal the contribution of the Tcr and chains to the combining site(s) of the Tcr molecule. Understanding of the Tcr in mature T cells could (also) provide a molecular framework to apply to potential Tcr binding during such developmental events as the induction of tolerance and T cell education.

T 346 TRANSFECTION ANALYSIS OF THE CONTRIBUTIONS OF TI α AND β CHAINS TO T CELL SPECIFICITY. T. Saito, A. Weiss, J. Miller, and R.N. Germain, Laboratory of Immunology, NIAID, NIH, Bethesda, MD 20892
To determine the contribution of the clonotypic Ti $\alpha\beta$ heterodimer to the antigen and/or MHC specificty of mature T cells, we have transfected expressable forms of cloned Ti α and/or β genes into either human or mouse T cells, and analyzed the transfected cells for Ti-T3 expression and responses to various combinations of antigen and Ia molecules. Introduction of either the Ti α or β gene of the 2B4 T hybridoma (pigeon cytochrome C responsive in the context of EβkEα) into T3$^-$ human Jurkat T cells led to re-expression of surface T3, and the ability to induce IL-2 production by stimulation with anti-clonotypic or anti-T3 antibodies reactive with the human-mouse Ti-T3 complexes. However, neither of these mixed Ti molecules mediated specific responses to pigeon cytochrome plus EβkEα. Jurkat cells transfected with and expressing both the mouse α and β gene products from 2B4, in contrast, responded to antigen with the same fine specificity and MHC restriction as the donor mouse T cell. Modulation experiments established that only the expected Ti $\alpha\beta$ heterodimers were associated with T3 on these Jurkat cells. These data thus establish that the a single receptor structure (the Ti$\alpha\beta$ heterodimer) is necessary and sufficient to define the dual specificity of Ia-restricted T cells. Additional transfection experiments in which the 2B4β gene was expressed in mouse T hybridomas of related but distinct specificity demonstrated the formation of new receptors with a predictable mixed specificity, consistant with the β gene product playing a major role in the MHC restriction specificity of both the parent and the transfected cell. These latter results have important implications for single vs. dual site models of T cell receptor function.

T 347 FINE SPECIFICITY AND T CELL RECEPTOR β-CHAIN GENE REARRANGEMENTS OF FIVE H-2D^b SPECIFIC CYTOTOXIC T CELL CLONES. Marco Schilham, Rosmarie Lang, Robbert Benner and Hans Hengartner. Erasmus University, Rotterdam, The Netherlands and The University of Zurich, Zurich, Switzerland.

A panel of H-2^b allospecific cytotoxic T cell clones of BALB/c origin have been produced. Six different clones were distinguished according to the following criteria. First, the fine specificity was determined by testing cytotoxicity and proliferation against target cells of different haplotypes. One clone (221) recognized H-2K^b, the other clones recognized H-2D^b. One of the D^b-specific clones (433) also recognized I-E^k. This cross-reactivity seemed to be of a higher affinity than the original specificity as could be demonstrated by cold target competition experiments as well as by inhibition of cytotoxicity with α-Lyt-2 monoclonal antibodies. Second, the clones were analyzed with anti-clonotypic antibodies directed against T cell clone 3F9 (BALB/c anti-H-2D^b). Only one clone (653) reacted with the anti-clonotypic antibodies. The third criterion was the pattern of rearrangements of the DNA coding for the β-chain of the T cell antigen receptor. This pattern was different for each of the clones, which means that all clones have been derived from different precursors. Interestingly, clone 653 (which reacts with the 3F9 specific monoclonal antibody) has deleted the $C_{\beta 1}$ gene, whereas this gene is functionally expressed in 3F9.

T 348 ANALYSIS OF T-CELL RECEPTOR STRUCTURES SPECIFIC FOR MINIMAL PEPTIDE/Ia DETERMINANTS. Nilabh Shastri, Joan Kobori, Cheryl Davis, Emma Saffman and Leroy Hood, California Institute of Technology, Pasadena, CA 91125.

To analyze the antigen/Ia recognition function of the T-cell receptor, we have generated panels of T-cell clones specific for peptide 74-96 of hen lysozyme and four different Ia molecules (A^k, E^k, A^b and A^{bm-12}). Fine specificity analysis using synthetic variant peptides and appropriate antigen presenting cells, showed that the Ia molecules restricting recognition had a profound influence on the minimal peptide determinants recognized. We have cloned and sequenced the genes of the α and β-chains of the T-cell receptor from genomic or cDNA libraries constructed from several of these T cells. The expression of these genes among the panels of T-cell clones and following gene transfer into other T-cell lines is being analyzed to determine the correlations between the receptor structure and its recognition function.

T 349 EVIDENCE FOR TWO STATES OF LFA-1 AND THE T CELL ANTIGEN RECEPTOR: THEIR RELATIONSHIPS ON T CELL SURFACES. Tarek M. Shata, Urszula Krzych, and George K.Lewis. University of Maryland at Baltimore,Baltimore,MD 21201

Studies of monoclonal clonotype-anti-clonotype interactions have shown a temperature dependent increase in apparent avidity. No significant differences were observed in saturation at any of the tested temperatures. Q10 analysis from 4C to 42C revealed a sharp transition at 15C, above which the avidity became temperature independent. Similar temperature dependency was not found for binding of anti-class I antibodies to the same T cells. These studies, together with receptor alkylation studies, suggest two states of the antigen receptor. One state is characterized by low avidity anti-clonotype binding at temperatures <15C, and the other state is characterized by high avidity anti-clonotype binding at temperatures >15C. Further studies, using a new monoclonal antibody against LFA-1 or a LFA-1 like molecule also revealed low avidity and high avidity binding states that were temperature dependent. A relationship between clonotype expression and high avidity anti-LFA binding was suggested by binding studies using variant T cell clones which had been selected for loss of clonotypic structures. Loss of clonotypic structures correlated with loss of high avidity anti-LFA-1 binding. These experiments suggest a previously unknown relationship between LFA-1 and the T cell antigen receptor.

T 350 Jα AND Jβ REGIONS OF THE T CELL RECEPTOR HETERODIMER ARE INVOLVED IN ANTIGEN-Ia MEDIATED TRIGGERING OF T CELL HYBRIDOMAS, Bhagirath Singh, Jana Lauzon, Ester Fraga, Michel Boyer, Wallace Smart, Martien van Hoof, Pat Kilgannon and Arun Fotedar, University of Alberta, Edmonton, Alberta, CANADA T6G 2H7

Beef insulin specific T cell hybridoma A20.2.15 secretes interleukin-2 (IL-2) in response to beef insulin presented by I-A^d containing antigen presenting cells. We have recently reported the complete sequence of the β-chain of the T cell receptor of this hybridoma (PNAS (USA) 83:8163, 1985). The α-chain encoding the T cell receptor has also been sequenced (Fotedar et al., this volume). A previously identified $J_{\beta 1.1}$ sequence is used by the β chain whereas the α-chain uses a new Jα (Jα A20.2) sequence. Thus, both Jα and Jβ sequences for this hybridoma are known. In order to define the role of J region in antigen recognition and triggering of T cells, we have used antibodies generated against the Jα and Jβ regions of the A20.2.15 hybridoma. Heptapeptide containing $J_{\beta 1.1}$ sequence and undecapeptide containing Jα A20.2 sequence were synthesized by the solid phase peptide synthesis. HPLC purified peptides were cross-linked to KLH. New Zealand white rabbits were repeatedly immunized with these conjugates and antisera were collected at various time intervals. These antisera bound to the Jα and Jβ peptides and Jβ antisera precipitated TCR molecules from the A20.2.15 cells. In addition these antisera blocked the antigen-IA^d mediated IL-2 release from the A20.2.15 cells. Mixture of anti Jα and Jβ antisera completely inhibited the response of A20.2.15 cells whereas normal rabbit serum has no effect on these cells. We therefore conclude that Jα and Jβ regions are directly involved in antigen-Ia mediated triggering of T cell hybridomas.

T 351 COMPARISON OF AN ANTIGEN/MHC-SPECIFIC T CELL REPERTOIRE IN THREE CONGENIC STRAINS OF MICE, Simona B. Sorger, Louis A. Matis, Pamela J. Fink, Isaac Engel, and Stephen M. Hedrick, University of California, San Diego, La Jolla CA 92093.

We have previously described the B10.A pigeon cytochrome *c*-specific T cell response in terms of functional phenotypes and T cell receptor (TCR) gene usage. All B10.A T cell clones studied respond to antigen in association with syngeneic B10.A APCs, and crossreact to antigen in association with one or two allogeneic variants of the I-E encoded MHC molecules. In congenic strains of mice expressing these allogeneic MHC alleles [B10.A(5R) and B10.S(9R)], pigeon cytochrome *c*-specific T cells exhibit very similar MHC crossreactivities. Our goal was to determine whether the same MHC crossreactive T cell clones were expressed in each appropriate strain, or whether each T cell repertoire was unique. The results indicate that identical V_α-J_α and V_β-J_β combinations were expressed by the major pigeon cytochrome *c*-specific response phenotype in B10.A and B10.A(5R) mice. In contrast, B10.A and B10.S(9R) mice exhibit similar response phenotypes to pigeon cytochrome *c* but express distinctly different TCR genes. These results indicate that the TCR repertoire is highly selected during the development of the immune system. The data also show that for the response in each strain, all of the gene elements involved in the generation of diversity appeared to be limited and therefore selected and important in the determination of TCR specificity. We are currently working to determine whether other MHC-restricted responses as well as alloreactive responses show the same degree of structural selection.

T 352 STRUCTURE OF AN ARSONATE-SPECIFIC T CELL RECEPTOR AND THE PREDOMINANT USE OF ITS Vα GENE IN THE ARSONATE RESPONSE OF CAF$_1$ MICE.

Kut-Nie Tan, Barry Datlof, Allan M. Maxam, and Anjana Rao. Dana-Farber Cancer Institute and Harvard Medical School, Boston, Massachusetts 02115 USA

We have cloned and sequenced the α and β chain T cell receptor genes from the arsonate (ARS)/I-A^d specific helper cell clone Ar-5 isolated from a CAF$_1$ mouse. The β chain utilised Vβ2, Dβ2, and Jβ2-6, while the α chain utilised a member of the Vα3 family and an unusual Jα identical to Jα20 in the 3' region (39 bases) but differing in the 5' region (14 bases) which we termed Jα20'. (Nomenclature based on Kronenberg, et al, Ann. Rev. Immunol. 4:529, 1986.) One other arsonate-reactive T cell clone, Ar-7, rearranged the same Vα and Vβ genes as Ar-5, whereas another, Ar-4, did not rearrange either of these V segments. Expression of Vα3 α chain, but not Vβ2, is strongly correlated with arsonate recognition in CAF$_1$ mice. CAF$_1$ mice were immunised with ARS-KLH, and subsequently the immune spleen cells were stimulated with ARS-OVA, in the absence of exogenous serum proteins or growth factors. This protocol selects for survival of ARS-reactive helper T cells. Northern analysis shows that immune spleen cells stimulated with ARS-OVA in culture over 2-4 weeks express >4-fold more Vα3-hybridising mRNA than control cells stimulated with OVA alone (data were normalised to total Cα RNA). Expression of Vβ2 mRNA was not detected in these spleen cell cultures. Using oligonucleotide probes spanning the Ar-5 V-J junction, we are testing whether the Vα3-Jα combination expressed in the ARS mass cultures is the same as in clone Ar-5. We are also transferring the Ar-5 α gene into T cells with different β chains to test whether the Ar-5 Vα3-Jα20' encodes sufficient structural information for recognition of ARS.

The T Cell Receptor

T 353 REGULATION OF GENE EXPRESSION FOR THE 20kD GLYCOPROTEIN OF THE T3/T CELL RECEPTOR COMPLEX (T3-δ chain).

Peter van den Elsen*, Katia Georgopoulos and Cox Terhorst.
Lab. of Molecular Immunology, Dana-Farber Cancer Institute, Boston, MA 02115.

Transcription of the gene coding for the 20kD glycoprotein of the T3/T cell receptor complex is evident only in T-cells as assessed by Northern blotting experiments and not in other cell types. In order to study the mechanism of this apparent tissue-specific expression we have isolated and characterized the genes encoding both the human and murine T3-δ chain. Both genes have an identical organization and comprise 5 exons. Interestingly they do not contain the so-called TATA and CAAT boxes found in many eukaryotic promoters. DNA fragments comprising the putative promoter sequence have been isolated and fused to the gene coding for chloroamphenicol transferase (CAT). These hybrid plasmids were introduced into a variety of cells and tested for the production of CAT enzyme in a transcient assay. The results of these analysis have shown that the activity of the T3-δ promoter is not resticted to T-cells only. Nuclear run-off assays have indicated that initiation of transcription also occurs in non T-cells. These results indicate that events post the initiation of transcription are responsible for the tissue-specific appearance of the T3-δ mRNA.

*Present address:Department of Immunohaematology and Bloodbank, Academic Hospital Leiden, Postbus 9600, 2300 RC Leiden, The Netherlands.

T 354 THE EFFECT OF THE NZW Cβ1, Jβ2 and Dβ2 DELETION ON THE T CELL REPERTOIRE, David L. Woodland, Brian L. Kotzin and Ed Palmer, National Jewish Center for Immunology and Respiratory Medicine, the VA Hospital and University of Colorado Health Sciences Center, Denver, CO,

We are interested in determining the relative importance of Dβ and Jβ gene segments in the generation of the T cell antigen receptor (TcR) repertoire. To this end, we are well advanced in the generation of BALB/c and C57BL/6 mice which are congenic for the β chain allele of New Zealand White (NZW) mice. The TcR β chain locus in NZW mice carries a deletion of Cβ1, Jβ2 and Dβ2 gene segments and can generate only 30% of the possible Vβ-Dβ-Jβ rearrangements that can occur in normal mice. This reduction of β chain rearrangements might be expressed as a significant reduction of the precursor frequencies of T cells against certain antigens. Thus, precursor frequencies of T cells reactive against specific antigens will be determined by limiting dilution analysis. Mice congenic for this deletion of β chain segments may be unresponsive to some antigens and the extent of the limitation of antigen reactivities may reflect the relative importance of the J and D segments in the generation of the receptor repertoire in T cells.

Accessory Molecules

T 400 INTRATHYMIC T CELL DIFFERENTIATION AND THE THYMIC MICROENVIRONMENT, Becky Adkins and Irving L. Weissman, Stanford University, Stanford, CA 94305

To study the differentiation of thymic progenitor cells independently of their ability to home to the thymus, purified L3T4$^-$Lyt-2$^-$cells (donor cells) were injected directly into the thymuses of normal, non-irradiated animals. The injected donor cells homed selectively to thymic cortical areas, proliferated extensively, and gave rise to the normal proportions of L3T4/Lyt-2 phenotypic subsets within 5 days of injection. Analyses of donor cells at earlier times after injection suggested that the L3T4$^-$Lyt-2$^-$ thymocyte subpopulation may contain several distinct types of progenitor cells. Experiments to test this hypothesis are currently underway.
In other experiments, we have found that thymic cortical epithelial cells coexpress I-A antigens and an antigen (6C3-Ag) associated with B-lineage proliferation and differentiation. Among solid lymphoid organs, only the thymus contains 6C3-Ag+ cells and within the thymus, 6C3-Ag is found exclusively on epithelial cells and nurse cells in cortical regions. In addition, the expression of 6C3-Ag on thymic epithelium is developmentally regulated, appearing first at 1 week following birth. To test the role(s) of the 6C3-Ag and/or 6C3-Ag+ cortical epithelial cells on intrathymic T cell maturation, we have begun *in vivo* treatment of mice with anti-6C3-Ag monoclonal antibodies.

The T Cell Receptor

T 401 IN VIVO USE OF MONOCLONAL ANTI-T CELL ANTIBODIES, Hugh Auchincloss, Jr., Theodore G. Mayer, Rafik Ghobrial, and Henry J. Winn, Massachusetts General Hospital, Boston, Massachusetts 02114. Monoclonal antibodies directed at T cell differentiation antigens (L3T4 and Lyt2) have been used in vivo to modify immune responses. Initial experiments sought optimal conditions for treatment. Intraperitoneal injections were as effective as intravenous administration; thymectomy potentiated the effectiveness of L3T4 antibodies; more frequent administration of Lyt2 antibodies compared to L3T4 were required; antibodies of different classes (IgG2b vs. IgM) and different species (rat vs. mouse) were effective in mice. Additional experiments tested the effectiveness of different antibodies in prolonging skin graft survival of different antigenic combinations. Class I disparate grafts were variably prolonged by Lyt2 antibodies and both Class II and minor-antigen disparate grafts were prolonged by L3T4 antibodies. Finally, B cell responses to alloantigens presented on skin grafts were reduced by L3T4 antibodies. These experiments provide insight into the mechanisms of immune responses to alloantigens and may help to improve the use of monoclonal antibodies for modifying these responses.

T 402 EVIDENCE THAT LFA-3 ACTIVATES T CELLS VIA THE CD2 RECEPTOR. B.E. Bierer, A. Peterson, Y. Takai, J. Greenstein, S. Herrmann, B. Seed, S.J. Burakoff. Dana-Farber Cancer Institute and Massachusetts General Hospital, Boston, MA 02215.

Monoclonal antibodies (MAb) directed against the CD2 molecule either inhibit, or, in certain combinations of MAb, stimulate T cell proliferation. Recent evidence has suggested that CD2 may be a T cell receptor whose ligand is LFA-3, a broadly distributed glycoprotein of 60kD. MAb to LFA-3 inhibit by binding to the target or stimulator cell. Human PBL will rosette with autologous erythrocytes (E). We have demonstrated that proliferation of human PBL is augmented by costimulation with human E, most dramatically demonstrated at limiting doses of mitogenic anti-CD3 MAb or PHA. This augmented proliferation is inhibited by treatment with either anti-CD2 or anti-LFA-3 MAb, suggesting that the enhanced stimulation provided by coculture with human E may be mediated by CD2 on the T cell interacting with LFA-3 on the red cell. Recently, a xenogeneic murine T cell hybridoma that produces interleukin-2 (IL2) in response to JY, an EBV-transformed human B cell line, has been infected with a defective retrovirus containing the CD2 cDNA, and selected by resistance to the antibiotic G418. By indirect immunofluorescence, several clones express the CD2 molecule. In these clones, anti-CD2 and anti-LFA-3 MAb inhibit IL2 production stimulated by JY. Stimulation of these cells with combinations of anti-CD2 MAb and purified LFA-3 are currently being explored.

T 403 REGULATION OF EXPRESSION OF LYT 2 IN T CELL HYBRIDOMAS. Amy M. Carbone, Philippa Marrack and John Kappler. Department of Medicine, National Jewish Center for Immunology and Respiratory Medicine, Denver, Colorado 80206

We have studied the regulation of expression of the T cell surface marker Lyt2 by fusing Lyt2$^+$ cells with cells that do not express this marker. We then looked for a trans-acting factor that would positively or negatively regulate Lyt2 expression. We found that when the AKR thymoma BW5147 (Lyt2.1$^-$) was fused with SJL lymph node cells (96% Lyt2.2$^+$) the resulting hybrids expressed neither Lyt2.1 (BW5147 allele) nor Lyt2.2 (SJL allele). We concluded from this result that a trans-acting, negatively regulatory factor contributed by the BW5147 genome supresses the exprssion of Lyt2.2 from the normal SJL parent. Lack of Lyt2.2 expression was not due to chromosomal loss since Lyt2$^-$ hybrids tested by Southern analysis carried at least one Lyt2 allele originating from the SJL parent. Furthermore, negative regulation of Lyt2 expression was at the level of transcription as demonstrated by the lack of Lyt2 mRNA in these hybrids. We have shown that the Lyt2 gene in Lyt2$^+$ tumor cells is undermethylated, whereas this gene is heavily methylated in liver and in Lyt2$^-$ tumor cells. We are currently studying the methylation state of the Lyt2$^-$ hybridomas and of normal Lyt2$^+$ lymph node cells to determine whether methylation of the Lyt2 gene plays an important role in the regulation of Lyt2 expression.

The T Cell Receptor

T 404 cDNA CLONING AND PRIMARY SEQUENCE OF THE ALPHA SUBUNIT OF p150,95, Angel Corbi, Linda Miller, Karen O'Connor, Tom Roberts and Timothy Springer, Dana-Farber Cancer Institute, Boston, MA 02115. p150,95 is a non-covalently associated heterodimer with a 150 kd alpha and 95 kb beta subunit. The beta subunit is common to two other glycoproteins, LFA-1 and Mac-1. p150,95 is known to be involved in cell adhesion processes and is normally expressed on monocytes, neutrophils, tissue macrophages and CTLs. Tryptic peptide amino acid sequences from the immunoaffinity purified molecule were used to design oligonucleotide probes for the alpha purified subunit. Screening of a lambda gt10 size-selected cDNA library from PMA-induced HL-60 cells yielded 8 positive clones. A 4.9 kb clone was subjected to further study and its complete sequence revealed that: 1. it contains the whole coding region for the p150,95 alpha chain; 2. the alpha subunit of p150,95 is a transmembrane polypeptide with a classical signal peptide and a short cytoplasmic tail: 3. the homology with the alpha subunits of the vitronectin and fibronectin receptors is not restricted to the N-terminus part of the polypeptide. The complete amino acid sequence of the p150,95 alpha chain will be presented.

T 405 ROLE OF L3T4 IN T CELL ACTIVATION. Barbara Fazekas de St. Groth and Trevor Owens, Walter and Eliza Hall Institute, Melbourne, Victoria 3050, Australia.
It has been proposed that L3T4 interacts with MHC to increase the overall avidity of the T cell receptor (TcR) for antigen in association with MHC. We have used two experimental systems to further examine the role of L3T4 in the activation of T cells. In the first system, antigen recognition by two unusual class-II MHC-restricted, $Lyt\text{-}2^+ L3T4^+$ clones was shown to be inhibited by anti-L3T4 mAbs, but not by anti-Lyt-2 mAbs. These results suggested that L3T4 and the TcR were physically close during antigen recognition, probably as part of a multimolecular complex from which Lyt-2 was excluded. We obtained further evidence of an interaction between L3T4 and the TcR using class II-restricted $L3T4^+ Lyt\text{-}2^-$ clones stimulated by non-MHC ligands such as the F23.1 mAb, Con A and PMA. Activation by F23.1 was maximal when the mAb was presented on a solid phase such as sepharose beads or tissue-culture plastic. Inhibition by anti-L3T4 mAbs was seen only at suboptimal concentrations of solid-phase F23.1. Similarly, anti-L3T4 mAbs inhibited responses to suboptimal, but not optimal, concentrations of Con A. However, the weak responses to soluble F23.1 or to PMA were not inhibited by anti-L3T4 mAbs. Such antibodies were therefore not inherently inhibitory, as has previously been suggested, but required TcR ligation for effect. These results suggest that there is an interaction between L3T4 and the TcR, independent of interaction of either molecule with the MHC. Only very high avidity TcR/ligand interactions (such as those produced by high concentrations of Con A or of F23.1 on plastic) are unaffected by the steric hindrance resulting from the ligation of L3T4 by mAbs.

T 406 AUTOCRINE REGULATION OF T CELL ACTIVATION Ly-6A/E MOLECULES BY INTERFERON-GAMMA, Francis J. Dumont, Merck Sharp & Dohme Research Laboratories, Rahway, NJ 07065.
The murine Ly-6A/E surface molecules are capable of transducing triggering signals in T cells and thus provide a potential alternative pathway for T cell activation. Expression of these molecules is augmented after mitogenic stimulation of T cells. We demonstrated (J. Immunol. 137, 201, 1986) that exogenous interferon (IFN)-gamma dramatically enhances Ly-6A/E in resting T cells. Since IFN-gamma is produced upon T cell stimulation, the possibility existed that increased Ly-6A/E in stimulated T cells reflects a response to IFN-gamma. This was explored using T cells from specific pathogen free C57Bl/6 (Ly-6A) and BALB/c (Ly-6E) mice stimulated with concanavalin A (Con A) or ionomycin + PMA. It was found that supernatants from Con A stimulated T cells could induce Ly-6A/E expression in resting T cells. Such activity was abrogated by treatment of Con A stimulated T cells with cyclosporin A, which blocks IFN-gamma production or by addition of a monoclonal antibody (MAb) anti-IFN-gamma to the supernatants. Addition of anti-IFN-gamma MAb to Con A or ionomycin + PMA stimulated T cells also prevented Ly-6A/E enhancement but not blastogenesis or expression of IL-2 and transferrin receptors. Therefore, endogenous IFN-gamma selectively upregulates Ly-6A/E expression through an autocrine circuit. The use of anti-IFN-gamma MAb to generate activated T cells lacking Ly-6A/E molecules should prove useful to further define the functional role of these molecules.

T 407 ROLE OF L3T4 IN T CELL ACTIVATION, Denise Gay, John Kappler and Philippa Marrack, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO

It has been postulated that the murine T cell molecule L3T4 enhances T cell receptor interaction with antigen/MHC by binding to a monomorphic region of the antigen presenting cell Ia protein. We have used a planar membrane system to demonstrate that this L3T4:Ia interaction does take place. A rare T cell hybridoma which responds to class I D^d protein but is $L3T4^+$ has been shown to respond to isolated D^d protein supported on planar membranes. This T cell hybridoma exhibits an eight-fold higher response when Ia protein is included with D^d on the planar membranes. This enhanced response can be diminished in the presence of anti-L3T4 antibodies. In contrast, a variant of this T cell hybridoma which has lost surface L3T4 but retained the D^d-restricted TcR (and all other T cell markers examined) responds equally well to the isolated D^d protein in the absence or in the presence of Ia protein. Ia protein alone elicits no response from either T hybridoma but does stimulate other appropriate Ia-restricted T cell hybridomas to IL-2 production.

T 408 Idenification of a Ligand for the Murine LFA-1 Mediated Adhesion Interaction. W.T. Golde, J. Kappler, and P. Marrack. National Jewish Center, Denver,CO 80206

We have previously described the effects of anti-LFA-1 blocking antibodies on the Class II restricted responses of cloned T cell hybridomas to protein antigens. Specifically, we found such T cell hybridomas have a differential sensitivity to anti-LFA-1 and that the LFA-1 mediated adhesion interaction is nonfunctional when murine fibroblasts are used as the stimulus. Using these data we have generated a series of monoclonal antibodies that have the identical blocking pattern as anti-LFA-1. These antibodies precipitate a molecule of 45-60Kd molecular weight that is a single polypeptide chain with significant micro-heterogeneity between cell sources. The molecule is expressed on activated cells of the leukocytic lineage and expression is greatly reduced or absent on all mouse fibroblasts tested. Our data are consistant with the hypothesis that this molecule is a ligand for LFA-1.

T 409 HOMOLOGOUS PEPTIDES FROM HIV GP41 AND HLA CLASS II BIND CD4 ON HUMAN T CELLS. Hana Golding, Frank A. Robey, Frederick T. Gates III, Wolfgang Lindner and Basil Golding. Immunology Branch, NCI, NIH; DBP and DBBP, FDA; Bethesda MD 20892.
The CD4 molecule has been identified as the receptor for HIV envelope protein. Recently, the possible natural ligand for CD4 on antigen presenting cells has been localized to the N-terminal domain of the beta chain of MHC Class II. It was postulated that the MHC Class II and HIV bind the non-polymorphic CD4 via similar conserved regions. A hydrophilic septamer was identified displaying a high degree of homology between gp41 of HIV and the beta-1 domain of HLA-DR and -DQ. Both the HIV and MHC Class II derived septamers were synthesized. Incubation of these peptides, but not control peptides, with CD4 positive cells at 37 C for 45 min resulted in reduced binding of anti-CD4 antibodies (OKT4, OKT4a and Leu3) to the cells. This peptide mediated reduction of binding to CD4 could be blocked in the presence of chloroquin. The binding of antibodies directed against other surface antigens, were unaffected by pre-incubation with the peptides. The temperature requirement and the sensitivity to chloroquin suggest that the peptides induced partial modulation of the CD4 molecules via receptor mediated endocytosis. In addition, flow cytometry showed that biotinylated chicken albumin conjugates of the peptides can bind directly to CD4 bearing CEM cells, but not to a CD4 negative CEM mutant or to B cell lines. This binding could be partially inhibited in the presence of mouse monoclonal anti-CD4 antibodies. Therefore, these findings suggest that, the homologous regions of HIV and MHC Class II, which we have identified, may be the sites involved in binding of AIDS virus and MHC Class II antigens to CD4 on human T cells.

T 410 T CELL ACTIVATION BY RECEPTOR-MEDIATED SIGNALS. K.S. Hathcock, J.A. Bluestone, D.M. Segal, and R.J. Hodes. NIH, Bethesda, MD 20892.

Experiments were performed to examine T cell activation by antibodies directed to the T cell receptor (TcR) complex and to analyze accessory cell (AC) signals required for these responses. Soluble monoclonal antibody to the murine T3-ε chain (143-2C11) activated both resting L3T4^{+}Lyt2^{-} and L3T4^{-}Lyt2^{+} T cells to proliferate when T-depleted spleen cells were employed as AC. In contrast, soluble F23.1 (a monoclonal antibody specific for $V_{\beta}8$ of the TcR) in the presence of T-depleted spleen cells predominantly induced proliferation in L3T4^{-}Lyt2^{+} and not L3T4^{+}Lyt2^{-} T cells in the presence of exogenous IL-2. However, heteroconjugate antibodies composed of F23.1 covalently linked to antibodies specific for AC surface molecules (class I, class II, or the modifying hapten TNP) induced proliferation in both L3T4^{+}Lyt2^{-} and L3T4^{-}Lyt2^{+} T cells. When F23.1^{+} T cells were activated with soluble heteroconjugate antibody and exogenous IL-2, virtually any lymphoid cell, including Ia^{-} T cells, could serve as an AC if it expressed the appropriate cell surface molecule for targeting by the heteroconjugate. When F23.1^{+} T cells were activated with heteroconjugate anibodies in the absence of exogenous IL-2, both subpopulations of resting T cells again proliferated, but now only when T-depleted spleen cells are used as AC, and not in the presence of other AC populations such as Ia^{-} T cells. These results suggest that, in the presence of exogenous IL-2, proliferation in response to heteroconjugates by both L3T4^{+}Lyt2^{-} and L3T4^{-}Lyt2^{+} T cells may only require AC as a means of cross-linking the TcR. More complex AC-derived signals may be required, however, for triggering of proliferation in the absence of exogenous lymphokines, since more limited cell populations act as AC under these conditions.

T 411 DIRECT EVIDENCE THAT LFA-3 IS A LIGAND FOR THE CD2 RECEPTOR. Steven H. Herrmann Yasuyuki Takai, Yvonne Rosenstein, Martha L. Reed, and Steven J. Burakoff, Division of Pediatric Oncology, Dana-Farber Cancer Institute, Boston, MA 02115

Cell-cell adhesion is required for T lymphocyte activation as well as effector function. The role of the T cell receptor for antigen in T cell-target cell adhesion is not clear. Indirect evidence suggests that the LFA-3 glycoprotein on target cells is responsible in part for antigen independent conjugate formation between lymphocytes and other cells. To directly investigate this we used artificial target cells (ATC) (spherical cell size nylon vesicles with an outer lipid membrane) that had been reconstituted with purified LFA-3. Conjugate formation was found between ATC expressing LFA-3 and intact T cells expressing CD2. These conjugates were blocked by the addition of monoclonal antibody against either CD2 or LFA-3. CD2 expressing cells did not form conjugates with ATC lacking surface protein or reconstituted with class I MHC proteins. These results indicate that LFA-3 is a ligand for the CD2 receptor. Current experiments are addressing whether this receptor-ligand pair functions in cell-cell adhesion only or if this interaction plays a role in T cell activation as well.

T 412 STRUCTURAL AND FUNCTIONAL STUDIES ON T11TS, THE LIGAND OF CD2. Thomas Hünig, Georg Tiefenthaler, Ellen Abernethy, Rita Mitnacht, Christine Köhler, Katja Prowald, Friedrich Lottspeich, and Stefan C. Meuer*, Genzentrum, D-8033 Martinsried, FRG, and *I. Med. Klinik u. Poliklinik der Universität, D-6500, Mainz, FRG.

Human T-Lymphocytes can be polyclonally activated by pairs of monoclonal antibodies (mAbs) to the CD2 molecule, classically known as the "E-receptor" for its capacity to bind sheep or human erythrocytes. We have identified the sheep form of the ligand of CD2 with a mAb that blocks the binding of sheep red blood cells (SRBC) to human and sheep T-cells. This molecule, which we call T11 target structure or T11TS, is a glycoprotein of 42kD mW expressed on all types of blood cells and some other tissues. We have purified T11TS to homogeneity and determined some of its biochemical properties as well as its partial amino acid sequence. T11TS carries 2 or 3 N-glycosydically linked carbohydrate chains and no O-linked sugars. The protein moiety has an app. MW of 32 kD. The partial sequence indicates that T11TS is a protein of hence unknown sequence. A rabbit antiserum to homogeneous sheep T11TS blocks the binding of human RBC to human T-cells. In Western blotting, it detects a broad band around 60 kD in lysates from human RBC and DAUDI cells. Experiments carried out in collaboration with Dr. T. Springer and colleagues indicate that this antiserum reacts with LFA-3, which was recently identified as the human ligand of CD2. A role for T11TS in T-cell activation is likely because a) anti-T11TS mAb blocks the sheep MLR and b) T11TS as expressed on SRBC is strongly costimulatory with individual anti-CD2 antibodies. Molecular cloning of T11TS employing oligonucleotide probes and the anti-T11TS antiserum is in progress.

T 413 TARGET SEQUENCES OF T-CELL ACCESSORY MOLECULE (CD4, CD8) INTERACTION WITH MHC CLASS I AND CLASS II MOLECULES, Tim Hunkapiller, Joan Goverman and Leroy Hood, California Institute of Technology, Pasadena, Ca. 91125; Doug Williams and Michel Klein, University of Toronto, Toronto, Ontario Canada M5s1A8.

We have recently published a model of T-cell antigen recognition and function that suggests that individual T cells are not MHC haplotype restricted (Goverman et.al., Cell 45,475). Direct interaction of CD8 and CD4 with MHC molecules is invoked to explain MHC class restriction. Furthermore, we have previously suggested that a short sequence conserved in both class I and class II polymorphic regions is a key for T-cell/target-cell interaction (Malissen et.al., Science 221,750). Our recent model proposes that this sequence is a binding target of CD4 and CD8. To test this hypothesis, we have generated a series of peptides that are to various degrees similar to the proposed target sequence. We are currently testing the ability of these peptides to inhibit T-cell activation by interfering with the binding of the native sequence. A helper T-cell line is being used that is sensitive to anti-CD4 antibody for which there is a non-sensitive mutant clone. T-cell activation is assayed through a calcium flux-dependent flourescence reaction. In this way, the kinetics of activation can be more finely followed than in a standard IL-2 release assay.

T 414 INTERACTION OF EFFECTOR CTL WITH CLASS I ALLOANTIGEN IS NECESSARY AND CAN BE SUFFICIENT FOR TRANSMEMBRANE SIGNALLING, Kevin P. Kane, Steven A.N. Goldstein and Matthew F. Mescher, Medical Biology Institute, La Jolla, CA 92037.

Purified Class I alloantigen on cell-size (5 micron), bead-supported artificial membranes (pseudocytes) is effectively recognized by primed precursor CTL and stimulates specific secondary *in vitro* responses (*J. Immunol.* in press). Current studies show that interaction of *mature* effector CTL *with H-2 on* pseudocytes results in antigen-specific binding and transmembrane signal generation. Pseudocytes stimulate specific, IL 2 dependent proliferation of cloned allogeneic CTL. Furthermore, pseudocytes stimulate release of serine esterase by effector CTL, a process which appears to involve rapid (1 to 2hr) degranulation by the CTL in response to antigen-dependent signalling. Allogeneic CTL clones release 25 to 40% of their total extractable serine esterase activity (measured using BLT substrate) upon interaction with either target cells or pseudocytes bearing the appropriate Class I proteins, but less than 5% upon interaction with cells or pseudocytes bearing inappropriate H-2. Using this approach to measure a relatively immediate consequence of antigen binding and transmembrane signalling, the roles of H-2 density on the membrane, the involvement of Lyt-2/3 interactions and the roles of additional CTL "accessory" proteins are being examined. The results obtained thus far demonstrate that Class I antigen is both necessary and sufficient for specific binding and transmembrane signalling to occur. While interaction of CTL surface components with other target cell ligands may contribute to the response under some conditions (*e.g.*, low antigen density) they are clearly not an absolute requirement.

T 415 THE β SUBUNIT OF LFA-1, Mac-1, AND p150,95: GENE CLONING, HOMOLOGY TO A FIBRONECTIN RECEPTOR, AND THE MOLECULAR BASIS OF LEUKOCYTE ADHESION DEFICIENCY. T. K. Kishimoto[1], T.M. Roberts[1], D.C. Anderson[2], and T.A. Springer[1]. [1] Dana Farber Cancer Institute, Boston, MA. and [2] Baylor College of Medicine, Houston, TX.

LFA-1, Mac-1, and p150,95 constitute a family of heterodimeric adhesion proteins which share a common β subunit of 95,000 daltons. The common β subunit is of particular interest because 1) MAb against the common β subunit are among the most potent inhibitors of immune cell adhesion and 2) Patients with leukocyte adhesion deficiency (LAD) lack cell surface expression of LFA-1, Mac-1, and p150,95 and are thought to have a defect in the common β subunit.

We have isolated cDNA clones which encode the 769 aa β subunit polypeptide. The deduced amino acid sequence shows a signal sequence, a large extracellular domain containing six potential N-glycosylation sites, a transmembrane domain, and a cytoplasmic domain of 46 aa. A 187 aa cysteine-rich domain (20%) contains four tandem repeats. The β subunit of the leukocyte adhesion proteins shows 45% amino acid identity with band III of integrin, a chick fibronectin and laminin receptor. Homology is found throughout the entire polypeptide; many of the mismatches are conservative amino acid changes. This homology suggests that the leukocyte adhesion proteins and the extracellular matrix receptors constitute a novel supergene family of adhesion proteins. Limited N-terminal sequences from the α subunits of the two families support this conclusion.

The β subunit cDNA was used to probe RNA from LAD patients. Four of five unrelated patients expressed detectable levels of the β subunit message. An antiserum against the denatured β subunit was produced and used to identify β subunit precursor in biosynthetically labeled cells from LAD patients. A number of abnormalities were detected by SDS-PAGE. The inheritance of the aberrant precursors correlated perfectly with the expected cell surface expression and the disease state. These results provide the strongest evidence to date that the primary genetic lesion resides in the common β subunit.

T 416 ABSTRACT OF CELL SURFACE LFA-1 AS A MECHANISM OF ESCAPE FROM IMMUNE SURVEILLANCE, Alan M. Krensky, Ann Wright, L. Jeffrey Medeiros, Teri D. Koller, Michael P. Link, Stephen D. Smith, Roger A. Warnke, and Carol Clayberger, Stanford University, Stanford, CA 94305.

Little is known about the mechanisms by which tumor cells evade detection by the immune system. One possible mechanism is alteration in the expression of cell surface molecules. During our studies of T cell recognition of autologous tumor cells, we observed that a number of tumor cells derived from patients with lymphoma were poor stimulators of both autologous and allogeneic T cell responses. Analysis of the tumor cell surface molecules showed that expression of LFA-1 was lacking, whereas normal leukocytes from these patients expressed normal levels of LFA-1. Examination of other lymphoid tumors revealed that the great majority of high grade, but not low or intermediate grade, lymphomas do not express the LFA-1 molecule. Furthermore, in an initial survey, 8/12 non-relapsing large cell lymphoma patients had tumors which expressed LFA-1, while only 3/18 relapsing patients had tumors which expressed LFA-1 ($p = 0.005$). These data suggest that some tumors may escape from immune surveillance by selective absence or loss of the LFA-1 cell surface molecule and that LFA-1 expression is of prognostic significance.

T 417 CLONING OF THE ALPHA SUBUNIT OF HUMAN LFA-1, Richard S. Larson, Angel Corbi, Timothy Springer, Dana-Farber Cancer Institute, Boston, MA 02115. LFA-1, Mac-1 and p150,95 are heterodimers possessing different alpha subunits and a common beta subunit. These proteins have recently been shown to be members of a large supergene family which includes the platelet protein IIbIIIa, fibronectin receptor and vitronectin receptor. LFA-1 has been implicated in a wide range of leukocyte functions including CTL-mediated killing, ADCC, and natural killing. The alpha subunit of human LFA-1 was first isolated by immunoaffinity chromatography and preparative SDS PAGE. Material purified by electrolution was used to produce tryptic peptides. The sequence of one peptide, L69, was used to synthesize an oligonucleotide probe. A lambda gt10 library produced from PMA induced HL60 cells was screened, and 21 clones were isolated. A 2.5 kb clone was contained nucleotide sequence corresponding to the peptide, L69, confirming its identity as a bona fide LFA-1 clone. An overlapping 4.4 kb clone was later isolated. These studies as well as further sequencing work will be presented.

T 418 CTL RECOGNIZE CONSERVED DETERMINANTS ON CLASS I MHC PROTEINS, Matthew F. Mescher and Steven A.N. Goldstein, Medical Biology Institute, La Jolla, CA 92037.

Studies of CTL recognition using cell-size, supported artificial membranes bearing Class I MHC proteins have provided direct evidence for enhanced responses resulting from recognition of conserved determinants on the H-2 protein. The response of primed precursor allogeneic CTL was found to be critically dependent on the density of alloantigen on the membrane. At suboptimal alloantigen density, incorporation of self or third party Class I, but not Class II, proteins onto the membrane dramatically enhanced the response. This enhancement was eliminated in the presence of anti-Lyt-2 antibody. The results directly demonstrate recognition of conserved Class I determinants and strongly suggest that Lyt-2/3 mediates this recognition. Furthermore, the suggested reciprocal relationship between antigen density and the contribution of Lyt-2/3 to triggering is directly confirmed. Finally , it appears that Lyt-2/3 interaction leading to enhanced response does not require binding of the same Class I (alloantigen) molecule that is bound by the T cell receptor.

T 419 FUNCTION OF ACCESSORY T CELL MOLECULES IN CD3 REDIRECTED CYTOTOXICITY. Ralph R. Quinones, David M. Segal, Ronald E. Gress. NIH, Bethesda, MD 20892

Cytotoxic T lymphocytes (CTL) can be activated to lyse target cells (TC) by antibody conjugates of monoclonal antibody (MAb) reactive with the CD3 antigen of the T cell receptor complex (OKT3) cross-linked to an antibody specific for the TC (T3-redirecetd cytotoxicity, T3RDC). T3RDC with an OKT3-anti-DNP conjugate and TNP-coated TC allowed investigation of the role of the accessory T cell surface molecules CD2 (E rosette receptor, LFA-2), CD8, and LFA-1 (CD 11a/18) in the lysis of TC not expressing nominal antigen. MAb which were specific for these molecules and inhibited CTL lysis of nominal nominal antigen (+) TC were evaluated for their ability to block T3RDC by class I and class II specific CD8(+) CTL. The level of lysis varied with the titer of T3 conjugate. We observed that: 1. LFA-1 MAb inhibited T3RDC even at levels of T3 conjugate resulting in maximal lysis; 2. CD2 and CD8 MAb resulted in inhibition when T3RDC was titered to yield suboptimal lysis; 3. CD8 MAb inhibited lysis of both human and murine TC deficient in class I expression; 4. A class II specific, CD8(+) CTL clone, insensitive to CD8 MAb in killing nominal anigen (+) TC, was inhibited by CD8 MAb in T3RDC. T3RDC provides a useful means of studying CTL-TC interactions by allowing the use of a variety of TC independent of nominal antigen expression. This system should be of use in assessing the role of transfected molecules as putative ligands on the TC for T cell accessory cell molecules.

T 420 FUNCTION OF T8 (CD8) IN A MURINE T CELL HYBRIDOMA, S. Ratnofsky, A. Peterson, J. Foran, B. Seed, S. Burakoff, and J. Greenstein. Dana-Farber Cancer Institute and Massachusetts General Hospital, Harvard Medical School, Boston, MA 02115.

The T8 (CD8) molecule is expressed on human T cells whose MHC restriction is for HLA class I gene products. We have expressed T8 in a murine T hybridoma specific for HLA class II gene products. Such a system might allow the separation of the ligand requirement of the T cell receptor from the putative liand of the T8 molecule, thereby providing insight into the biological role of T8. We employed a novel gene transfer system to facilitate these studies. A T8 cDNA clone was inserted into a retroviral vector containing a covalently linked selectable marker gene capable of conferring G418 resistance to cells carrying the provirus. All G418 resistant cell lines derived from infection with the recombinant retrovirus were positive for T8 expression as assessed by indirect immunofluorescence. The level of expression attained was equivalent to that on IL-2 dependent human T cell lines and was stable. $T8^+$ hybridomas express T cell receptor (F23.1) and LFA-1 at levels equal to the parent cell line. When presented with stimulator cells that express both class I and II HLA antigens, transfectants produced 10-20 fold greater levels of IL-2 than either a G418 resistant $T8^-$ hybridoma or the parent line. Stimulation of $T8^+$ cells was blocked by the addition of anti-T8 antibody. The increased sensitivity of transfectants was also reflected in their response to suboptimal cell numbers or plasma membranes expressing the relevant antigens. Taken together these data suggest that the T8 molecule is capable of functioning in a murine T hybridoma to augment its response to antigen.

T 421 THE CD4 MOLECULE IS ASSOCIATED WITH THE T CELL RECEPTOR, Kaj M. Saizawa, Jose Rojo, Sakhina Haque and Charles A. Janeway, Jr., Section of Immunobiology, Howard Hughes Medical Institute at the Yale University School of Medicine, New Haven, CT 06510.

The CD4 (L3T4 in the mouse) molecule is found on the surface of about 60% of peripheral T cells. T cells that recognize foreign antigens in the context of self class II MHC proteins always express CD4 and never CD8; anti-CD4 antibody blocks the response of CD4 positive T cells to antigen presented in the context of self class II MHC molecules. For this reason, it has been proposed that CD4 binds to a non-polymorphic portion of class II MHC molecules and increases the binding of T cells to antigen presenting cells.

We have studied the effect of anti-CD4 antibodies on the response of a cloned T cell line to a variety of stimuli, and have used anti-CD4 in monovalent Fab, Fab'2, IgG and multivalent forms to affect these responses. We find that responses to antibodies directed at a particular epitope on the T cell receptor are inhibited by anti-CD4, even in monovalent form, while responses to antibodies directed at other epitopes are unaffected or augmented by anti-CD4. The augmentation, and the inhibition of responses to mitogenic lectins, thought to represent a negative signalling role of CD4, are not seen with the monovalent Fab form of anti-CD4. Thus, we can distinguish negative signalling from steric inhibition, and can also show steric blocking of responses to antibodies directed at a particular T cell receptor epitope.

These data suggest that CD4 is part of the T cell receptor complex. This is supported by finding that CD4 co-modulates with the T cell receptor complex in some systems. Supported by the Howard Hughes Medical Institute and NIH grants CA-29606 and AI-14579.

T 422 CD4$^-$CD8$^-$ PROGENITOR CELLS IN PBL OF MULTIPLE MYELOMA PATIENTS GENERATE CLONES OF CD4$^+$ OR CD8$^+$ LYMPHOCYTES IN VITRO, Horacio Serra, Michel J. Mant, Ben A. Ruether, and Linda M. Pilarski, University of Alberta, Edmonton, Alberta CANADA T6G 4R7.
In previous work we reported the apparent presence of CD4$^-$CD8$^-$ progenitor cells in the PBL of myeloma patients as identified by an unexpectedly high T cell clonogenic efficiency of PBL. Two to three times more T cell clones were observed than would have been expected based on the number of CD4$^+$ or CD8$^+$ T cells in the PBL sample cloned. Using these same conditions, PBL from normal donors have a T cell clonogenic efficiency of 1.0. To demonstrate this abnormal lymphocyte progenitor subset more directly, we have treated PBL samples from normal donors or myeloma patients with a mixture of anti-CD4 and anti-CD8 plus complement. The cells remaining after treatment were analyzed by immunofluorescence and shown to be negative for expression of CD4 or CD8. These cells were then cultured at limiting dilution with irradiated feeder cells, PHA, and TCGF for 3-4 weeks. Any clones arising in these cultures were typed for CD4 or CD8. PBL from normal donors, treated with anti-CD4 and anti-CD8, generated no clones. In contrast 25% of treated PBL from myeloma patients generated CD4$^+$ clones. Thus in these patients 30 to 40% of CD4$^+$ clones arise from CD4$^-$CD8$^-$ cells. These experiments demonstrate conclusively that there exist CD4$^-$CD8$^-$ lymphocytes that cannot differentiate in vivo, but efficiently give rise to T cell clones in vitro. Studies are in progress to determine the surface phenotype of this subset and the T cell receptor α and β genes rearrangements.

T 423 ANTI-LYT-2 INHIBITION OF CYTOLYSIS THAT IS INDEPENDENT OF CLASS I RECOGNITION. Linda Sherman, Claire Langlet and Gary Neil. Department of Immunology, Research Institute of Scripps Clinic, La Jolla, California 92037
It has been proposed that antibodies specific for accessory molecules such as L3T4 and Lyt2 inhibit T cell function by two distinct mechanisms. One is interferance with the putative role of the accessory molecule in binding MHC molecules and the other is the delivery of a specific "off" signal to the T cell. In order to investigate the latter, we have used a system in which CTL recognition is mediated by hybrid antibodies which bridge the CTL's receptor with an appropriate ligand on the target cell in a manner that is independent of class I expression. In this system we have observed inhibition of lysis by anti-Lyt2 antibodies. In screening a number of CTL clones it was observed that clones varied significantly in their degree of inhibition. This variability appears to be a function intrinsic to the CTL clone and is independent of the concentration of ligand on the target cell. The mechanistic basis for this inhibition will be reported.

T 424 FUNCTION AND EXPRESSION OF T4 IN MURINE XENOGENEIC T CELL HYBRIDOMAS
Barry P. Sleckman, Andrew Peterson, William K. Jones, Judith A. Foran, Julia L. Greenstein, Brian Seed and Steven J. Burakoff. Dana Farber Cancer Institute and Massachusetts General Hospital, Boston, MA 02115.
In order to investigate the role of T4 in the cellular immune response, a xenogeneic murine T cell hybridoma that produces interleukin 2 (IL2) in response to HLA-DR was infected with the defective retrovirus MNST4 DAMP. As a provirus MNST4 DAMP is capable of conferring T4 expression and resistance to the antibiotic G418 upon the host cell. As compared to the non T4 expressing hybridoma, the resulting T4 expressing cells are capable of responding to a log fewer HLA-DR expressing Daudi cells, suggesting that T4 may function to enhance responsiveness under conditions of limiting antigen. Furthermore this response is blocked by the monoclonal antibody OKT4, whereas the non T4 expressing cells are unaffected. Several T4 mutants have been generated including ones in which the cytoplasmic domain of the T4 molecule has been truncated and one in which it has been replaced with the cytoplasmic domain of the human IL2 receptor. T4/T8 hybrid molecules have also been constructed. In addition to the HLA-DR responsive hybridoma, a human class I responsive murine T cell hybridoma has been developed. Both of these hybridomas expressing both the wild type and mutant T4 molecules will be used to study the mechanisms by which T4 influences T cell responsiveness.

T 425 ASIALO GM1 AS AN ACCESSORY MOLECULE FOR T CELL RECEPTORS OF ALLOREACTIVE CTL.
Chou-Chik Ting, Myrthel E. Hargrove, and Yeon-Sook Yun. Immunology Branch, NCI, NIH. Bethesda, MD. 20892.
The expression and function of asialo GM1 (AsGM1) in alloreactive CTL was studied. AsGM1 was expressed by a majority of L3T4$^-$ (either Lyt-2$^+$ or Lyt-2$^-$) CTL clones (8 out of 9), independent of their class specificity (class I or class II). In contrast, all L3T4$^+$ CTL (3) and T helper clones (4) were AsGM1$^-$. The cytotoxic reactions mediated by AsGM1$^+$ CTL were blocked by αAsGM1. The blocking effect was largely dependent on the expression of AsGM1 on the effector cells in a dose dependent fashion, and to a much lesser degree, on the expression of AsGM1 on the target cells. Addition of purified AsGM1 alone also blocked the cytotoxic reactions. Addition of other structurally similar but antigenically different glycolipids did not affect the CTL-mediated cytotoxicity. Furthermore, adding both the αAsGM1 and AsGM1 at proper doses inhibited the blocking (deblocking), indicating that the blocking effect was specific for AsGM1. The expression of AsGM1 also appeared to be associated with the antigen-nonspecific NK-like activity exerted by the alloreactive CTL. All CTL which expressed AsGM1 possesed NK-like activity against antigen unrelated tumor targets, and the AsGM1$^-$ CTL displayed only antigen specific allo-reactivity. These findings indicate that the AsGM1 expressed on alloreactive CTL may function as the accessory molecules for T cell receptors in the antigen specific alloreactive cytotoxicity mediated by AsGM1$^+$ CTL. The expression of AsGM1 may also be related to the activation of NK-like apparatus in these CTL. Therefore, the AsGM1 expression may modulate both the activity and the specificity of the CTL cytotoxicity.

T 426 MOUSE L3T4 GENE SEQUENCE, STRUCTURE AND EXPRESSION IN T CELLS AND BRAIN,
Tourvieille, Beatrice, Scott D. Gorman, Elizabeth H. Field and Jane R. Parnes, Medicine/Immunology, Stanford University Medical Center, Stanford, CA 94305
L3T4 is a T cell surface protein that is expressed on most mouse thymocytes and on mature mouse T cells that recognize class II (Ia) major histocompatibility complex proteins. Such cells are predominately of the helper/inducer phenotype. We have isolated and sequenced mouse cDNA clones homologous to human CD4. After insertion into an expression vector a full-length cDNA clone was transfected into L cells and shown to transfer expression of L3T4. The protein sequence predicted from the sequence of these cDNA clones shows that L3T4 is a member of the immunoglobulin (Ig) gene superfamily. It is encoded by a single gene that does not require rearrangement prior to expression. This gene has been isolated and sequenced. It is composed of at least ten exons spanning a minimum of 16 kb. In contrast to other Ig-like genes, the L3T4 gene has an intron in the NH_2-terminal domain which is homologous to Ig variable regions. The cDNA encoding L3T4 has been transfected into functional cytotoxic T cells and their function is being evaluated. mRNA encoding L3T4 has been demonstrated not only in T lymphoid cells, but also in brain where the predominant mRNA is 1 kb smaller. The difference in this mRNA is in protein coding sequence at the 5' end. In situ hybridization studies and monoclonal antibody staining are being used to evaluate the cells in brain which express L3T4.

T 427 EXPRESSION AND FUNCTION OF THE LYT-2 T CELL DIFFERENTIATION ANTIGEN,
Rose Zamoyska and Jane R. Parnes, Medicine/Immunology, Stanford University Medical Center, Stanford, CA. 94305, USA
The Lyt-2/3 molecule is a glycoprotein expressed predominantly on subsets of T lymphocytes which are restricted by class I major histocompatibility complex molecules and it has been postulated to be involved in the recognition between T cells and their target cells. We recently cloned the gene coding for the Lyt-2 polypeptide chains (Zamoyska et al., 1985 Cell 43:153) and demonstrated that both polypeptide chains bearing the Lyt-2 determinant are encoded by a single gene and result from alternative modes of mRNA splicing. The difference between the two Lyt-2 polypeptides is in the lengths of their cytoplasmic tails which results from alternative splicing of the mRNAs to include or exclude an entire exon which encodes 31 base pairs. The exclusion of this exon causes premature termination of the polypeptide chain. RNAse protection experiments have demonstrated that both forms of mRNA are present in approximately equal amounts in thymus, lymph node and spleen and in all cytotoxic T cell clones examined so far, regardless of whether or not their function is inhibitable by anti-Lyt-2 antibodies. Transfection of expression vectors containing cDNA clones encoding each of the polypeptides individually into T cells results in stable expression of disulphide-bonded homodimers on the cell surface. Both chains have been individually transfected into an L3T4$^+$ T cell hybridoma specific for hen egg lysozyme to examine whether expression of the Lyt-2 molecule per se can confer susceptibility to blocking with anti-Lyt-2 antibodies i.e. whether a negative signal can be transmitted to the cell via these molecules. Results to date show that the antigen specific response of these transfectants cannot be blocked by anti-Lyt-2 antibodies suggesting that anti-Lyt-2 antibodies do not block responses simply by transmitting a negative signal to the cells via these molecules.

Control of Ig and T Cell Receptor Gene Expression

T 428 **IDENTIFICATION OF PRE-B CELL NUCLEAR PROTEINS WHICH SPECIFICALLY INTERACT WITH THE IMMUNOGLOBULIN V-J RECOMBINATION SEQUENCES,** Renato J. Aguilera and Hitoshi Sakano, University of California, Berkeley, CA 94720.

Immunoglobulin and T-cell receptor gene rearrangements are mediated by basically the same recombination mechanism. Adjacent to these gene segments are two consensus sequences CACTGTG and GGTTTTTGT separated by either a 12 or 23 base pair (bp) long spacer. Rearrangement takes place between two pairs of the conserved sequences, with one pair separated by a 12-bp spacer and the other by a 23-bp spacer (12-23 bp spacer rule). Using the gel-migration retardation assay we have detected in a pre-B cell nuclear extract a DNA-binding protein which interacts specifically with a double stranded oligonucleotide containing the recombination sequences of the Ig light-chain variable region gene V_{K21} with the conserved sequences separated by 12-bp spacer. The DNA-binding protein recognizing the V_{K21} recombination sequences was shown to interact with the heptamer sequences by both DNaseI and DMS footprinting. This protein can be competed with increasing amounts of cold V_{K21} competitor DNA but not by other oligonucleotides encoding J_K or irrelevant sequences. The protein appears to be present in nuclear extracts from B and T-cell lines but could not be detected in Hela or L-cell nuclear extracts. Recently, a distinct factor to the one previously descrived was found to interact with the J_{K1} consensus sequences (23-bp spacer). Interestingly, a point mutation at the heptamer abolishes the ability of this factor to bind to the J_{K1} sequences.

T 429 **MINIMUM SEQUENCE REQUIRERMENT FOR IMMUNOGLOBULIN AND T-CELL RECEPTOR GENE REARRANGEMENTS,** Shizuo Akira, Kenji Okazaki and Hitoshi Sakano, University of California, Berkeley, CA 94720.

In order to understand the molecular mechanisms of the antigen receptor gene rearrangement, we have investigated the essential and minimum sequence requirement for the V-J gene recombination using a retroviral vector system. In this system, site specific V-J recombination will activate an inverted neo gene by placing it into the proper orientation relative to the viral LTR promotor. We have introduced the recombination substrate into pre-B cell lines as well as B-cell progenitors derived from bone marrow culture, which are actively rearranging their Ig gene segments in culture. We have shown that the putative V-J recombinase can undergo the rearrangement just with two sets of 7mer and 9mer sequences without any coding sequences. We have also analyzed which point mutation(s) in the consensus sequences affect the recombination.

T 430 IMMUNOGLOBULIN V_H GENE DIVERSITY, ORGANIZATION, AND EXPRESSION IN HUMANS, Jeffrey E. Berman, S. Mellis, K. Nickerson, C. Kowal, H. Suh, R. Collum, R. Pollock, B. Heinke, C. Smith, C. Cantor, L. Chess, and F. Alt, Columbia University College of Phys. & Surg. New York, NY 10032.

We have identified four Hu V_H gene families using two strategies: 1) A panel of mouse V_H family probes was used to isolate homologous Hu V_H genes, 2) A Hu J_H probe was used to isolate V_H-D-J_H rearrangements, which were subsequently used to identify homologous germline genes. Twenty isolated germline V_H genes were sequenced, allowing characterization of the basic structure and relatedness of Hu V_H genes from all four families and to deduce evolutionary relationships between Hu and mouse V_H genes. Phage cloning and pulsed field gradient gel analysis demonstrated intermingling of all four Hu V_H families, in striking contrast to the mouse where the families are usually segregated in discrete clusters. Currently, we are studying Hu V_H gene usage and expression in various types of Hu B lineage tumors. A-MuLV-transformed pre-B cells have proven very useful in studies of V_H rearrangement, expression, and utilization patterns in the mouse. We have begun similar studies with human pre-B cell lines, including EBV-transformed Hu fetal liver lines, some of which have undergone D to J_H rearrangement and may append VH segments during growth in culture. It has been suggested that germline expression of mouse V_H genes may target rearrangement. Our preliminary evidence suggests germline V_H expression also occurs in humans.

The T Cell Receptor

T 431 ANALYSIS OF THE T CELL RECEPTOR IN CYTOCHROME C SPECIFIC HYBRIDOMAS VIA CLONING, SEQUENCING AND TRANSFECTION, Isaac Engel, David McElligott, Pamela J. Fink, Louis A. Matis and Stephen M. Hedrick, University of California, La Jolla, CA 92093.
We are attempting to understand the relationship between the T cell receptor primary structure and MHC/antigen specificity of the cytochrome c specific helper T hybridomas 9R-1 and 9R-2 (Hedrick et al, 1982), as well as of other cytochrome c specific T cell clones. We have constructed genomic libraries for 9R-1 and 9R-2 from which we have isolated the unique productively rearranged β chain genes of both hybridomas, and have identified the VDJ_{β}combinations they use as well as their junctional region sequence. In addition, the VJα combination used by 9R-2 has been determined. Sequence comparisons between the β chains of 9R-1, 9R-2 and several other T cell clones studied (Fink et al, 1986) reveals the conservation of an aspartate residue at position 100 (within the junctional region) between the two hybridomas and all clones which share with them the ability to respond to $I\text{-}E^{s}_{\beta}/^{k}_{\alpha}$ plus cytochrome c. We would like to directly test the importance of this aspartate residue by way of site directed mutagenesis and transfection. Recently we have introduced via electroporation a 12kb genomic fragment containing the entire 9R-2 β chain along with 5.5kb of upstream flanking region into the T cell clone D10 (Kaye et al, 1982). Results to be presented indicate that we are able to obtain clones that transcribe the transfected gene at high levels in only a small percentage of stable transfectants. Fink et al, Nature 321, p. 219-226 (1986)
Hedrick et al, Cell 30, p. 141-152 (1982)
Kaye and Janeway, JEM 159, p. 1397 (1984)

T 432 T cell disfunction of scid mouse is dependent on lack of rearrangement of TcR genes, S.Habu,M.Kimura,M.Katsuki and K.Hioki,Tokai Univ. Japan.
It is known that the T cell specific properties initiate to be expressed in ordered manner on the lymphoid cells immediately after their migration into the thymus. However, it is remained whether the sequential expression is regulated by developing thymic stromal cells or by the gene products which have been activated in the thymic lymphocytes. The scid mouse, T and B cell deficient mutant, may be a model to clarify these questions. In the adult thymocytes of scid mouse, more than 90% of them were $Thy\text{-}1^{+}$ and $IL\text{-}2R^{+}$. However, Lyt-2 or L3T4 was not detectable on thymocytes as well as spleen cells. In addition, Southern blott analysis using a cDNA probe 86T5 demonstrated no rearranged pattern of β-chain genes of T cell receptor(TcR) in the scid thymocytes. Since rearrangement/expression of TcR genes is proved to precede Lyt-2/L3T4 expression but follows Thy-1/IL-2R expression in the normal embryonic mouse, the scid mouse thymocytes are considered to be arrested their differentiation in the early stage of T cell ontogeny. Of the littermates of scid mice, the mouse with low or medium level of serum Ig showed some evidence of the rearranged pattern of TcR B-chain genes in parallel with serum Ig amount. In addition, $Lyt\text{-}2^{+}$ and $L3T4^{+}$ thymocytes appeared in these mice. Taken together, it is strongly suggested that the scid mouse impairs a common process which is required for differentiation both of T and B cells after they branch into the independent cell lineage.

T 433 ANALYSIS OF PROMOTER FUNCTION IN CLONED HUMAN T LYMPHOCYTES, John E. Hambor, Robert R. Getty, Diane C. Farhi, David R. Kaplan, and Mark L. Tykocinski, Case Western Reserve University, Cleveland, OH 44106.
In order to establish a gene transfer system for expression of high levels of selected mRNAs in human T lymphocyte clones, we screened a panel of five eukaryotic promoters to identify ones with maximal activity. Plasmids in which various promoter/enhancer elements are linked to the prokaryotic chloramphenicol acetyltransferase (CAT) gene were introduced into two of our nonneoplastic human T cell (CD4+) clones, V1 and CH.4, and the neoplastic human T cell lymphoma MOLT 4 by protoplast fusion. Analysis for transient CAT expression (48 hr) by an enzymatic assay showed that the Rous sarcoma virus (RSV), the leukotropic papovavirus and the rat p3C5 gene ionophore-inducible promoters drive high levels of CAT expression, whereas the human metallothionein II_A and the simian virus 40 early promoters are substantially weaker in all cell lines. The RSVCAT DNA segment was subcloned into the plasmid p220.2, a high copy number episomal replication vector which contains the Epstein-Barr virus (EBV) ori, a portion of the EBV nuclear antigen (EBNA-1) gene and a resistance gene for the eukaryocidal antibiotic hygromycin (hyg). Transient CAT expression assays demonstrated that RSV promoter function is maintained in this episomal replicon. We devised an efficient protocol for the derivation of stable cloned human T cell transfectants. Human T cell clones stably transfected with RSV-CAT/220.2 continued to express high levels of CAT activity after 17 days of hyg selection. In situ hybridization using a ^{35}S-labeled anti-sense CAT RNA probe confirmed that these stable transfectants were uniformly expressing high levels of CAT mRNA.
(Supported in part by grants AI22505 and CA36189)

T 434 REARRANGEMENT AND EXPRESSION OF T CELL RECEPTOR GENES IN PRE-T CELLS. Yasuhiro Hashimoto, Erica Sibinga, Ann Haberman and Kenneth J. Blank. Department of Pathology, University of Pennsylvania, PA 19104

The study of T cell receptor gene rearrangement and expression during development frequently involves the analysis of hybridomas. Such studies may be complicated by use of the fusion partner, BW5147, which expresses the TcR alpha and beta chain genes. Fusion with thymocytes might result in the rearrangement and expression of previously inactive TcR genes. We have circumvented this problem by the isolation of several unique murine thymomas representing various phenotypically immature stages of T cell development. Experiments within our lab and with Dr. Mark Davis have allowed the analysis of T cell receptor gene expression and rearrangement patterns within these pre-T cells. The results of these experiments suggest:

1) TcR gene rearrangement may precede and/or be disassociated from Thy 1 gene expression.
2) Beta and gamma chain gene rearrangement may precede alpha chain gene rearrangement.
3) Beta and gamma chain gene transcription is not necessary for alpha chain rearrangement.

T 435 EXPRESSION OF CLONED T CELL RECEPTOR GENES, Fredrik Ivars, Leslie Berg, Christopher Goodnow, Henri-Jean Garchon, Susan Gilfillan and Mark Davis. Stanford University, Stanford CA 94305

We have prepared constructs of T cell receptor (TCR) alpha and beta chain genes from the pigeon cytochrome c specific T cells 2B4 and 5C.C7. For the TCR alpha chains, the expressed variable region genes were constructed from a combination of genomic and cDNA clones. The variable region gene of the 2B4 beta chain was previously described. For expression studies in tissue culture cells, two approaches were followed: (1) transient expression was assayed by fusing the coding regions of the constructs to the human cytomegalovirus (CMV) immediate early promoter/enhancer, and (2) the entire genes were linked to selectable genetic markers for stable introduction into lymphoid cells. RNase protection assays indicated that RNA transcribed from the CMV-TCR constructs introduced into L cells is processed identically to transcripts of the TCR genes in the original T cells. TCR gene constructs with or without the immunoglobulin heavy chain enhancer inserted into the TCR J-C intron were stably introduced into both T cells (EL4) and B cells (J558L). RNA analysis indicates that the immunoglobulin heavy chain enhancer increases expression of TCR genes by severalfold in both T and B cells. In addition, transfected lines were found to produce the 2B4 TCR alpha chain protein as detected by immunofluorescence and immunoprecipitation using a monoclonal antibody specific for the protein. Alpha and beta chain constructs have also been introduced into fertilized mouse eggs by microinjection. Offspring carrying the transgenes are currently being analyzed for expression of the introduced TCR genes.

T 436 GENE TRANSFER OF T CELL ANTIGEN:Ia RECEPTORS USING RETROVIRAL VECTORS, Jonathan Kaye, Isaac Engel, Pamela J. Fink and Stephen M. Hedrick, University of California, La Jolla, CA 92093.

Despite the elucidation of the gene elements which encode the antigen: Ia receptor on helper T cells, the structural basis of the receptor-ligand interaction is still poorly understood. We are addressing this problem using gene transfer techniques to examine the relationship between the structure of the receptor chains and the resulting recognition properties of the T cell. A series of cloned α and β chain cDNA's derived from cloned helper T cell lines or hybrids specific for pigeon cytochrome C and I-E^k molecules, but differing in fine specificity, have been cloned into retroviral vectors. Such vectors lack retroviral structural gene sequences but contain viral regulatory elements necessary for the expression of the inserted cDNA, as well as contain a dominant selectable marker, in this case resistance to compound G418. We have used these constructs to produce infectious viral particles containing either α and β chain genes. Both helper T cell hybrids, and a cloned helper T cell line specific for conalbumin and I-A^k, have been successfully infected with these recombinant viruses, transferring G418 resistance to the recipient cell. However, the level of mRNA expression of the transferred receptor genes has been variable. In one instance we have infected a cloned T cell line with a mix of recombinant viruses containing α chain and β chain genes. The recipient infected cells expressed both α and β chain genes encoded by the viruses. We are currently cloning these infectants to look for cells expressing high levels of both receptor chains, and to characterize the resulting antigen: Ia recognition properties.

T 437 REARRANGEMENT OF T CELL RECEPTOR BETA CHAIN GENES IN TRANSFORMED HUMAN CELL LINES THAT HAVE B CELL CHARACTERISTICS AND PRODUCE IMMUNOGLOBULIN,
Thomas J. Kindt and Mary Ann Robinson, Laboratory of Immunogenetics, NIAID, NIH, Bethesda, MD 20892

Lymphoid cell lines were derived by Epstein-Barr virus (EBV) transformation from 60 individuals in eight different families for use in a molecular genetic study of HLA and T cell receptor genes. Transformation of peripheral blood lymphocytes by co-incubation with EBV is thought to produce B lymphoblastoid cell lines, however, rearrangement of T cell receptor (TCR) β chain genes was observed in a cell line from one of these individuals. Examination of DNA from peripheral blood lymphocytes of this individual showed germline configuration of both TCR β genes. A second transformation of cells from this individual yielded a line that also had a rearranged TCR β gene, however, the pattern of rearrangement was different from that of the original line. The rearrangement events in both cell lines involved the maternal TCR β haplotype. It was possible to derive cell lines from this individual with nonrearranged T cell genes by cloning of cells from an early passage of the EBV transformation culture. The cell lines with the rearranged T cell genes, cells from the same individual and from those from a sibling that did not carry rearranged T cell genes were compared. Clonality of lines was ascertained by unique rearrangement patterns for immunoglobulin H and κ chain genes. All lines contained rearranged immunoglobulin genes, secreted immunoglobulin and carried B and not T cell surface markers. These results support the hypothesis that a common recombinase mediates rearrangement events in both T and B cell lines; a rationale for its activity on certain T cell genes in B lymphoblastoid cell lines is being sought.

T 438 DECREASE IN T CELL RECEPTOR (TcR) γ RNA IN MOUSE THY-1+ DENDRITIC EPIDERMAL CELLS (dEC) AFTER CON A STIMULATION.
William A. Kuziel, Robert Tigelaar, Akira Takashima, Paul Bergstresser and Philip W. Tucker. U.T.H.S.C.D. Dallas, Texas 75235.

Thy-1+ dEC are bone-marrow-derived, located in the mouse epidermis, and characterized by a striking dendritic morphology in situ and expression of large amounts of cell surface Thy-1. Thy-1+ dEC have a phenotype similar to fetal or immature thymocytes ($L3T4^-$, $Lyt\text{-}2^-$). Con A-stimulated Thy-1+ dEC proliferate, secrete an IL2-like factor, and exhibit MHC-nonrestricted, NK-like cytotoxicity. Clones established by limiting dilution microculture of FACS-purified Thy-1+ dEC retain these phenotypic and functional properties. Northern blot analysis of four clones showed that each expressed abundant levels of two TcR γ RNAs: two forms of Cγ1 (differing in the 3'-untranslated region) in association with the proximal variable region, V3, and either V1.1-Cγ4 or V1.2-Cγ2. None of the clones expressed TcR α RNA and only one expressed TcR β RNA at a very low level. Two clones were analyzed at the DNA level and showed rearrangements of TcR γ and β genes; all four β genes in both clones were involved in a rearrangement/deletion event. Con A stimulation led to the rapid appearance of IL-2 RNA with a short-lived peak occurring between 6-9 hrs; IL-2 receptor RNA also increased within 6 hrs and remained elevated for longer than 24 hrs. While RNA for the δ chain of T3 increased, Con A stimulated a rapid decrease in the steady state level of TcR γ RNA, which had not returned to normal levels by 24 hrs. The regulated expression of V3-Cγ1 RNA suggests an important relationship between expression of the V3 variable region and the function of these unusual cells, which may represent a murine intraepidermal equivalent of recently described peripheral CD3+,$CD4^-$,$CD8^-$, TcR γ+ T cells in humans.

T 439 DEVELOPMENTAL REGULATION OF T CELL ANTIGEN RECEPTOR EXPRESSION,
H. Robson MacDonald, Ralph C. Budd, Guido Miescher, Rosemary K. Lees, Rawleigh C. Howe, Ludwig Institute for Cancer Research, Lausanne Branch, 1066 Epalinges, Switzerland.

The T cell antigen receptor (TCR) complex consists of a disulphide-linked alpha/beta heterodimer non-covalently associated with a molecular complex known as CD3. Genes coding for the polymorphic alpha and beta chains of the TCR complex undergo somatic re-arrangements similar to those of immunoglobulins and are initially expressed in a developmentally ordered fashion in the thymus. Thus immature thymocytes (lacking expression of the differentiation antigens CD4 and CD8) express high levels of beta chain transcripts but very low levels of alpha chain transcripts, whereas more mature thymocytes (and peripheral T cells) express both. We have now identified a rare subpopulation corresponding to ~10% of immature ($CD4^-$ $CD8^-$) mouse thymocytes which transcribe abundant amounts of TCR alpha chain mRNA and express low levels of TCR protein (as assessed by monoclonal antibodies KJ16-133 and F23.1) on the cell surface. Interestingly, the proportion of $KJ16^+$ or $F23^+$ cells in this thymocyte subpopulation is significantly (2-fold) higher than in mature thymocytes or peripheral T cells. Since KJ16 and F23 react exclusively with TCR proteins containing products of a particular family of beta chain variable region genes, these data strongly suggest that utilization of TCR beta chain gene segments is developmentally regulated. Whether this regulation is linked to the expression of products of the major histocompatibility complex is currently under investigation.

The T Cell Receptor

T 440 EVOLUTION OF MURINE IMMUNOGLOBULIN Cλ2 AND Cλ4 GENES. Fathia Mami and Thomas J. Kindt, Laboratory of Immunogenetics, NIAID, NIH, Bethesda, MD 20892

A genomic clone containing the λ constant (C) region genes, λ2 and λ4, has been isolated from a genomic library from the mouse strain SPE. SPE is an inbred strain derived from progenitors trapped near Grenada in Spain and has been classified as mus 3 or mus spretus. The structure of the Cλ2-Cλ4 gene cluster in SPE has been conserved relative to laboratory mouse strains. Sequence comparison of SPE Cλ2 and Cλ4 genes indicates that these genes have evolved under different selective pressures. The BALB/c and SPE Cλ2 genes are highly conserved (99% sequence identity) and the SPE Cλ2 gene appears to be functional. By contrast, the Cλ4 genes have significantly diverged in sequence and the SPE Cλ4 contains a large deletion relative to the BALB/c counterpart. This gene, which is not expressed in laboratory mouse strains presumably because of a fault in the joining (J) λ4 sequence, appears to have been under less stringent evolutionary pressure and is certainly a pseudogene in the SPE strain by virtue of the observed C region defects. By contrast to BALB/c, the SPE Jλ4 gene includes all sequences required for a functional J gene. It appears that the Cλ4 gene became nonfunctional in an ancestral mouse species and that evolutionary drift may account for the accumulation of additional defects in this pseudogene.

T 441 TRANSCRIPTIONAL REGULATION OF THE T CELL RECEPTOR BETA CHAIN LOCUS Skye McDougall and Kathryn L. Calame, Department of Biological Chemistry and Molecular Biology Institute, UCLA, Los Angeles, CA 90024.

The transcriptional regulation of a murine T cell receptor beta chain gene is being examined in order to understand how this family of genes is expressed in a regulated manner. The T cell receptor beta chain locus has been examined for the presence of a transcriptional enhancer. The region spanning Jβ1 thru Cβ2 has been extensively subcloned into the traditional CAT vector pA10CAT2 and assayed for enhancer activity by transient transfections into beta-expressing murine T helper cell lines. No enhancer activity has been detected utilizing either the SV40 promoter or a Vβ promoter. This result suggests that there is no enhancer in the region of the beta chain locus studied and that transcriptional regulatory mechanisms may not be as highly conserved between the T cell receptor genes and immunoglobulin genes as is their structure and ability to rearrange. The transcriptional start site of the Vβ3 promoter has also been determined and studies are in progress to determine the functionally important regions of this promoter for baseline and, possibly, T-cell specific expression.

T 442 CYCLOSPORIN A INHIBITS THE INDUCTION OF T-CELL RECEPTOR mRNA, Miles Wilkinson & Carol MacLeod, Cancer Center, University of California, San Diego, 92093.

The mechanism by which the immunosuppressant cyclosporin A (CsA) inhibits T-cell function is not clearly understood. Recently, the expression of the α chain of the T-cell antigen receptor (TCR) has been shown to regulate and limit TCR expression in maturing thymocytes. Here, we show that CsA inhibits the induction of TCR-α mRNA elicited by calcium ionophore A23187 treatment of the murine T-lymphoma SL12.4 and RS4.2 cell clones. CsA exerts a partially reversible inhibitory effect on both early and late phases of TCR-α mRNA induction. The transcription of the TCR-α gene is not inhibited by CsA; instead CsA inhibits TCR-α mRNA induction by regulating specific post-transcriptional mechanisms. CsA only exhibits an abrogatory effect in response to A23187; it does not inhibit TCR-α mRNA induction elicited by phorbol myristate acetate or cycloheximide. CsA does not significantly inhibit TCR-α mRNA accumulation in T-lymphoma clones which constitutively express this transcript. CsA has no effect on the constitutive expression of TCR-β, Thy-1, Lyt-2 and actin mRNA in T-lymphoma clones. However, the amount of T3-δ mRNA is depressed in RS4.2 cells treated with both CsA and A23187, but not when cells are treated with either agent alone. Our results demonstrate a specific inhibitory effect of CsA on calcium dependent activation events in T-cells, including the induction of TCR mRNA expression. Supported by NIH CA37775 and T32 HL07107.

Class II MHC Structure/function

T 443 MOLECULAR BASIS FOR DEFECTIVE CELL SURFACE EXPRESSION OF THE E_α:E_β POLYPEPTIDE, A.B. Begovich, F.M. Tacchini, T.H. Vu, P.P. Jones, Stanford University, Stanford, CA 94305. Four of the eleven independent inbred haplotypes (b, s, f and q) and approximatley 15-25% of the chromsomes found in wild Mus populations fail to express the I-E molecule. To date the only E^- defect that has been characterized is the $E_\alpha{}^0$ mutation, a 627 bp deletion at the 5′ end of the E_α gene in b, s, and many wild derived haplotypes. We are examining the nature of the E^- defects in the f, q, and w17 haplotypes. The w17 haplotype of the H-2 congenic strain B10.CAS2 was derived from the species *Mus castaneus*, while the b, s, f , and q haplotypes were derived from the species *Mus domesticus*. These two species are thought to have separated over 2 million years ago. We will show that the $E_\alpha{}^{w17}$ gene contains the $E_\alpha{}^0$ mutation and that failure to express the $E_\beta{}^{w17}$ gene is due to a single base insertion in the 5′ donor splice site of the first intron. We will also show that $E_\beta{}^q$ has the same splicing defect as $E_\beta{}^{w17}$, an interesting finding in light of the genetic distance between the two species that gave rise to these haplotypes. Finally, we will report our progress on the characterization of the defects in the $E_\alpha{}^q$ and $E_\beta{}^f$ genes.

T 444 A MOLECULAR DISSECTION OF IA STRUCTURE-FUNCTION RELATIONSHIPS IN IMMUNE RECOGNITION. N. S. Braunstein, A. J. Sant, F. Ronchese, R. I. Lechler, and R. N. Germain. Laboratory of Immunology, NIAID, NIH, Bethesda, MD 20892.

Using DNA mediated gene transfer into mouse L cells, the effects of Ia α and β polymorphisms on heterodimer expression, serologic epitopes, and T cell stimulation were analyzed. The results demonstrate a segregation of function between two distinct segments of the β1 domain. Thus polymorphic residues in the NH2-terminal half of the β1 domain determine with which α chain a particular β chain can achieve efficient cell surface expression. Alterations in this region of β1 affect antibody binding to distally encoded β and α epitopes, apparently through conformational effects on the assembled heterodimer. T cell co-recognition of antigen and Ia is highly sensitive to the conformational changes induced by varying the polymorphic residues controlling this αβ interaction. Allelic variable residues in the COOH-terminal half of the β1 domain, on the other hand, show only minor effects on cell surface Ia expression and overall conformation but instead define epitopes that seem to be directly involved in the binding events related to antibody recognition of Ia and restricted recognition by T cells of Ia plus antigen. These data suggest a segmental model of Ia structure-function in which all Ia molecules have a similar overall structure determined by the interaction of the α and β chains, a process critically dependent on the NH2-terminal polymorphic regions of β. Other regions, including HV3 of the β chain, are postulated to remain exposed on the surface of the molecule and together with portions of the α chain, form the structures directly involved in both T cell and antibody recognition events.

T 445 STRUCTURAL MUTATIONS AFFECTING INTRACELLULAR TRANSPORT AND SURFACE EXPRESSION OF MURINE CLASS II MOLECULES. L.H. Glimcher, Z. Ghogawala, M. Rodriguez*, D.J. McKean* and I.J. Griffith, Harvard School of Public Health and Harvard Medical School, Boston MA 02115 and *Mayo Clinic Medical School, Rochester MN 55905.
Our laboratory has generated a panel of Ia-negative variant cell lines in order to define regions of the murine class II molecules important for intracellular transport and cell surface expression. These variants were all derived from the M12.4.1 H-2^d B cell lymphoma by γ-irradiation (1,000R) and several rounds of negative immunoselection with an anti-Ia monoclonal antibody (mAB) plus complement. It was possible to identify variants that did not express one or both Ia isotypes on their cell surface, that is they were I-A^-/I-E^+, I-A^+/I-E^- or I-A^-/I-E^- (Glimcher et al. 1985. J. Immunl. 135:3542). Further analysis allowed us to select variants that contained mutations affecting post-translational Ia gene expression. It was possible using immunoelectronmicroscopy to observe accumulations of the appropriate Ia polypeptides in distinct intracytoplasmic compartments. Transfection studies indicated that the transport defects result from structural mutations in the Ia genes. Sequence analysis will be presented.

T 446 CONTRIBUTION OF NZW GENES TO LUPUS-LIKE AUTOIMMUNE DISEASE. Brian L. Kotzin and Ed Palmer, University of Colorado, VA Medical Center and National Jewish Center for Immunology and Respiratory Medicine, Denver, CO.

New Zealand Black (NZB) x New Zealand White (NZW) F_1 mice exhibit an autoimmune disease characterized by high serum levels of IgG antibodies to double-stranded DNA and histones and a fatal immune-complex glomerulonephritis. In contrast, only rare parental NZB or NZW mice express this lupus-like disease. We have previously identified a unique NZW T-cell receptor beta-chain gene complex distinguished by a large deletion of DNA containing $C_{\beta 1}$, $D_{\beta 2}$, and $J_{\beta 2}$ cluster. NZW mice also differ from NZB and other strains at the T-cell receptor alpha-chain locus and at the major histocompatibility (H-2) locus. We studied the contribution of these 3 NZW gene loci to (NZBxNZW) F_1 disease by backcrossing F_1 mice to NZB mice and correlating the genotype with the expression of lupus-like disease in backcross mice. Mice were identified as either heterozygous for the NZW allele or homozygous for the NZB allele for each gene by Southern analysis of genomic DNA.

Forty-five percent of 174 female backcross mice developed severe proteinuria prior to 11 mo of age compared with 95% of (NZBxNZW) F_1 mice (N=40), and less than 5% for NZB (N=50) and NZW (N=30). Of 47 randomly-selected backcross mice with severe renal disease, 89% carried the NZW $H-2^z$ allele while approximately half carried the NZW T-cell receptor alpha- or beta-chain alleles. Of backcross mice without evidence of renal disease (N=45), 85% were homozygous for the NZB $H-2^d$ allele. Approximately half carried the NZW alpha- or beta-chain genes. Similar percentages and correlations were also observed for IgG autoantibody production. Thus, the NZW gene contribution to lupus-like (NZBxNZW) F_1 disease appears to be related to one dominant gene linked to the $H-2^z$ locus. In contrast, the unusual NZW T-cell receptor gene complexes do not appear to be involved.

T 447 CYTOTOXICITY OF MYCOBACTERIAL ANTIGEN INDUCED CD_4^+ HUMAN T CELL CLONES AGAINST ANTIGEN PRESENTING CELLS. RELATION BETWEEN CYTOTOXICITY AND OTHER FUNCTIONS, Abu S. Mustafa[1], Richard A. Young[2] and Tore Godal[1]. [1]Institute for Cancer Research, N-0310 OSLO 3, [2]Whitehead Institute for Biomedical Research, Cambridge, MA 02142, USA.

A total of 120 BCG induced and 42 M. leprae induced human CD_4^+ T cell clones were raised from vaccinated healthy volunteers. About one third of the clones were specific in proliferative and lymphokine production assays to the antigens against which they were raised, others were limited to broadly crossreactive. When tested for cytotoxicity, the T cell clones were capable of killing BCG and M. leprae pulsed monocytes/macrophages and EBV transformed B cell lines pulsed with PPD. Optimal cytotoxicity occured at T cell to antigen presenting cell (APC) ratio of 10:1, whereas optimal proliferation and lymphokine production was observed at T cell to APC ratio of 1:1. Recombinant antigens isolated by antibody probes from genomic DNA library of M. leprae and M. tuberculosis were screened against T cell clones to define the antigens recognized in proliferative responses. Five M. leprae specific T cell clones responded to 18 k recombinant antigen of M. leprae and one crossreactive T cell clone proliferated to 65 k recombinant antigen of both M. leprae and M. tuberculosis. Proliferative and cytotoxic activities of these T cell clones paralleled closely with respect to antigen specificity vis a vis crossreactivity and class II MHC restriction. Relevance of these findings in the regulation of immune response against mycobacteria will be discussed.

T 448 RECOGNITION OF NOVEL MHC CLASS II ANTIGENS BY T LYMPHOCYTE CLONES, E. Nisbet-Brown, M. Letarte, J. Lee, J.A. Falk and E. Gelfand. The Hospital for Sick Children and Canadian Red Cross Blood Transfusion Service, Toronto, Canada.

We used antigen-specific human T cell clones to study the relationship between major histocompatibility complex (MHC) and antigen recognition specificities expressed by T cells. Tetanus toxoid (TT)-specific T lymphocyte clones were derived from an immunized donor by limiting dilution from peripheral blood mononuclear cells (PBM) restimulated with TT in vitro. Clones were screened for MHC-restricted antigen recognition against HLA-typed antigen-presenting cells (APC) using an in vitro T cell proliferation assay. Several distinct patterns of antigen recognition were identified. In addition to T cells which recognized TT in association with donor Class II MHC antigens, we found clones which simultaneously expressed self-restricted antigen recognition and alloreactivity, and other clones which recognized TT specifically with HLA Class II nonidentical APC. This was confirmed in inhibition studies using monoclonal antibodies against Class II MHC antigens to block proliferative responses. We propose that these clones may be restricted by "hybrid" HLA Class II MHC antigens and we suggest a possible structure for these molecules. These results argue that the T cell antigen receptor can undergo random or antigen-dependent changes in vitro, and that somatic diversification of the T cell repertoire may occur in vivo.

T 449 A MOLECULAR MECHANISM FOR THE UNRESPONSIVENESS OF B10.A(5R) MICE TO PIGEON CYTOCHROME C. F. Ronchese, B.S. Fox, R.H. Schwartz and R.N. Germain. Laboratory of Immunology, NIAID, NIH, Bethesda, MD 20892.

B10.A mice are responders to pigeon(P) and moth(M) cytochrome C(CC) in the context of EβkEα, whereas B10.A(5R) mice respond to MCC but not PCC together with EβbEα. The majority of T cell clones derived from PCC primed B10.A mice mimic these in vivo response patterns, i.e., they respond to either P or M CC with self-Ia(EβkEα), but only to MCC with allogeneic Ia (EβbEα). To investigate the molecular basis for these response patterns, variant Eβ genes encoding either polymorphic residue 29 from Eβk associated with the polymorphic residues 87,89, and 93 from Eβb (Eβkb) or vice-versa (Eβbk) were generated by hemi-exon shuffling, and these or wild-type Eβ genes cotransfected with Eα into L cells. Both recombinant and wild-type molecules expressed by the transfectants were fully competent in presenting antigen to a panel of EβbEα/EβkEα degenerate or restricted T cell clones and hybridomas of various antigen specificities. However, Eβbk was unable to present PCC to specific T cell clones and hybridomas, although it retained the ability to present MCC to the same T cells. The importance of residue 29 in presenting PCC was confirmed when T cells with less frequent patterns of fine specificity were analyzed: EβbkEα transfectants presented only MCC to a T cell hybridoma restricted to only EβkEα, and presented PCC to a T cell clone restricted to only EβbEα. These data indicate that a single residue in the HV2 region of Eβ can affect the fine specificity of Ia-dependent antigen presentation (desetope function) in a manner concordant with IR gene control without altering the restriction phenotype (histotope function) of the same class II molecule. This functional independence of desetope and histotope implies that each may play a unique role in determining the immune response potential of an individual.

T 450 FUNCTIONAL EPITOPES OF I-A^k INVOLVED IN ANTIGEN PRESENTATION - Edward Rosloniec, Denise Gay and John Freed, Nat'l. Jewish Ctr. for Immunol. & Resp. Med., Denver, CO 80206

In order to probe the interaction of T-cell receptor, Ia and antigen during antigen presentation, monoclonal antibodies (mAb) to I-A^k were used to block the activation of I-A^k restricted T-cell hybrids by antigen presenting cells (APC) and lysozyme fragments. Three T-cell hybrids, each specific for a different antigenic peptide of lysozyme, were found to have differential sensitivities to the blocking effect of mAb to nominal Ia specificities 1,2,15,17 and 19. While the majority of mAb tested were inhibitory, mAbs 17/227 (Ia.15) and H116-32 (Ia.19) did not block T-hybrid h4Ly7.5 However, mAb 17/227 was strongly inhibitory for h4Ly18.2 and 50.5, whereas 18.2 was not blocked by mAb 11-5.2 (Ia.2) and 10-3.6 (Ia.17). Fab' fragments, prepared from several of the mAb, showed the same pattern of blocking as the intact mAb. However, the Fab's from H116-32 failed to inhibit presentation to 50.5 implying that the epitope bound by this mAb is distal to site(s) of interaction with the T cell receptor. Peptide specificity was not responsible for the differences in anti-I-A blocking as 4 T-cell hybrids specific for the same lysozyme peptide also showed differential patterns of anti-I-A blocking. Measurements of L3T4 expression by the T-cell hybrids did not explain the differences observed in a T-cell hybrid's sensitivity to mAb blocking. Studies using mutant APC suggest that each T-cell hybrid used in these studies expresses a different T-cell receptor, indicating that the mAbs in the blocking experiments may be interfering with T-cell receptor-I-A interactions. Experiments are currently underway to address this possibility directly.

T 451 THE IR TO BEEF AND SHEEP INSULIN IS CONTROLLED AT THE LEVEL OF ANTIGEN PRESENTATION AND NOT THE T CELL REPERTOIRE, Christine K. Rudy and Brigitte T. Huber, Tufts University School of Medicine, Boston, MA.

B6 and the Ia mutant bm12 mice show a reciprocal qualitative difference in responsiveness to beef and sheep insulin; namely, the former are high responders to beef insulin and low responders to sheep insulin, while the latter show the opposite response patterns, as measured by T cell proliferation. Using T cell hybridomas (THy) this response was further analyzed at the clonal level. Both the heterocliticity of the response and the results obtained in THy titration studies support the hypothesis that the T cell receptor (Tcr) recognizes a shared antigenic determinant on beef and sheep insulin. The response pattern, therefore, is a reflection of differentiatial antigen presentation of the two species of insulin; i.e. Ia^b presents beef insulin more efficiently than sheep insulin and vice-versa for Ia^{bm12}. Beef and sheep insulin differ from each other by a single amino acid in the A chain loop. This allows us to dissect the immune molecule into its functional parts and to directly test our hypothesis by: a) blocking presentation with peptides containing the A chain loop which is thought to contain the agretope, the Ia-antigen interaction site; and b) by direct activation of the THy with N-terminal peptides which supposedly contain the epitope, the Tcr-antigen interaction site.

The T Cell Receptor

T 452 MOLECULAR AND FUNCTIONAL ANALYSIS OF MURINE IA ASSEMBLY AND EXPRESSION.

Andrea J. Sant, Ned S. Braunstein, and Ronald N. Germain. NIAID, NIH, Bethesda MD. 20892

Cell surface expression of Ia antigens is dependent on association of the α and β chains into a stable heterodimer. Using recombinant DNA technology and an L cell transfection system, the effect of polymorphism on α β dimer formation and co-expression have been investigated. In analyzing the requirements for inter-isotype dimer expression, we found that allelic polymorphic residues in Aβ chains have a profound impact on the ability of Aβ to be co-expressed with Eα. By using exon shuffled and hemi-exon shuffled Aβ genes, we determined that the differential capacity of Aβ to be co-expressed with Eα can be totally accounted for by polymorphic residues in the amino-terminal half of the β1 domain. To assess the importance of isotypic residues in α β co-expression, hybrid Aβ-Eβ genes were constructed to generate proteins consisting of Aβ in the first domain and Eβ in the second domain, transmembrane and intracytoplasmic regions. Hybrid or wild type β chain genes were co-transfected into L cells with either Aα or Eα. Flow cytometric analysis of the resultant transfectants indicated that the isotypic origin of the carboxy-terminal half of the β chain had no detectable influence on the efficiency of dimer formation and cell surface expression. In addition, when paired with Aα, the A-E hybrid β chains were comparable to wild type Aβ in the antigen specific, MHC restricted activation of T lymphocytes, suggesting that isotypically determined structures in the membrane proximal regions of Ia do not influence the specificity of antigen presentation and T cell recognition. Together, these results argue strongly that the selective expression of α β pairs and the structures important for antigen specific T cell recognition are determined almost exclusively by the amino terminal domains of Ia molecules.

T 453 EXPRESSION OF TRUNCATED CLASS II I-A ANTIGEN CHAINS, Paul Travers and Hugh McDevitt Stanford University Medical School, Stanford, CA 94305

Polymorphic residues within the MHC class I and class II antigens are believed to influence the interaction of these antigens with both nominal antigen and the T cell receptor. To attempt to understand these interactions at the molecular level we have been constructing a soluble and potentially secretable form of the class II I-A antigen for both biochemical and crystallographic studies. Premature stop codons have been inserted into both the alpha and beta chain cDNA's encoding the I-Ak allele, and these cDNA's have been expressed under the control of a strong cellular promoter in a variety of lymphoid cell lines. In the absence of the transmembrane domains the two chains do not pair and are not exported from the cell. In addition the beta chain is found in association with a 73K cellular protein which we believe may be BiP/GRP78. Various approaches to circumvent the failure of chain association are being attempted. We believe the mechanism underlying the failure of the chains to associate to be a conformational change in the alpha chain to a state in which it cannot subsequently bind the beta chain. Experiments to test this hypothesis are in progress.

Antigen Processing

T 500 MAPPING OF ANTIGEN EPITOPES INTERACTING WITH CLASS II MHC PRODUCTS AND WITH THE ANTIGEN RECEPTOR OF T LYMPHOCYTES, Luciano Adorini, Michael Darsley*, Ettore Appella* and Gino Doria, Laboratory of Pathology, ENEA C.R.E. Casaccia C.P. 2400, 00100 Roma Italy, *Laboratory of Cell Biology, NCI, NIH, Bethesda, MD 20292, U.S.A.

We have analyzed a T cell hybridoma (3A1) obtained from hen egg-white lysozyme (HEL)-primed BDF1 mice, activated to produce IL-2 by HEL and by the HEL synthetic peptide 105-120 associated to I-E^d molecules. The HEL region 105-120 represents the immunodominant HEL epitope recognized in the context of Ia^d molecules. This hybridoma responds to duck lysozyme, carrying a substitution at position 116 as compared to HEL, but it does not respond to ring-necked pheasant lysozyme, different from HEL at residues 113 and 114 and it is stimulated weakly by human lysozyme, different from HEL at five residues in the region 105-120. Synthetic peptides of the HEL sequence 105-120 substituted at residues 113 and 114 do not induce IL-2 production by the 3A1 hybridoma indicating that these two contiguous residues are both critical for 3A1 cell activation. Studies on competitive inhibition between peptide 105-120 and its analogues indicate that residue 113 is recognized by the 3A1 T cell receptor and that residue 114 is involved in binding to I-E^d molecules. Morever, experiments with truncated peptides indicate that residues in the N-terminal and C-terminal regions of the sequence 105-120 are also involved in binding to I-E^d molecules suggesting a complex pattern of interspersed residues in the antigen molecule interacting with the T cell receptor and with class II MHC products.

T 501 ADSORPTIVE PINOCYTOSIS, Gerard Andlauer, BNL, New York, 11973. Antigen molecules bind to receptor proteins located on the surface of T cells: the antigen - receptor complexes will be induced to aggregated by at least divalent antigen. The patches collect over one pole of the T cell to form a cap preliminary to a coated pit that initiates a vesicle for internalizing the antigen - receptor complexes near the Golgi appara tus where the lysis occurs. This script may be suitable to other lymphocyte surface molecules such as the IL 2 receptor complexes.

T 502 ANTIGEN PRESENTATION AND PROCESSING REQUIREMENTS OF POLY 18-SPECIFIC T CELL HYBRIDOMAS, Michel Boyer, Arun Fotedar, Wallace Smart and Bhagirath Singh, University of Alberta, Edmonton, Alberta, CANADA T6G 2H7
The processing requirements of two Poly 18 reactive T cell hybridomas were examined. These hybridomas recognize different parts of the synthetic polypeptide antigen Poly 18, Poly Glu-Tyr-Lys-(Glu-Tyr-Ala)$_5$ in association with IA^d molecules on antigen presenting cells (APC). Hybridoma A.1.1 responds to Glu-Tyr-Lys-(Glu-Tyr-Ala)$_3$-Glu-Tyr-Lys as the minimum antigenic sequence while hybridoma B.1.1 recognizes Glu-Tyr-Ala-(Glu-Tyr-Ala)$_3$-Glu-Tyr-Lys/Ala. It was found that these hybridomas responded to antigen presented by glutaraldehyde and chloroquine treated APC, suggesting that antigen processing is not a requirement for the activation of these cells. Synthetic peptide analogs of the minimum peptide sequences were also tested and found to be stimulatory on similarly treated APC. The reactivity pattern of hybridoma B.1.1 in the presence of glutaraldehyde fixed APC revealed that antigens containing lysine were presented with much less efficiency than antigens without lysine. Since this effect was not seen with chloroquine treated APC, these results suggest that lysine residues on the antigen interact with the APC surface, presumably with the Ia molecule. We also propose that alanine residues in the α-helical Poly 18 line up to form a hydrophobic ridge spiraling up the outside of the molecule. This feature may be required for appropriate interaction between antigen, the T cell receptor and APC.

T 503 Ir GENE CONTROL OF THE T CELL EPITOPE REPERTOIRE AND FINE SPECIFICITY OF THE RESPONSE TO EQUINE MYOGLOBIN. S.J. Brett, K.B. Cease and J.A. Berzofsky NCI, NIH, Bethesda, MD 20892
In order to understand the molecular basis of Ir gene control we have characterized the major epitopes on equine myoglobin (EqMb) recognized by B10.S (H-2^s) and B10.BR (H-2^k) mice, both high responders to EqMb but the former a high responder and the latter a low responder to sperm whale myoglobin (SWMb). The major determinants recognized by B10.S polyclonal T cell lines and the majority of B10.S clones crossreacted with myoglobin variants which had Asp (not Glu) at position 109 whereas B10.BR polyclonal T cell lines and clones appear to be uniquely specific for EqMb and not crossreactive with a panel of 18 myoglobins from other species. We found that $^{17}/18$ EqMb-specific B10.S (I-A^s restricted) T cell clones responded to a synthetic peptide of residues 102-118. This peptide was not immunodominant for B10.BR T cells as it stimulated a proliferative response less than 10% of that to EqMb in polyclonal T cell lines and only $^3/15$(I-A^k restricted) clones. The fact that some I-A^k restricted clones did respond to this peptide suggests however that the low responsiveness to this determinant was not due to an absence of T cells specific for this region in the repertoire. There also appears to be some overlap in the repertoire of fine specificities recognized by B10.S and B10.BR 102-118-specific clones. Thus, although B10.BR and B10.S strains are both high responders to EqMb the haplotype influences the expressed T cell repertoire and the relative immunodominance of various sites among those which can be recognized.

T 504 THE INTERACTION BETWEEN IMMUNOGENIC PETIDES AND Ia. S. Buus, A. Sette and H.M. Grey. Nat'l. Jewish Ctr. for Immunol. & Resp. Med., Denver, CO 80206.

Using gel filtration, the capacity of purified IA^d, IE^d, IA^k and IE^k molecules to bind 11 different immunogenic peptides was examined. In every instance, a specific, saturable binding to the relevant restriction element was found. The K_D of these bindings were typically in the 5-50 µM range. Most of the peptides bound weakly or not at all to non-restriction elements, but in one instance such binding was stronger than the binding to the restriction element. Thus, in order for a protein antigen to be a T cell immunogen, it is an essential, but not sufficient requirement, that a peptide derived from the antigen binds to Ia. Using the panel of peptides to inhibit the binding of radiolabeled peptide to Ia data was obtained suggesting that there is only one peptide binding site on each Ia; and glutaraldehyde crosslinking of radiolabeled peptide to Ia suggested that this binding site is composed of both the α- and the β-chain of Ia. The functional relevancy of the detected bindings of peptide to Ia was demonstrated by the complete correlation between the capacity of the panel of peptides to inhibit the binding of a given peptide to Ia and the capacity of the same panel to inhibit the presentation of that peptide to T cells. Complexes of Ia and immunogenic peptide could be isolated from unbound peptide and shown to be very potent stimulators of T cells even in the absence of exogenous peptide. Thus, T cells recognize a preformed complex of Ia and immunogenic peptide.

T 505 REGULATION OF T CELL ACTIVATION BY IMMUNOMODULATORS OF CLASS II ANTIGENS ON MONOCLONAL PRESENTING CELLS, Michael J. Daley*, Jean-Girard Guillet**, and Malcolm Gefter**. American Cyanamid Co, Princeton, NJ* and MIT, Cambridge, MA**.

Monoclonal populations of B cell and macrophage tumors were used to study the effects of various immunomodulators on the ability of these cells to present antigen to discrete T cell hybridomas. The hybridomas were selected on the basis of different antigenic specificities to a defined region of the lambda repressor protein. Class II antigen induction was followed by quantitative flowcytometry; mRNA induction was studied by Northern blot analysis. These quantitative measurements were followed after various times and doses in vitro with a number of immunomodulators and then correlated to the biological activity of IL-2 production upon appropriate addition of antigen to the treated presenting cells. The effects of cloned gamma interferon (G-IFN), LPS, indomethicin and Cyclosporin-A (Cyclo-A) were all examined. G-IFN demonstrated a dose dependent enhancement or suppression of biological activity, depending upon the endogenous state of Class II expression and the T cell hybridoma used to assay IL-2 secretion. However, at all doses tested, G-IFN enhanced Class II antigen expression. Conversely, Cyclo-A inhibited Class II antigen expression in high doses, but in very low doses enhanced Class II antigen expression. Antigen presentation was significantly inhibited at high doses, but carry over of Cyclo-A into the IL-2 assay can not be ruled out. Low doses of Cyclo-A, although capable of increasing Class II antigen mRNA expression, only showed minimal effects on hybrids for IL-2 production enhancement. The density of Class II expression and presentation is not a direct correlation, and non-specific factors may mediate selective immune responses.

Abstract Withdrawn

T 507 THE T CELL RECOGNITION OF A PHYLOGENETICALLY DISTANT PROTEIN ANTIGEN A. Finnegan J.A. Smith, M.A. Smith, D.H. Sachs and R.J. Hodes. NCI, NIH, Bethesda, MD 20892

To determine whether tolerance to self proteins is responsible for the limited number of determinants recognized by T cells on foreign but closely related proteins T cell clones specific for the bacterial antigen staphyloccocal nuclease (Nase) were derived. T cell clones generated from ($H-2^b$ X $H-2^k$)F1 mice were shown to be specific for Nase in association with either $I-A^b$ or $I-E^k$. The fine specificity of these T cell clones was analyzed using a series of 20 amino acid overlapping peptides spanning the Nase sequence. The $I-A^b$ restricted clones responded to peptide 91-110 and not to other Nase peptides while the $I-E^k$ restricted clones responded to peptide 81-100 and not to 91-110 or to other Nase peptides except for a weak cross reaction to a structurally similar peptide 51-70. The T cell repertoire to a phylogenetically distinct protein is therefore also limited to a small number of antigenic epitopes. The importance of peptide size and amino acid composition were also analyzed. Peptides of 15 amino acids but not 10 amino acids were sufficient to stimulate the clones. Substitutions of one amino acid in peptide 86-100 uncovered a fine specifity difference between $I-E^k$ restricted clones. One clone 24.3 was unable to respond to a peptide substituted at residue 91 while a substitution at residue 95 had no effect. Conversely, clone G53 responded very weakly to a substitution at residue 95 but was unaffected by a substitution at residue 91. These results suggest that residues 91 and 95 may be involved in peptide recognition by the T cell receptor. In addition, although T cell recognition is limited to a particular region of the antigenic molecule there appear to be differences in fine specificity of the determinants recognized within that region.

T 508 A SECOND SITE ON THE CYTOCHROME C T CELL DETERMINANT THAT INTERACTS WITH THE Ia MOLECULE. B.S.Fox, F.Carbone, Y.Paterson, F.Ronchese, R.N.Germain and R.H.Schwartz, Laboratory of Immunology, NIAID, NIH, Bethesda, MD 20892 and Scripps Clinic and Research Foundation, La Jolla, CA 92037.

The dominant T cell determinant in cytochrome c has previously been shown to contain two critical sites: residue 99 interacts with the T cell receptor and residue 103 interacts with the Ia molecule. Synthetic peptide antigen analogs were made containing single amino acid substitutions at position 95. These substitutions caused a shift in antigenic potency, defining residue 95 as critical to the T cell response. Upon immunization with these analogs, a heteroclitic response was observed to the parent antigen. This suggested that residue 95 did not contact the T cell receptor. In addition, a change in relative potency was observed for antigen analogs when antigen - presenting cells expressing two different Ia molecules were compared, $E\beta^k$:$E\alpha^k$ and $E\beta^b$:$E\alpha^k$. This suggested that residue 95 directly interacted with a polymorphic site on the Ia molecule. A different pattern of antigenic potencies was observed, however, on L cells transfected with $E\beta^k$:$E\alpha^k$ and $E\beta^b$:$E\alpha^k$. Furthermore, fixation with paraformaldehyde to block antigenic processing again changed the relative potencies of the antigen analogs. L cells, splenocytes expressing $E\beta^k$:$E\alpha^k$ and splenocytes expressing $E\beta^b$:$E\alpha^k$ were all affected differently by fixation. Substitutions at position 95 can therefore detect an antigen processing difference between splenocytes and L cells.

T 509 TRANSITORY STAGES IN THE PRESENTATION OF INFLUENZA VIRUS ANTIGENS TO HELPER T CELLS, Charles J. Hackett and Laurence C. Eisenlohr, The Wistar Institute, Philadelphia, PA, 19104.

The route by which antigens on viral particles are presented to helper T cells (T_H) is being studied using influenza virus. As a T cell antigen, influenza virus is efficiently presented *in vitro* to T_H specific for either internal or external virion proteins: Only picomolar concentrations of the relevant determinant introduced on intact virus is required for T_H stimulation; this effect is independent of virus replication. Kinetic studies show that only 2-10 min of virus exposure to the antigen-presenting cell (APC) suffices for maximum T_H stimulation. However, a lag period is required after the pulse for the expression of T_H determinants on the APC surface. Three stages have been identified which lead to this high-efficiency presentation: 1. Rapid binding of virus via its hemagglutinin molecule to cell-surface sialic acid focuses the virion onto the APC. 2. The bound virus enters a cellular compartment from which it is no longer removable by neuraminidase. Transition to this compartment may occur at 10°C. 3. A further period at temperature >20°C is needed to complete expression of T_H determinants. Temperature-shift studies indicate that if virus is allowed to accumulate at 10°C after APC binding, the required exposure to the elevated temperature is abbreviated, suggesting that entry into the neuraminidase-resistant compartment is a required stage in antigen presentation.

The T Cell Receptor

T 510 A NEW PATHWAY TO ANTIGEN PRESENTATION. Ellen Heber-Katz, Eiji Watari and B Dietzschold. The Wistar Instititute. Philadelphia, PA 19104.

There has been much data supporting the idea that T cell antigenic determinants have two distinct functional sites, that which interacts with the T cell receptor or the epitope and that which interacts with the Ia molecule or the agretope. Our recent experiments with a peptide determinant derived from the glycoprotein D of herpes simplex virus (HSV) have allowed us to define a third functional site on a T cell antigen. By addition of a fatty acid to this peptide, we have maintained the original two sites but have now created a structural characteristic that can induce an Lyt 2 + T cell population. These cells appear to be peptide specific and upon adoptive transfer protect an animal from a lethal HSV infection. Exactly how this molecular structure functions in T cell activation is under investigation and its implications in antigen presentation will be discussed.

T 511 FUNCTION OF ANTIGEN-SPECIFIC IMMUNOGLOBULIN RECEPTORS IN T CELL-B CELL INTERACTIONS, Nobumichi Hozumi, Roland Tisch, Masahide Yoshikawa and Motoo Watanabe*, Mount Sinai Hospital Research Institute, 600 University Avenue, Toronto, Ontario M5G 1X5 (*On leave from Central Research Laboratory, Mitsubishi Petrochemical Co. Ltd., Ibaraki, Japan)

The cellular properties of antigen presentation have been well-studied. In contrast, the biochemical and molecular aspects of antigen presentation are still not well understood. In order to investigate anitgen presentation at a biochemical and molecular level, we have established monoclonal B cell lines expressing $sIgM_{Tnp}$ specific for the hapten Tnp through recombinant DNA and gene transfer techniques. The B cell transformants were shown to present antigen (Tnp-proteins) to Th cells very efficiently (Watanabe et al., Proc. Natl. Acad. Sci. USA 83:5247, 1986). Currently we are studying mechanisms involved in specific antigen presentation and processing. This work includes the behavior of sIg receptors during T-B interaction, experiments using chemical reagents which block antigen processing, assessing the association of antigen with Ia molecules and experiments using antigens with different epitope densities so as to determine the role of Ig crosslinking in this process. Furthermore, we have constructed transformants expressing sIgD with Tnp specificity ($sIgD_{Tnp}$). This system now allows us to elucidate differences between sIgM and sIgD in their roles involving antigen presentation, B cell activation and differentiation. Work focusing on antigen presentation will be presented (supported by the MRC and the NCIC).

T 512 INFLUENCE OF T CELL SPECIFICITY ON THE ANTIBODY RESPONSE TO THE ACETYLCHOLINE RECEPTOR. Keith A. Krolick, University of Texas Health Science Center, San Antonio, TX 78284.

Lymph node T cells were obtained from rats immunized with purified acetylcholine receptor (AChR) or isolated receptor subunits (α,β,γ, or δ). The immune T cells were then analyzed for their ability to respond to native AChR or AChR subunit challenge in an in vitro proliferation assay, as well as to perform as helper T cells (T_H) in an in vitro anti-AChR antibody response. Results indicated a significant degree of subunit-to-subunit crossreactivity at the T cell level. Moreover, helper function could be generated by stimulation of T cells immune to any of the AChR subunits, although α-immune T_H cells appeared to provide a quantifiably increased level of specific antibody production by AChR-immune B cells in vitro when compared to antibody produced by the same B cells in conjunction when β, γ, or δ-immune T_H cells.

T 513 ANTIGEN-INDUCED INTERNALIZATION OF ANTIGEN RECEPTORS IN B AND T CELLS.
L.D. LESERMAN, Centre d'Immunologie INSERM-CNRS de Marseille-Luminy, Case 906, 13288 Marseille cêdex 09, France.

I propose that the antigen-induced internalization of antigen receptors of B or T lymphocytes serves to assure the interaction of those receptors, in intracellular compartments, with the major histocompatibility complex (MHC)-encoded molecules of the cells, that are themselves internalized constitutively. Antibody and T cell receptors have conserved regions which promote the association of their unique amino acid sequences ("idiotypes") with MHC molecules as a consequence of exposure to the acidic environment of endocytic vesicles. If the antigen is internalized with the antigen receptor it may associate with MHC molecules that it encounters after internalization, if it has sequences permitting this association. Thus, B or T cells may express either "idiotype"-MHC or antigen-MHC complexes, or both, after the re-expression at the cell surface of the complexes which form intracellularly, permitting the recognition of these cells by T cells specific for these structures. This mechanism permits the organism to respond to antigens for which it has affinity, in a manner which does not depend on the structure of the antigen, by the use of its own receptors. Antigen receptors co-evolve with the MHC molecules of the species in a way that preserves the possibility of their interaction.

T 514 CELL SURFACE EXPRESSION AND FUNCTION OF CLASS II MHC MOLECULES IN THE PRESENCE AND ABSENCE OF INVARIANT CHAIN, Jim Miller, Andrea Sant, and Ronald N. Germain, LI, NIAID, NIH, Bethesda, MD 20892

During biosynthesis, the highly polymorphic Class II MHC (Ia) molecules are complexed with a nonpolymorphic polypeptide chain, the invariant chain (Ii). The Ia-Ii complex is formed in the rough endoplasmic reticulum and remains throughout cytoplasmic transport to the cell surface where dissociation occurs. This specific interaction between Ia and Ii and their coordinant expression in all Ia-positive cells has led to the hypothesis that Ii is required to direct Ia to the cell surface, perhaps through a special compartment in which Ia-antigen association can occur. To address this possibility, cDNA clones encoding these proteins were tranfected into BALB/c 3T3 cells, which express neither of these molecules. We have found that the Ia-Ii complex is not required for the transport of either Ia or Ii through the Golgi apparatus, as evidenced by the addition of complex carbohydrate side chains, including chondroitin sulfate to Ii. Nor is Ii required for efficient cell surface expression of Ia. In addition, preliminary data indicate that Ii is not required for the functional recognition of Ia and antigen by T lymphocytes. However, efficient antigen processing was not evident in the 3T3 transfectants whether or not Ii was present. Thus, it remains possible that Ii plays a role in antigen processing and/or antigen association with Ia, but that this role was not evident in the 3T3 cells because they lack (an) additional component(s) necessary for complete processing function. Additional transfection experiments are in progress to better define the possible role(s) of Ii in Ia function.

T 515 EVIDENCE FOR A SECOND Ia-INTERACTION SITE WITHIN THE PIGEON CYTOCHROME c T_h CELL DETERMINANT. Yvonne Paterson, Francis Carbone and Uwe Staerz, Department of Immunology, Research Institute of Scripps Clinic, La Jolla, CA 92037.

The murine T cell proliferative response to pigeon cytochrome c is I-E^k restricted and directed to a single site in the cytochrome c molecule located at the carboxy terminus within the sequence 95-104. In the T cell specificities so far described the interaction of the antigen with the I-E^k molecule is clearly dependent on the presence of a lysine residue at position 104. This residue is also effective at position 103. Thus deleting the alanine that is in that position in pigeon cytochrome c results in either an equal or better T cell response to the antigen, depending on the I-E^k molecule expressed by the antigen presenting cell. (R.Schwartz, Ann. Rev. Immunol.,3,237-262). We have recently cloned a T cell line 22D11 from B10.BR mice with a specificity also restricted to the complete sequence 95-104 but which does not recognise antigen that contains the alanine deletion at position 103. This is the case whether the antigen is presented by syngeneic antigen presenting cells or presenting cells from B10.A, B10.A5R, or B10.S9R strains of mice. We postulate that the Ia interaction site for this rare T cell specificity to pigeon cytochrome c must occur in some other region of the molecule within the sequence 95-104.

The T Cell Receptor

T 516 Ia-ANTIGEN COMPLEXES CAN BE DETECTED IN THE SUBCELLULAR COMPARTMENT, M. Laurie Phillips, Cecil C. Yip and T. L. Delovitch, University of Toronto, Toronto, ONT M5G 1L6. Using the technique of photoaffinity labeling, we have previously demonstrated that beef insulin binds to Ia molecules on antigen presenting B hybridoma cells (APCs). In the present study, we show that complexes between Ia and antigen can also be found in the subcellular compartment. Ia-antigen complexes are detectable in the plasma membrane fraction, a Golgi and smooth vesicle (SV) enriched fraction, and in an endoplasmic reticulum enriched fraction shortly after exposure of the cells to antigen. Treatment of the cells with monensin following crosslinking, but prior to fractionation, results in a reduction of label in the Golgi-SV fraction relative to the plasma membrane fraction. While this suggests that some of the complexes found in that fraction have been internalized from the cell surface, the initial Ia-antigen complex may originate subcellularly. It is possible that Ia molecules are involved not only in the routing of antigen from the cell surface to the cytoplasm but also from the cytoplasm to the cell surface.

T 517 T HYBRIDOMA CELL RECOGNITION OF APAMIN, A 18 AMINOACID NEUROTOXIC PEPTIDE. Michel Pierres, Anne Regnier-Vigourous, Mohammed El Ayeb and Claude Granier, Centre d'Immunologie INSERM-CNRS de Marseille-Luminy, Case 906, 13288 Marseille cédex 9, France.
Apamin is a neurotoxin of 18 aa isolated from bee venom. Because of its structural features (2 disulfide bonds) and the availability of synthetic analogs, we have used it as a model system to study both the recognition and the processing of a peptide antigen. T cell proliferative responses elicited by 7 nmoles of apamin were found to be under _H2_-linked Ir gene control (responder haplotypes : $H\text{-}2^d$ and $H\text{-}2^b$), and involved A^d and A^b as restriction elements. Analysis at the clonal level was carried out using a series of IL-2-producing T hybridoma cells derived from BALB/c or C57BL/6 apamin-primed lymph node T cells. These cells recognized apamin presented by A^d- or A^b-positive B lymphoma or transfected L cells. The use of synthetic analogs of apamin revealed that 2 sites were critical for T cell recognition, involving the first 6 NH_2-terminal residue stretch, and the hydrophobic leucine 10. All the T hybridoma cells examined required antigen processing, as shown by the inability of paraformaldehyde-fixed APC to present native apamin. The IL-2 responses of the majority of the hybridomas tested could, however, be restored when reduced apamin was used with fixed APC, thus indicating that at least one step of the processing of this peptide involved a disruption of its 2 disulfide bonds. Experiments are underway to investigate the interaction of class II molecules with reduced and labeled apamin, as well as the biochemical basis of its processing by APC.

T 518 PRESENTATION OF MEASLES VIRUS TO DR1-RESTRICTED CTL CAN OCCUR IN ABSENCE OF THE INVARIANT CHAIN. Rafick P. Sekaly, Steve Jacobson and Eric O. Long, Laboratory of Immunogenetics, NIAID and Neuroimmunology Branch, NINCDS, NIH, Bethesda, MD 20892.
Measles virus specific CTLs are primarily restricted by class II MHC antigens and belong to the T4 positive subset of T lymphocytes. It is not known which viral antigens are presented to the CTLs and whether there is preferential restriction by either DR, DQ or DP antigens. Since measles virus will not infect murine lines, a human fibroblast line was transfected with expressible cDNAs for α and β chains of HLA-DR1 and was infected _in vitro_ with measles virus. These cells were efficiently lysed by measles virus specific CTL clones. Lysis was dependent upon concomitant expression of DR1 antigens and infection with measles virus and it was inhibited specifically by mAbs to DR in a dose dependent manner. No RNA transcript of the invariant chain gene could detected in the DR1 transfectants before or after measles virus infection. These data show that presentation of viral antigens can occur in the absence of invariant chain. To determine which viral antigens are presented to the CTLs individual viral genes in eukaryotic expression vectors were introduced by transfection into murine cells previously transfected with DRα and DRβ cDNAs and expressing high levels of DR1 on their surface. Colonies of transfectants which expressed both DR1 and the transfected viral gene were identified by RNA dot blot analysis. T cell populations primed _in vitro_ with measles virus were able to lyse specifically the DR1-positive cells transfected with the matrix or the nucleocapsid viral genes. Studies are in progress to define the precursor frequency of T cell subsets responding to the various viral antigens and their requirement for accessory molecules.

T 519 STRUCTURAL REQUIREMENTS FOR THE INTERACTION BETWEEN $I-A^d$ AND IMMUNOGENIC OVALBUMIN-DERIVED PEPTIDE. A. Sette, S. Buus and H.M. Grey. Natl. Jewish Ctr. for Immunol. & Resp. Med., Denver, CO, 80206.

Using a recently described gel filtration method, we have studied the interaction between a radiolabeled immunogenic peptide representing amino acids 323-339 of ovalbumin (Ova 323-339) and its restriction element ($I-A^d$). This interaction is specifically inhibitable by unlabeled Ova 323-339 and by other $I-A^d$-restricted peptides derived from unrelated proteins. Analysis of these $I-A^d$ binding peptides reveal certain structural similarities which allowed us to tentatively identify the amino acid residues involved in the interaction with $I-A^d$. To define more precisely the region within Ova 323-339 responsible for Ia interaction on the one hand, and T cell receptor interaction on the other, we have synthesized a series of truncated peptides which were tested for their capacity to bind to Ia and their capacity to stimulate 3 Ova 323-339 specific T cell hybrids. The binding to Ia was largely accounted for by Ova 325-335. However, this peptide failed to stimulate 2 of the 3 T cell hybrids tested suggesting that some of the residues required for T cell receptor interaction are found outside the region involved in Ia interaction. The fine specificity of the interaction between $I-A^d$, Ova 323-339, and T cell receptor will be further addressed by the analyses of a series of peptide analogs.

T 520 NO MHC CLASS DISTINCTION BY AN MHC RESTRICTED T CELL CLONE, Elizabeth Simpson, Kyuhei Tomonari and Andrew Mellor, Clinical Research Centre, Watford Road, Harrow, Middlesex, HA1 3UJ.

A T cell clone specific for $H-2K^k$ + non H-2(X) has been established from a C3H mouse immunised with CBA T cells. This clone cross reacts with E^k + non H-2(Y) and E^d + non H-2(Z). If it expresses only one T cell receptor this does not distinguish class I from class II MHC molecules and recognises three separate but cross reacting epitopes created by the association of different non H-2 gene products with different MHC molecules. Rearrangements and expression of TCR α β and γ chains of this clone are being examined.

T 521 HUMAN T CELL CLONES TO (NANP)3 P.FALCIPARUM CIRCUMSPOROZOITE PEPTIDE: MONOCLONAL ANTIBODIES TO (NANP)3 DO NOT BLOCK THE T CELL RESPONSE, Francesco Sinigaglia, Hugues Matile, Dieter Gillessen, Arnold Trzeciak, Edgar P. Heimer*, Nathalie Boulanger, John R.L. Pink, F. Hoffmann-La Roche & Co. Ltd., Central Research Units, 4002 Basle, Switzerland, *Hoffmann-La Roche Inc., Peptide Research Dept., Roche Research Center, Nutley, N.J. 07110, USA

The circumsporozoite protein of P.falciparum contains multiple repeats of the sequence Asn-Ala-Asn-Pro (NANP). A T cell line specific for (NANP)3 was obtained from a volunteer immunized with (NANP)3 coupled to tetanus toxoid and absorbed to alum. The cell line was maintained by repetitive antigen stimulation and cloned by limiting dilution.
Using monoclonal antibodies to the non-polymorphic regions of human HLA class II antigens, the proliferative response of a (NANP)3 specific T cell clone (FN-220) was shown to be restricted to HLA-DP region encoded molecules. In addition 5 different monoclonal antibodies specific for (NANP)3 failed to inhibit proliferation of clone FN-220. Also, glutaraldehyde fixation and chloroquine treatment of the autologous Epstein-Barr virus-transformed B cell line used as antigen-presenting cell did not affect antigen presentation to the T cell clone. These results suggest that (NANP)3 must undergo a conformational change before it is recognized by clone FN-220. Furthermore the presence of anti-(NANP)3 circulating antibodies should not prevent (NANP)3-specific T cell induction in vivo.

The T Cell Receptor

T 522 T CELL HYBRIDOMAS REACTIVE WITH THE ACETYLCHOLINE RECEPTOR AND ITS SUBUNITS, Joseph Tami, Olgui Urso, Keith Krolick, University of Texas Health Science Center at San Antonio, TX. 78284-7758.
A panel of thirty cloned rat-mouse T cell hybridomas was prepared by fusion of acetylcholine receptor (AChr)-reactive rat T cells with the mouse thymoma, BW5147. The T cell hybrids were demonstrated to be AChR-reactive by their ability to secrete IL-2 in response to either AChR itself or by purified AChR subunits. Various patterns of AChR subunit reactivity were observed, suggesting a predominant recognition of the alpha subunit, and also considerable cross-reactivity from one subunit to another. Herein lies an interesting correlation, in that the predominant antibody response to the acetylcholine receptor is to an immunodominant region of the alpha subunit in the disease myasthenia gravis.

Lymphokines and Lymphokine Receptors

T 523 IL-4 HAS THYMOCYTE GROWTH FACTOR ACTIVITY, Simon Carding and Kim Bottomly, Yale University School of Medicine and Howard Hughes Medical Institute, New Haven, Ct. 06510.
The T-cell derived B-cell stimulatory factor I (BSF-1/IL-4) lymphokine has been described as a factor that acts as a B-cell co-mitogen with anti-immunoglobulin antibody or SAC, and induces expression of class II (Ia) major histocompatability antigens on B-cells. In our studies, we have utilized the IL-4 producing murine helper T-cell clone D10 as a source from which to isolate IL-4. We have shown that the proliferative activity of thymocytes cultured with recombinant IL-2 and sub-mitogenic concentrations of PHA is increased 3-10 fold in the presence of IL-4 as determined by [^{3}H]-thymidine incorporation after culture for 72h. In contrast, IL-4 alone is unable to induce proliferative activity of thymocyte cultures. This synergistic activity of IL-4 is inhibited by inclusion in thymocytes cultures of the anti-IL-4 monoclonal antibody 11B11. Furthermore, potentiation of the IL-2-induced thymocyte proliferation is not seen with γ-interferon, IL-1 or IL-3. In addition, this activity of IL-4 appears to be specific for thymocytes as highly purified preparations of T-cells did not respond to IL-4 in the presence or absence of IL-2. These findings suggest that IL-4 may play an important role during thymocyte maturation in the thymus, perhaps to enhance the proliferation and expansion of thymocyte populations responsive to IL-2.

T 524 LACK OF IL-2 DEPENDENT PROLIFERATION DESPITE SIGNIFICANT EXPRESSION OF HIGH AFFINITY IL-2 RECEPTOR ON MURINE T LYMPHOCYTE CLONES LATE AFTER ANTIGENIC STIMULATION, Albert M. Churilla and Vivian Lam Braciale, Washington University School of Medicine, St. Louis, MO 63110
We examined the expression of IL-2 receptors on class I and class II MHC restricted, influenza specific, murine T lymphocyte clones at early (day 3) and late (day 8-12) times after antigenic stimulation. IL-2 receptor expression on the three clones examined increases to peak levels early and subsequently decays ten to fifty fold during this time period, as evidenced by monoclonal anti-IL-2 receptor antibody binding. However, in IL-2 binding site studies these clones retain high levels of high affinity IL-2 receptors (46% to 97% of day 3 levels) at the later time points, despite their inability to proliferate in response to IL-2 in the form of supernatant from Con A stimulated rat splenocytes. In order to elucidate where the potential block may be which prevents these CTL clones from proliferating in an IL-2 dependent manner, the ability of the cells to internalize bound IL-2 at late times after activation is examined. All three clones late after activation are able to internalize bound IL-2 with efficiencies equivalent to that seen at day 3. Additionally, two of the three clones late after activation are able to upregulate expression of IL-2 receptors in response to picamolar concentrations of IL-2, therefore, for the CTL clones examined, at late times after antigenic stimulation, engagement of IL-2 by the high affinity receptor seems not to be a sufficient signal to induce cells to transit through the cell cycle. (supported by grants AI-15608, AI-15353, HL-33391, and AI-07163 (A.M.C.))

T 525 I-A-POSITIVE ANTIGEN PRESENTING CELLS, IL-1, IL-2, AND GAMMA-INTERFERON ARE REQUIRED FOR FETAL THYMOCYTE DEVELOPMENT IN VITRO, D. DeLuca, Department of Biochemistry, Medical University of South Carolina, Charleston, SC 29425.
We studied the development of T-cells from 14d gestation murine fetal thymus rudiments in organ cultures. We found that anti-IA monoclonal antibodies inhibited development of Lyt2+/L3T4- and Lyt2-/L3T4+ T-cells in the cultures. Ia positive non-lymphoid cells were lost after anti-IA treatment. Recombinant IL-1 reversed this inhibition and restored the Ia-positive cells. Anti-IL-1, anti-IL-2, anti-IL-2 receptor, and anti-gamma interferon also inhibitied the development of T-cells. IL-1, gamma interferon and antigen presenting activity were detected in the cultures incubated at 37°C, but not in lymphocyte depleted cultures incubated at 20°C. Antigen presenting activity in the cultures was sensitive to anti-dendritic cell monoclonal antibody (33D1) plus complement. These data are consistent with the idea that the development of thymocytes requires recognition of class II molecules on antigen presenting cells to induce the production of IL-1. IL-1 may then activate thymocytes to produce IL-2 receptor, IL-2, and gamma-interferon. These substances may sustain the proliferation of thymocytes, and cause the further expression of class II molecules on thymic epithelial cells which is important in further thymocyte differentiation.

T 526 INDUCTION OF CLASS I & CLASS II MHC GENE EXPRESSION BY IFN-γ AND TUMOR NECROSIS FACTOR, Yvonne R. Freund & Patricia P. Jones, Stanford University, Stanford, CA. 94305.
Recognition of antigen by the T cell receptor requires the presence of class I or class II MHC molecules. The expression of these molecules is regulated by two lymphokines: IFN-γ and tumor necrosis factor (TNF_α & TNF_β). We are analyzing the mechanisms by which IFN-γ stimulates increases in basal expression of H-2 antigens, and synergizes with TNF to induce Ia expression on the murine macrophage cell line, WEHI-3. Increases in surface expression are the result of elevations in levels of mRNA of all of the component chains of Ia and H-2 antigens. Nuclear run-on experiments indicate that IFN-γ induces transcription of Ia and H-2 genes but exerts its primary effect on H-2, β_2-microglobulin and invariant chain post-transcriptionally. TNF_α and TNF_β, products of activated macrophages and T cells, respectively, synergize with IFN-γ to increase levels of surface Ia antigens. Neither TNF exerts any effect on H-2 expression, nor do they induce Ia expression in the absence of IFN-γ. TNF_α and TNF_β shorten the lag time before Ia induction by IFN-γ and increase the maximal levels of induction. The effect of TNF on WEHI-3 cells is stable, since synergy is observed when cells are briefly exposed to TNF 24 hours before IFN-γ is added. Experiments are underway to determine, at the molecular level, the site of action of TNF and the sequence of events involved in the synergy.

T 527 IMMUNE RESPONSIVENESS OF CYTOTOXIC T LYMPHOCYTE PRECURSOR IS DEPENDENT ON A NEW LYMPHOKINE MODULATING FUNCTIONAL INTERLEUKIN-2 RECEPTOR EXPRESSION: Conny Hardt, Jörg T. Epplen (*) and Hermann Wagner, Institute of Medical Microbiology and Immunology, Ulm, FRG and (*) Max-Planck-Institute for Immunobiology, Freiburg, FRG.
We provide evidence that three signals are involved in cytotoxic T cell activation, each signal representing one restriction point in cytotoxic T cell function: The first signal is supplied by interaction of a specific T cell receptor with the responding antigen and can be delivered by mitogens, allogeneic MHC determinants, viral proteins in conjunction with self MHC structures or by crosslinking T cell receptor molecules by specific antibodies. The second signal necessary for T cell activation is the expression of functional Interleukin-2 (Il-2) receptors. Functional Il-2 receptor expression can be induced by antigen presenting cells via secretion of soluble products operationally termed Il-2 receptor inducing factor (RIF). This signal however is not provided by "nonimmunogenic" stimulator cells such as resting B lymphocytes or certain tumor cells; addition of RIF conveys immunogeneity. The third signal is clonal expansion. Cell proliferation is induced and controlled by the growth promoting effect of Il-2 in T cells expressing high affinity Il-2 receptors. Here we define the nature, functional activity and biochemical characteristics of the lymphokine RIF which controls the induction of the second signal. The influence of receptor cross linking, RIF and Il-2 on the induction of Il-2 receptor message is described. In the presence of all three signals mRNA expression occurs and in magnitude it parallels the peak of cell proliferation.

T 528 ROLE OF GROWTH FACTORS IN LEPROSY PATIENTS, T. Jayaraman, R.S.Mishra*, A.K.Sharma* and I.Nath, Department of Pathology and Dermatology*, All India Institute of Medical Sciences and Safdarjang Hospital, New Delhi-110 029, India.
Since leprosy is associated primarily with a T cell defect and T cell growth factors have been shown to play an important role in the expansion and development of the immune response, we have analysed the ability of macrophages and lymphocytes of 69 leprosy patients to generate and/or respond to Interleukins 1 and 2. Whereas nomral levels of IL-1 was produced in leprosy subjects on stimulation with the mitogen PMA, 6/14 lepromatous individuals failed to generate IL-1 to *M. leprae* antigens. Similarly IL-2 production to antigen specific stimulation was reduced at the lepromatous ple of the spectrum. With a view to reconstituting this defect exogenous IL-2, from stimulated JR4 cells, constitutively produced from MLA gibbon cell line and recombinant sources was added to PBMC of these individuals. Heterogeneity of *in vitro* responses was observed. Whereas 1/3 of individuals failed to respond to IL-2, the others showed moderate to high proliferation in the presence of *M. leprae* and IL-2.

T 529 DIFFERENTIAL ACTIVATION REQUIREMENTS OF HUMAN B CELL SUBSETS. Diane F. Jelinek and Peter E. Lipsky. UTHSCD, SW Medical School, Dallas, TX. 75235.
To examine the role of interleukin 2 (IL2) in human B cell responsiveness, the capacity of IL2 to promote proliferation and differentiation of B cell subsets was examined. Unfractionated highly purified peripheral blood B cells stimulated with Staphylococcus aureus (SA) and mitogen activated T cell factors (TF) or recombinant IL2 (rIL2) proliferated extensively and generated large numbers of Ig secreting cells (ISC). Although no absolute requirement for interleukin 1 (IL1) was observed, concentrations of IL1 as low as 1 pM markedly augmented both growth and differentiation of B cells stimulated with SA+rIL2. Analysis of spleen and lymph node (LN) B cells indicated that both populations responded to SA+TF. However, only spleen B cells responded effectively to SA+rIL2. To examine the responsiveness of B cell subsets in greater detail, blood δ+ and δ- B cells were separated with the fluorescence activated cell sorter. Both subsets responded optimally to SA+TF, whereas only the δ- B cells responded effectively to SA+rIL2. Diminished responsiveness of the δ+ B cells to IL2 could not be corrected by the addition of IL1 nor attributed to a failure of the induction of IL2 receptor expression as assayed by staining with monoclonal antibodies. Responses of SA+rIL2 stimulated δ+ B cells could be augmented by addition of B cell growth factor but still remained suboptimal. Moreover, addition of small numbers of δ- B cells augmented the ability of the δ+ cells to respond to SA+rIL2. These results indicate that responsiveness of human B cell subsets to IL2 varies. SA-activated B cells bearing a more mature phenotype (δ-) respond to IL2 in the absence of other lymphokines. Production of an additional factor by the δ- B cells may facilitate IL2 responsiveness.

T 530 AUTOADOPTIVE IMMUNOTHERAPY FOR BRAIN TUMORS USING AUTOLOGOUS INTERLEUKIN-2 STIMULATED LYMPHOCYTES, Carol A. Kruse, Sharon Waldrop, Phillip Jewett and Paul C. Bunn, Divisions of Surgical Oncology and Medical Oncology, University of Colorado Health Sciences Center, Denver CO 80262.
Clinical trials with recurrent primary brain tumor patients have been instituted where two separately-activated populations of T lymphocytes are generated and implanted into the tumor bed of the patients at craniotomy. Autologous stimulated lymphocytes (ASLs) are ten-day cultured nonadherent T lymphocytes that are responsive to Phytohemagglutinin and to human recombinant Interleukin-2. The lymphokine activated killer (LAK) cells are produced by exposure of T lymphocytes, isolated from a Ficoll-Paque gradient, to higher concentrations of Interleukin-2 only, for three days. The ASL and LAK populations are phenotypically distinct when compared by fluoresceinated monoclonal antibodies against T lymphocyte surface markers. Both activated populations are also distinct when compared to the starting lymphocytes obtained from the peripheral blood (PBL) of the brain tumor patients. The helper/inducer to suppressor/cytotoxic ratio (T4/T8) of the LAK was generally 2:1 and very much like that of the starting PBL. A complete reversal of this ratio was obtained for the ASL. Very low percentages of NKH-1 were present in all, however, the T11 marker increased from the PBL to the LAK to the ASL and reached almost 99% in the ASL. The T11 is thought to represent a second NK cell population distinct from OKM1. The activated T cell marker, Ta1, also increased from PBL to LAK to ASL preparations. This research was supported by the American Cancer Society grants CH339 and IN5Z.

T 531 Rapid down-regulation of Il-2 expression by mitogen activated T cells after removal of the stimulant.
A. Schimpl, R. Swoboda, U. Bommhardt, E. Serfling and E. Wecker, Institut für Virologie und Immunbiologie, Versbacher Str. 7, D-8700 Würzburg, F.R.G.

Mitogen stimulated L3T4$^+$ Lyt2$^-$ and L3T4$^-$ Lyt2$^+$ T Cells transiently express Il-2. Maximal mRNA levels are reached at 4 - 6 hrs (phorbolesters plus Ca^{++} ionophores) or 8 - 12 hrs (e. g. ConA). The half life of Il-2 mRNA is approximately 30 min, it can be extended manyfold after addition of cycloheximide. Kinetic and mRNA stabilization experiments indicate that the Il-2 mRNA signal decreases late in G_1 and that the virtual absence of an Il-2 mRNA signal at later times is not due to increased RNA degradation, but rather to cessation of transcription. When -methylmannose is addid to ConA activated cells at the peak of Il-2 expression, Il-2 mRNA disappears within 1 - 2 hrs. Since the Il-2 mRNA half life remains unaltered, the data suggest that withdrawal of the mitogenic signal leads to a rapid stop of transcription of this gene.

T 532 THE ROLE OF SPECIFIC ANTIGEN RECOGNITION AND LYMPHOKINES IN B CELL PROLIFERATION AND DIFFERENTIATION,
Susan L. Swain, University of California, San Diego, La Jolla, CA, 92093.
Using the in vivo CH12 B cell lymphoma line specific for sheep red blood cells (SRBC), we have investigated the role of Antigen (Ag) and T cell derived lymphokines IL2, BSF_1 (IL4) and $BCGF_{II}$ (IL5) in proliferation and differentiation of this B cell line. We find that Ag (SRBC) and $BCGF_{II}$, but not the other lymphokines, synergize markedly to promote the proliferation and differentiation of the CH12 B cells. The synergistic effects are especially marked when low numbers of CH12 cells are cultured. At cell numbers in the range of 600-3000 per well, significant proliferation occurs, and recovered PFCs are in the range of 10-50% of initial cell numbers. A role for contaminating non-B cells or for autocrine production is very unlikely in cultures of so few cells. Since CH12 B cells are non-resting B cells which differentiate to give maximum numbers of Ig secreting cells on day 3, these results suggest that Ag and $BCGF_{II}$ are two essential signals for the proliferation and maturation of B cells in the latter part of the immune response. Although it is widely assumed that Ag plays a selective role in the B cell response, it has been hard to rule out the possibility that such a role is due only to secondary effects of Ag-presentation to helper T cells. These studies support the hypothesis that interaction of specific Ag with Ig-receptor provides a direct signal which is required repeatedly at several phases of the B cell response.

T 533 TRIGGERING OF CYTOLYTIC ACTIVITY AND CYTOTOXIC LYMPHOKINE RELEASE BY PERTURBATION OF Ti-CD3 COMPLEX ON HUMAN T CELL HYBRIDOMAS.
C.F. Ware, L.M. Green, F. Coffman, D.L. Haviland, M.H. Grayson, J.L. Reade and A.E. Burger. Division of Biomedical Sciences, University of California, Riverside, CA and the Upjohn Co., Kalamazoo, MI.

CEM.TETI and lectin-activated T lymphoblasts were used to construct functionally active human T cell hybridomas. One hybrid, the II23.D7 line, was characterized for its ability to mediate direct cytotoxicity and secrete lymphokines. The II23.D7 expressed CD4, CD3-Ti complex and LFA-1 and was CD8$^-$. Cytotoxicity, as measured in a ^{51}Chromium release assay, was inducible with anti-CD3 monoclonal antibodies or with phorbol ester. Perturbation of the Ti-CD3 complex also initiated IL-2 and cytotoxic lymphokine synthesis and secretion. Specific antibodies indicated the toxin produced by II23.D7 was antigenically similar to lymphotoxin (LT). The kinetics of direct cytotoxicity mediated by II23.D7 required 10-12 hrs to lyse 50% of the target cells and correlated with the induction and secretion of LT by the hybridoma. Target cells susceptible to killing by the II23-D7 exhibited a parallel sensitivity to the cytotoxic activity of soluble LT. Target cells lysed by the II23.D7 hybrid or LT underwent DNA fragmentation characteristic of CTL-mediated killing. In comparison, IL-2 dependent allospecific CTL clones which exhibited rapid, specific lysis of lymphoblastoid targets ($\leq$4 hrs) required 10-12 hr to induce 50% target cell lysis of the LT sensitive target cells. The II23.D7 line had no detectable granules or esterase activity. These results suggest that the II23.D7 T cell hybridoma contains a distinct cytotoxic mechanism functionally separate from the granule-mediated mechanism reported for murine CTL. Supported by CA35638, NCI.

T 534 GM-CSF AS A T CELL GROWTH FACTOR. A. Woods, J. West, R. Rasmussen and K. Bottomly. Yale University School of Medicine and Howard Hughes Medical Institute, New Haven, CT. 06510

It is widely accepted that T cells carrying L3T4 molecules on their surface are helper T cells (Th). However, we have shown that Th cells are functionally heterogeneous with some cloned T cells failing to activate antigen-specific B cells to secrete. We have shown that functionally distinct T cells differ in the lymphokines they produce upon stimulation with antigen/mitogenic lectins. Of the 50 cloned lines analyzed, it was observed that all T cells that helped antigen-specific B cells also produced the lymphokine BSF-1/IL-4. Cloned lines that failed to help in this system did not make IL-4. Furthermore, the "non helper" cloned T cell lines characteristically produced lymphotoxin and interferon gamma. All of the cloned T cell lines analyzed made factors that induced proliferation of the T cell line HT-2. However, not all the HT-2 proliferative activity could be accounted for by IL-4, IL-2 or both. As shown by HPLC fractionation and by inhibition of T cell growth factor with monoclonal antibodies to IL-2 and IL-4 there is an additional HT-2 stimulating activity. Further studies using bone marrow, DA-1, CTLL and FDC-P1 proliferation assays indicated that this lymphokine correlates with the activity of GM-CSF. All clones studied produced considerable quantities of GM-CSF regardless of whether they produced IL-2, IL-4. Preliminary data suggest that GM-CSF contributes to the proliferation of concanavalin A stimulated normal T cells at low cell density. The precise role of GM-CSF in these cultures is currently being investigated.

T 535 MOLECULAR CLONING OF HUMAN INTERLEUKIN-3 (IL-3) GENE. Y-C. Yang and S.C. Clark, Genetics Institute, Cambridge, Mass. 02140

We recently isolated a cDNA clone encoding a novel primate colony-stimulating factor (CSF) from a gibbon ape T-cell line. This factor was identified by its ability to stimulate the proliferation of blast cells from patients with chronic myelogenous leukemia in the presence of antiserum which neutralized the biological activity of GM-CSF in the same assay system. The nucleotide sequence of cDNA clone for this novel activity proved to have significant homology with the nucleotide sequence encoding murine IL-3. Moreover the recombinant protein expressed in the monkey cos-1 cell system proved to be capable of supporting the proliferation of erythroid as well as myeloid progenitors from normal human bone marrow. Thus this novel growth factor is both structurally and functionally related to murine IL-3.

We have used the gibbon IL-3 cDNA clone as a hybridization probe to identify clones for the corresponding human IL-3 gene. The gene structure between human and mouse IL-3 genes is very similar and all the transciptional controlling elements are quite conserved between the two genes. The human protein sequence predicted by combining all of exons from the human gene proved to be closely related to the gibbon IL-3, differing in 11 of 152 amino acids. The overall homology between the human and murine IL-3 sequences at the nucleotide level is 53%. Interestingly, the homology between the two genes is greater in non-coding sequence elements than it is in the coding regions.

T 536 CLONING OF GENES THAT ARE INDUCED UPON STIMULATION OF HUMAN T LYMPHOCYTES Peter F. Zipfel, Steven G. Irving, Kathy Kelly and Uli Siebenlist, NIAID, NCI National Institutes of Health, Bethesda, MD 20892

We have constructed a subtracted cDNA library in lambda gt10 from mRNA isolated from human peripheral T cells which were stimulated for 4.5 h with PHA and PMA in the presence of cycloheximide. This library contains about 45,000 clones and is enriched for induced genes, as determined by the frequnecy of c-myc to microglobulin clones (9:1) in the unamplified library. After amplification, 20,000 phage were screened with a subtracted cDNA probe, and 528 positively hybridizing clones were isolated. These cDNA clones were determined to contain induced genes by several criteria. First, we performed differential screening using cDNA probes derived from unstimulated and stimulated T cell mRNA. Second, we ascertained the presence of the induced c-myc and IL-2 receptor gene in our population of induced clones. Third, we isolated randomly several of these clones as probes and confirmed their mitogenic stimulation by Northern blot analysis. Crosshybridization studies between the 528 clones revealed the presence of two large sequence families and the presence of about 60 individual genes. Judging by the kinetics of mRNA levels during the early stimulation phase many of these genes fall into two broad categories: those which are expressed rapidly and disappear quickly in a manner analogous to the induction of the c-fos oncogene; and those genes whose expression is slightly delayed but which are present for a prolonged period. The time course of the latter group resembles the expression of the c-myc oncogene.

The T Cell Receptor

Influences on the Peripheral Repertoire

T 537 T CELL RECEPTOR REPERTOIRE OF B10 ANTI-Bm12 REACTIVE HYBRIDS - Jerome Bill, Jordi Yague, Janice White and Ed Palmer, National Jewish Center for Immunology and Respiratory Medicine, 1400 Jackson, Denver, CO 80206.

We find apparent preferential usage of particular V α and V β genes in the T-cell response of C57BL/10 (B10) mice to the alloantigen, I-A bm12. I-A bm12 reactive blasts were converted into T-cell hybridomas by fusion with BW5147 and examined for expression of specific T-cell receptor Vα and Vβ chain genes using Northern analysis. Preliminary results on 36 bm12 reactive hybridomas from B10 mice indicate that multiple Vα and Vβ gene segments are used in the B10 anti-bm12 response. Surprisingly, 19 hybridomas can be accounted for by usage of only 7 Vα/Vβ pairs, including one pair (Vα2/Vβ8) which is present in 6 hybridomas. These data are consistent with the idea that there may be predominant clonotypes in the T-cell response to an alloantigen. Additional hybridomas are being generated, including some in H-2 disparate mice, to confirm preferential use of specific Vα/Vβ pairs and to determine any effect of thymic H-2 type on this preference. Individual hybrids will be further studied to determine the contribution of junctional and N-region diversity to bm12 reactivity.

T 538 DETECTION OF A NOVEL TcR-BETA GENE IN THE LEWIS-RESISTANT (Le-R) RAT
Wm. F. Hickey, Univ. of Pennsylvania, and E.P. Blankenhorn, Hahnemann Univ. Philadelphia, PA 19104.

The Le-R rat was discovered in a closed colony of Lewis rats. It differs from the Lewis in that it is resistant to a number of T-cell mediated autoimmune diseases (encephalomyelitis, uveitis, orchitis, neuritis) to which the Lewis is highly susceptible. Lewis derived T-helper cells capable of passively transfering autoimmune encephalomyelitis (EAE) or uveitis (EAU) to Lewis hosts also produce disease in the Le-R. Immunogenetic studies have shown that Le-R resistance is determined by one or two non-MHC-linked genes. We have detected a novel TcR-beta allele (TcR-beta^{avl}) in the Le-R which is distinct from Lewis and all other rat strains examined. The difference between Lewis and Le-R in the region detected by cDNA probes RBL-5 and KdD64 probably represents a mutation in the TcR-beta gene. The Le-R is identical to Lewis by all other cDNA probes tested and by a number of phenotypic characteristics. This change in the T-cell receptor constant region may be related to the Le-R rat's resistance to T-cell mediated autoimmune conditions by altering the Le-R T-cell repetoire or responsivity to certain antigens.

This work was supported by Nat'l M.S. Soc. Grant #RG-1685-A1

T 539 THE T CELL REPERTOIRE IN Ig TRANSGENIC MICE. Jean LANGHORNE, Marinus C. LAMERS, Georges KÖHLER, and Klaus EICHMANN, Max-Planck-Institut für Immunbiologie, D-7800 Freiburg, Federal Republic of Germany
In addition to MHC, the other gene complex which has some influence on the expressed T cell repertoire is that of the immunoglobulin (Ig) genes. It is now clear, however, that the antigen-receptors of T and B cells are distinct, thus other explanations must be sought for the apparent influence of B cells on the T cell repertoire. In this regard, it has been suggested that T cell idiotypes are selected by the available B cell and antibody repertoire. Transgenic mice in which rearranged μ and K genes coding for an IgM (SP6) specific for TNP have been introduced into the germ line (Rusconi and Köhler (1985) Nature 314, 330-334) are being studied in order to determine whether the T cell repertoire is modified by the presence of the transgenic immunoglobulin idiotype. This modification can be envisaged as occurring in at least 2 ways. 1) There is a selection for T cells whose receptors share idiotopes with those of SP6 and 2) there is a selection for T cells which recognise SP6 idiotopes. By limiting dilution analyses the frequencies of T cells specific for TNP-modified syngeneic cells are similar in transgenic mice and normal littermate controls. In order to determine whether TNP-reactive T cells from transgenic mice share idiotopes with SP6, the effects of 2 monoclonal antibodies specific for SP6 idiotype on the functional capacities of T cells were investigated. The proliferative and cytotoxic T cell response of cells cultured at low cell numbers could not be inhibited by either monoclonal antibody. However the helper capacity of TNP-specific T cell lines (L3T4$^+$, Lyt2$^-$) established from transgenic but not normal mice could be partially inhibited by these antibodies. The implications of these data for the generation of the T cell repertoire will be discussed.

T 540 THE COMPOSITION OF THE T CELL RECEPTOR REPERTOIRE IN NUDE MICE. James R. Maleckar, Gary L. Gilmore and Linda A. Sherman, Department of Immunology, Research Institute of Scripps Clinic, La Jolla, CA 92037.
Previous results from several laboratories have demonstrated the presence of functional T lymphocytes in congenitally athymic (nude) mice. The present study represents an analysis of the T cell receptor repertoire exhibited by such cells. Clones of H-$2K^b$ specific cytotoxic T lymphocytes (CTL) were generated under primary limiting dilution conditions using spleen cells from nude mice. These clones were analyzed on a panel of K^b mutant target cells in order to assess the receptor specificity of each clone. Unlike thymic bearing mice whose CTL repertoires are exceedingly diverse, it was found that in most cases the vast majority of clones from each individual exhibited the same reactivity pattern. The particular pattern varied from individual to individual. Clones from several animals that exhibited this phenomenon were further analyzed using a monoclonal antibody that can detect utilization of the gene products of the $V_\beta 8$ family. In one animal all clones were $V_\beta 8$ positive whereas in the others, all clones were negative. We conclude that the T cell receptor repertoire in nude mice is extremely limited and represents in vivo expansion of a relatively small number of functional precursors. We are currently analyzing the extent of receptor diversity in nudes by molecular analysis.

T 541 SOMATIC MUTATION OCCURS EARLY IN THE PRIMARY ANTI-PHOSPHORYLCHOLINE RESPONSE. Ursula V. Malipiero and Patricia J. Gearhart, Johns Hopkins University, School of Hygiene and Public Health, Baltimore, MD 21205.
Anti-phosphorylcholine (PC) antibodies are encoded by one variable heavy chain gene, $V_H 1$ from the S107 subfamily, and three variable light chain genes, V_KT15 from the $V_K 22$ subfamily, V_KM3 from the $V_K 8$ subfamily and $V_K 167$ from the $V_K 24$ subfamily. Previous studies showed that antibodies after secondary immunization are diversified by somatic mutation. To determine the onset of somatic mutation, we analyzed the immunoglobulin genes during the primary response to PC. Hybridomas were made at 7 and 11 days after immunization with PC-bovine serum albumin, and cDNA clones were sequenced by the dideoxynucleotide method and compared to the germline sequences. In hybridomas from day 7, no substitutions out of 1100 nucleotides were found. However, in hybridomas from day 11, a rise in mutation was seen. The V_H genes had three substitutions out of 1260 nucleotides, or 0.2% mutation, and the V_K genes had two substitutions out of 1130 nucleotides, or 0.2% mutation. Most of the light chains were encoded by the V_KT15 gene and no mutations were detected. In one antibody which used the V_KM3 gene, four nucleotide substitutions in the light and heavy chain genes caused four amino acid replacements and one mutation was silent. The mechanism of somatic mutation seems to be activated between day 7 and day 11 after primary immunization. (Supported by NIH grant CA-34127 and a Swiss National Science Foundation Fellowship.)

T 542 SELECTION AND MAINTENANCE OF T-CELL REPERTOIRE: SELECTIVE INFLUENCES OF NATURALLY ACTIVATED B LYMPHOCYTES. C.Martinez-A.,A.Coutinho, A. de la Hera, C.Marquez,M. Toribio and M.A.R. Marcos. Dep.Immunol. Clinica Puerrta de Hierro, S.Martin de Porres, 4 28035 Madrid, Spain.
Idiotype sharing between anti-TNP T_H cells and antibodies in Balb/c mice results from immunoglobulin-dependent selection of the T-cell repertoire. We have tested the hypothesis that mutual repertoire selection by B and T_H cells take place in the comportament of naturally activated lymphocytes.
Naturally activated L3T4$^+$ T-cells as opposed to resting L3T4$^-$ lymphocytes display effector helper activity upon coculture with syngeneic hapten-derivatized B cells. However, after continous "in vitro" stimulation of resting L3T4$^+$ T-cells with hapten-coupled syngeneic spleen cells, effector T_H cells lines with the same specificity can be derived. Using hapten specific helper cell assays for the expression of the clonotype defined by the F6(51) anti-idiotypic antibody it was found that idiotype expression by the helper cells is only restricted to the pool of "naturally" activated T-cells.
These results demonstrated repertoire differences between the set of resting and internally activated T lymphocytes in normal individuals. They also suggest the importance of idiotypic network interactions in the compartment of activated T and B cells.

T 543 STUDIES ON A MECHANISM FOR SELF-TOLERANCE M. McDuffie, N. Roehm, P. Marrack, and J. Kappler, Natl. Jewish Ctr. for Immunol. & Resp. Med., Denver, CO 80206

A murine monoclonal anti-T-cell receptor antibody, KJ23-588 (KJ23) binds to a βchain variable region associated with receptors of relatively limited specificity. This unusual property of these receptor molecules allowed us to examine the specificities of the receptor repertoire on thymocytes during maturation. KJ23 binds to receptors on immature thymocytes in three I-E$^-$ strains of mice and in C57BR mice which express I-E^k. While the mature thymocytes and T cells in the I-E$^-$ strains also express KJ23$^+$ receptors, mature KJ23$^+$ T cells in C57BR thymus and periphery are rare. In addition, F_1 animals heterozygous for KJ23 and a permissive H-2 region but bearing I-E molecules on their antigen presenting cells invariably have very low numbers of KJ23$^+$ mature T cells. In order to localize the site of suppression of the KJ23$^+$ phenotype, we made fetal thymus organ cultures from a permissive strain (SWR, H-29) and a non-permissive one (C57BR, H-2^k) at a stage in development when receptor was beginning to be expressed. Monoclonal antibodies against Class II molecules were added to some of the cultures, and they were allowed to develop until T cells of mature phenotype were present in the thymic lobes. αI-A exposure during maturation decreased both L3T4$^+$/Lyt2$^-$ and mature KJ23$^+$ cell numbers in cultures of SWR and C57BR thymus lobes, as predicted from our data and that of others. αI-E exposure had no effect on SWR thymocyte development (I-E$^-$) but resulted in a 2- to 3- fold increase in the number of mature KJ23$^+$ cells. Similar experiments _in vivo_ have resulted in an 8-fold increase in the numbers of KJ23$^+$ cells. We are currently investigating the reactivity patterns in the KJ23$^+$ repertoire of untreated and anti-class II-treated C57BR mice.

T 544 FACS ANALYSIS OF T CELL RECEPTOR USAGE IN THE DBA/2 RESPONSE TO SPERM WHALE MYOGLOBIN. Penny Morel, Alexandra Livingstone and C. Garrison Fathman, Stanford University, Stanford CA 94305.

The relationship between structure and function of the T cell receptor in its interaction with MHC and antigen remains unclear. In an attempt to investigate this further, we have established a series of sperm whale myoglobin-reactive T cell clones from individual DBA/2J mice. These clones were previously shown to fall into three distinct groups: 1) I-E^d restricted, 112-118 specific; 2) I-E^d restricted with epitope not identified; and 3) I-A^d with varying specificities between residues 106-118.

This panel of clones was examined by fluorescence activated cell sorter using the antibodies KJ16 and F23.1 These antibodies stain overlapping populations of T cells whose $V_β$ genes fall into the $V_β8$ (C5) family. Of the clones stained to date, 8 clones that are I-E^d restricted and 112-118 specific stain with both F23.1 and KJ16. Of 4 I-A^d restricted clones, 3 were negative for both antibodies and 1 was F23.1 positive but KJ16 negative. Of 4 I-E^d restricted clones with unknown specificity, 3 were F23.1 positive, one of which was also KJ16 positive, and another was KJ16 negative.

Thus, using these two antibodies, it was possible to show that I-E^d restricted 112-118 reactive T cell clones from DBA/2J mice show a preferential usage of the $V_β$ gene for the $V_β8$(C5) family. This has also been confirmed by molecular analysis. Also, none of the I-A^d restricted clones used the same $V_β$ gene.

T 545 CULTURED THYMIC FRAGMENT IMPLANTATION IN NUDE RATS. Jan Rozing, Jef Vos & Henk J.Schuurman. TNO Inst.Exp.Geront., Rijswijk & Univ. Utrecht, Holland.

After CTF implantation nude rats develop T-cell reactivity. This is irrespective of RT1 differences between donor CTF and recipient. We studied the development of alloreactivity in WAG (RT1^u) nudes implanted with CTF of a variety of allogeneic donor origin and followed it from 2 to 18 weeks later. In MLR and CML the CTF-implanted nudes exhibited at 14-18 weeks responses of similar magnitude as immunocompetent littermates. The specificity was not related to the RT1 haplotype of the donor CTF. The alloresponse was even directed to the donor haplotype. However, skin transplants from donor and recipient haplotypes were accepted, whereas third-party grafts were rejected. In (immuno) histology, medulla-like areas in the CTF grafts were populated by RT1 class II positive cells of acceptor haplotype with a dendritic morphology. The cortex-like areas of the CTF grafts exhibited RT1 class II expression of the donor haplotype on epithelial cells. We conclude that CTF after implantation in athymic nude animals become populated not only by precursor T-cells, but also by dendritic cells from the bone marrow in the recipient. The influx of these two populations occurs almost simultaneously, starting two weeks after implantation. The dendritic cells of recipient origin may be involved in the generation of alloreactive T-cells after CTF implantation. The RT1 haplotype expression by donor epithelium is ignored in this process, but the grafted cells induce in vivo tolerance to the donor haplotype. This tolerance is not based on deletion of alloreactive T-cells, as in vitro alloreactivity to the donor RT1 haplotype is generated.

The T Cell Receptor

T 546 CLONAL DOMINANCE IN T LYMPHOCYTES CULTURED FROM HUMAN ARTHRITIC SYNOVIA. I. Stamenkovic, M. Stegagno, K.A. Wright, R.B. Colvin, E.P. Amento, S.M. Krane, R.J. Duquesnoy and J.T. Kurnick. Harvard Med. Sch. and Massachusetts Gen. Hosp., Boston MA 02114 and Univ. of Pittsburgh, Pittsburgh, PA 15213.

In an effort to elucidate possible oligoclonality of the activated T lymphocyte infiltrate in rheumatoid arthritis (RA) and other chronic destructive inflammatory arthritis, Interleukin 2 (IL2) responsive cells were propagated from synovia of 14 patients (11 RA and 3 osteoarthritis) undergoing surgery for advanced destructive arthritis. Clonality of the the infiltrating lymphocytes was evaluated using Southern blot analysis of rearrangements in T cell receptor B-chain genes. Fragments of synovia were cultured in 10u/ml of recombinant IL2. After expansion in vitro DNA from $2x10^7$ cells was digested with EcoRI, HindIII and BamHI before blotting and hybridization with a constant region cDNA probe derived from JurB1. All 14 cultures showed distinct rearrangements indicating that each was characterized by the predominance of a limited number of clones. Blood lymphocytes stimulated with IL2, PHA, tetanus toxoid or allogeneic cells and cultured for up to 90 days in IL 2 did not demonstrate the emergence of a similar oligoclonality. These results suggest that a limited number of T cell clones predominate at the site of tissue injury in arthritic synovia. Supported in part by NIH grants AM03564 and CA44324.

T 547 DISSOCIATION OF ALLOGENEIC CLASS II RECOGNIZING AND EFFECTOR FUNCTIONS OF NEONATALLY TOLERIZED MICE. J.W.Streilein, K.Mohler and P.J.Wood, U.of Miami Sch of Medicine, Miami, Fla.

In H-2 recombinant, congenic mice derived from the A/J background strain, neonatal transplantation tolerance of class II MHC antigens is revealed by indefinite acceptance of orthotopic skin allografts. In this strain combination, tolerance is achieved and maintained without complete clonal deletion/inactivation of lymphocytes capable of recognizing the tolerogens. Spleen and lymph node cells from the majority (70%) of A.TH mice tolerant of A.TL proliferate in vitro when cultured with irradiated A.TL stimulators. The responding cells have been demonstrated to be Thy 1+, L3T4+. and Lyt 2-; in addition, their proliferative response can be inhibited by monoclonal antibodies (anti-IAk and anti-IEk) directed at determinants present on the stimulator cells, suggesting that the responding cells resemble normal alloreactive cells. However, these responding cells rarely produce tolerogen-specific cytotoxic T effector cells in vitro, despite the fact that these cells produce normal amounts of IL-2 in response to tolerogen in vitro. Moreover, effectors of tolerogen-specific delayed hypersensitivity can not be identified in these mice. We conclude that the maintenance of skin graft tolerance in these animals results from a dissociation between antigen recognizing and effector potentials of normal, class II MHC-specific T cells. At present we can not distinguish whether the lack of class II-specific effector function results from clonal deletion of a distinct lineage of cells differentiated for this function, or whether cells with both helper and effector functions lose the latter property as a consequence of the tolerance-conferring stimulus.

T 548 FUNCTIONAL FLEXIBILITY OF HUMAN ALLOPROLIFERATIVE T CELL CLONES NOT ASSOCIATED WITH APPARENT CHANGES IN T CELL RECEPTOR GENE REARRANGEMENT, P. Wernet, E. M. Schneider, F. Kalthoff, F. Busch, M. Fili, and G. Pawelec, Eberhard-Karls-Universität, D-7400 Tübingen, FRG.

Alloreactive interleukin 2 (IL2)-dependent CD4+ proliferative human helper T cell clones, which systematically lost the ability to proliferate autonomously to specific antigen during culture, no longer upregulated IL2 receptors after challenge with antigen and failed any longer to secrete IL2. The continued presence and unchanged density of CD3 structures and determinants bound by antibody WT31 on the cell surface, however, suggested a retention of expression of T cell receptors for antigen. Southern blotting with a Cß and a C gamma probe showed that aged clones, as well as subclones, all manifested the same original T cell antigen receptor ß chain gene rearrangements. That such receptors were in fact still expressed and were specifically functional was shown by the retained ability of aged clones to respond in an antigen-specific fashion by secretion of factors with colony stimulating activity for granluocyte, macrophage, and eosinophil progenitors from normal human bone marrow cells. These results are consistent with the hypothesis that even within human monoclonal T helper populations, at least under the current conditions of in vitro clonal propagation, variants with different regulatory activities may continuously emerge, and might be selected for or against according to environmental conditions as well as genetic preprogramming. Nonetheless such T cell clones seem to remain true to their initial antigen specificity.

T 549 IDENTITY OF GAMMA AND BETA CHAIN-GENE REARRANGEMENT PROFILES IN T-ALL REVEALING A NOVEL PATHWAY OF INTRATHYMIC GENERATION OF THE T CELL RECEPTOR REPERTOIRE, P.Wernet, F. Kalthoff, M. Fili, B.-Ch. Yang, F. Herrmann, and E. M. Schneider, Eberhard-Karls-Universität, D-7400 Tübingen, FRG.

Twenty-two cases of T-ALL have been studied with respect to surface marker expression, rearrangement of the T cell-specific beta and gamma chain genes, as well as the Ig heavy and light chain genes. Eighteen of 22 cases had rearranged beta and gamma chain loci, whereas 4 cases revealed a germline configuration consistent with a phenotype of immature thymocytes. Most striking, however, was the observation that 13 cases could be clustered into distinct groups comprising 2, 3, or 4 T-ALL with identical or very similar rearrangement profiles of beta and gamma chain genes. Moreover, the distinct thymic differentiation stages defined according to qualitative and quantitative changes in the expression of T cell surface antigens did correlate to individual clusters of rearrangement profiles concerning the TCR beta and gamma chain genes. Results imply that early rearrangements of TCR genes might be relatively restricted and that the clonal diversification of the antigen receptor is an ongoing process during thymic T cell development. A model is presented suggesting that certain self-reactive thymocytes are not simply excluded from further differentiation but rather might be triggered to express novel antigen receptor idiotypes via alterations of their primary rearrangement.

T 550 MODIFICATION OF THE T CELL REPERTOIRE IN NEONATAL TOLERANCE. P. Wood, S. Socarras and J. W. Streilein. U of Miami Sch of Medicine, Miami, FL 33101

The prevention of self-reactivity among T cells is thought to occur through a mixture of clonal deletion/inactivation and suppression of potential self-reactive cells. One prediction of a clonal deletion hypothesis is that preferential deletion of high avidity self reactive cells occurs resulting in significant modification of the T cell repertoire and preferential escape of low avidity cells. We have investigated this prediction using neonatal tolerance as a model for self-tolerance. In mice rendered tolerant of major histocompatibility complex (MHC) alloantigens by the injection of semi-allogeneic hematopoeitic cells at birth, >90% of tolerogen specific cytotoxic T cell precursors (pT_cs) are functionally deleted. The residual tolerogen specific pT_cs can be activated in the presence, but not absence, of exogenous help. The specificity of the remaining tolerogen specific T_cs was investigated using a variety of techniques and evidence has been obtained that the tolerogen specific T_c repertoire has been profoundly changed. This includes the findings that (i) the primarily K^b directed T_c reponse of normal B10.A spleen stimulated with B10 is switched to a predominant anti D^b response in B10.A mice tolerant of B10 (ii) T_c lines from C57BL/6 (B6) mice tolerant of the K^b mutant bml show different cross reactivity patterns compared to normal B6 and (iii) bml specific T_cs from B6 mice tolerant of bml are more easily inhibitable with anti Lyt2 antibody than those from normal B6 mice. Thus, the results support the idea that modification of the fine specificity of the T cell repertoire occurs as a result of the introduction of alloantigen into the neonatal environment and provide the first evidence that avidity of cells for antigen plays a direct role in this modification.

NOTES