REGULATION OF THE IMMUNE SYSTEM

Eli Sercarz, Harvey Cantor and Leonard Chess, Organizers

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Plenary Sessions

Poster Sessions

Chemistry and Biology of Corecognition of MHC and Antigen by T Cells

0239 A COMPARISON OF DIFFERENT MECHANISMS OF Ir GENE CONTROL OF THE T CELL PROLIFERATIVE RESPONSE TO PIGEON CYTOCHROME C IN [B10.A(4R)xB10.PL]F1 MICE, Ronald H. Schwartz and Zdenko Kovač, Laboratory of Immunology, NIAID, NIH, Bethesda, MD 20205

Experimental evidence has been reported which suggests that MHC-encoded molecules can influence the immune response both through effects on the T cell repertoire and during T cell activation at the time of antigen presentation. If one accepts this conclusion, the next question to ask is whether either of these mechanisms exerts a more profound influence than the other. Although this is a question which cannot be answered fully until we have a complete understanding of the germ line encoded T cell repertoire, one experimental system exists which has allowed us to compare the impact of positive selection in the thymus to that of antigen presentation in the periphery. This system is the T cell proliferative response to pigeon cytochrome c, which is controlled by two Ir genes that encode the Eβ and Eα Ia molecules. Previous studies from our laboratory have demonstrated that the k, d, p and r alleles of Eα will complement with the k allele of Eβ to form a fully functional Ia molecule for an immune response. In contrast, the u allele of Eα does not complement i.e. the (B10.A(4R) x B10.PL)F1 [Eβ of k x Eα of u] is a nonresponder to pigeon cytochrome c. Biochemical studies by Pat Jones and colleagues have demonstrated that the Eα of u chain preferentially associates with the Eβ of u chain over the Eβ of k chain in this F1 strain. The result is a quantitative decrease in the expression of the Eβ of k:Eα of u Ia molecule on the surface of the F1 cells. This decrease manifests itself functionally in antigen presentation experiments in which it was demonstrated that 10 times as much pigeon cytochrome c was required to stimulate antigen-specific T cell clones and lines when (4RxPL)F1 APCs were used compared to B10.A (Eβ of k:Eα of k) APCs. In the experiments to be discussed at the meeting we have now addressed the question of which mechanism, thymic selection or antigen presentation, plays the major role in determining the nonresponder status of the (4RxPL)F1. To explore this question B10.A(b.m.) → (4RxPL)F1(irr) and (4RxPL)F1(b.m.) → B10.A(irr) chimeras were set up. The former creates an animal with normal B10.A APCs and quantitatively defective thymic selection environment while the latter creates an animal with defective (4RxPL)F1 APCs and a normal thymic selection environment. Immunization with pigeon cytochrome c revealed that the (4RxPL)F1(b.m.) → B10.A(irr) chimeras were still nonresponders to the antigen while B10.A(b.m.) → (4RxPL)F1(irr) chimeras were responders. However, the response of the latter chimeras was quantitatively lower than that of normal B10.A controls demonstrating that some effect of decreased thymic selection could be detected in this assay. Overall the experiments demonstrate that in this particular Ir gene-controlled system the effect of the Ia molecule during antigen presentation is the dominant mechanism for nonresponsiveness.

T Cell Activation and Differentiation

0240 CHARACTERIZATION OF cDNA CLONES ENCODING ONE CHAIN OF THE T CELL RECEPTOR FOR ANTIGEN. Stephen M. Hedrick, David I. Cohen, Ellen A. Nielsen, Yueh-Hsiu Chien, Joshua Kavaler, and Mark M. Davis, Laboratory of Immunology, NIH, Bethesda, MD; University of California, San Diego, La Jolla, CA; Stanford University, Stanford, CA.

A set of cDNA clones were isolated that code for membrane-bound or secreted proteins specifically expressed in T lymphocytes. Of eleven clones isolated one was shown to code for the thy-1 molecule. A second clone, TM36 hybridized to genomic fragments that rearranged in mature T cell DNA as compared to liver, or B cell DNA. Three homologous clones were isolated from a thymus cDNA library and the nucleotide sequence determined. The three clones T1, T3, and T5 shared a region of identical nucleotide sequence and a region of nonhomologous nucleotide sequence reminiscent of constant and variable regions of immunoglobulin genes. The T1 clone was 930 bp long with an open reading frame beginning in the variable region and extending to the 3' end of the clone. The translated amino acid sequence showed striking homology to conserved residues in both the variable and the constant region of immunoglobulin heavy and light chains. In particular, there were cysteines in the same positions as those seen in Ig heavy chains, and at the second v-region cyteine (residue 92) there were 11/12 amino acids in common with certain heavy chain v-regions. Finally, the junction between the v-region and the c-region comparing T1 and T3, with that of T1 and T5 shows a disparity of 46 nucleotides indicating a possible J-like gene segment. The amino acid sequences in this putative J region were found to be as similar to heavy and light chain J regions as they are to each other indicating a fourth family of J genes. Experiments are currently in progress to further characterize the structure and expression patterns of TM36.

0241 THE MHC-RESTRICTED ANTIGEN RECEPTOR ON T CELLS, John Kappler, James Allison, Kathryn Haskins, Charles Hannum, Ralph Kubo, Bradley McIntyre, Michele Pigeon, Ian Trowbridge Janice White and Philippa Marrack. Department of Medicine, National Jewish Hospital, Denver CO 80206, and University of Texas System Cancer Center, Smithville, TX, and Salk Institute, La Jolla, CA.

We have raised monoclonal antibodies in mice and rats against cloned T cell hybridomas or tumors from mouse and man. A number of antibodies have been identified which react only with the immunising T cell clone. Where possible these antibodies have been shown to interfere with the ability of the target T cell to interact specifically with antigen in association with the appropriate product of the MHC. By isolating other T cell hybridomas which react with a clone specific antibody we have demonstrated that these antibodies probably react with the entire receptor for antigen plus MHC on target T cells.

These antibodies precipitate an 80-85 kd heterodimer from T cells. In mouse reduction reveals two glycopolypeptide chains of 40-43 kd each, differing in charge. In man, again two chains are revealed after reduction, an acidic chain of about 45 kd, and a basic chain of 38 kd. Tryptic fingerprinting of ^{125}I surface labelled material reveals variable and constant peptides, present in both chains. No peptides are shared between the two chains. The overall structure of this molecule is strikingly reminiscent of immunoglobulin although no genes coding for this material are shared with immunoglobulin genes, and we can detect no antigenic crosseactivity between this protein and immunoglobulin (1-3).

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B Cell Activation and Control of B Cell Differentiation

0242 ACTIVATION AND IMMUNOREGULATION OF HUMAN B LYMPHOCYTE FUNCTION, Anthony S. Fauci, A. Muraguchi, John H. Kehrl, and Joseph L. Butler, Laboratory of Immunoregulation, NIAID, NIH, Bethesda, MD 20205.

An in vitro model system has been developed for delineating the minimal, optimal, and synergistic signals involved in the activation, proliferation, and differentiation of normal human B lymphocytes (1). B cells were activated by triggering with either anti-μ or Staphylococcus aureus Cowan strain 1 (SAC). Purified B cell growth factor (BCGF) with a MW of 18-20 Kd and an IEP of 6.6 (2) as well as purified B cell differentiation factor (BCDF) with a MW of 30-35 Kd and an IEP of 5.9 were obtained from human T-T cell hybrids developed from the fusion of peripheral blood T cells and a CEM-6 T lymphoblastoid cell line. Using countercurrent centrifugation elutriation, large numbers of small (G_0) B cells were isolated, and it was demonstrated that these resting cells could be driven by low concentrations of anti-μ (15 ug/ml) to progress from G_0 to G_{1a} phase and to express the activation antigen 4F2 which is not seen on resting B cells but is expressed on activated B and T cells. Higher concentrations of anti-μ (100 ug/ml) or SAC can drive G_0 cells through G_{1b} whereby they then express the transferrin receptor (defined by monoclonal antibody 5E9) and proceed to S phase. BCGF synergizes with anti-μ or SAC in the induction and maintenance of proliferation of resting B cells (1). Indirect evidence strongly suggests that activated B cells express receptors for BCGF since they respond by proliferation to this purified factor whereas G_0 cells do not. Furthermore, activated, but not resting, B cells can absorb out BCGF activity from the hybrid supernatant. Activated normal B cell blasts will not differentiate in the presence of BCGF alone; however, upon addition of BCDF to culture, SAC-activated cells differentiate to Ig-secreting cells. Likewise, BCDF activity, but not BCGF activity, can be absorbed out by CESS cells which differentiate in the presence of BCDF. Using this system, we have demonstrated that there are present in the normal human B cell repertoire cells in all stages of the B cell cycle from resting cells to activated, but not proliferating cells, to proliferating and/or differentiated cells. A variety of disease states such as the acquired immunodeficiency syndrome manifest profound abnormalities of B cell activation, and these will be discussed (3). Finally, pharmacologic agents such as cyclosporin A and inhibitors of adenyl cyclase have been employed to dissect out the earliest events in the B cell activation process.

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0243 CONTROL OF B CELL ACTIVATION, PROLIFERATION AND DIFFERENTIATION. William E. Paul, Laboratory of Immunology, NIAID, NIH, Bethesda, Md 20205

The responses of resting B lymphocytes to antigenic stimuli appear to involve an ordered series of independently controlled steps including activation, proliferation, and differentiation. A substantial body of data indicates that receptor cross-linkage by anti-IgM antibodies causes activation, as measured by increase in cell volume, RNA synthesis, and membrane depolarization. Others have reported that cognate MHC-restricted T cell-B cell interaction cause the activation of resting B cells. Recently, evidence has been obtained that "MHC-restricted" T cells may activate resting B cells through a mechanism that depends neither on the specificity of the B cell's receptor for antigen nor on the class II molecules expressed by the B cell. Thus, one must postulate the existence of non-specific B cell activating factors. The entry of B cells activated by anti-IgM into S phase has been shown to depend on the action of both interleukin-1 and a B cell growth factor, now designated BSF-p1. BSF-p1 is an ∿15,000 dalton molecule with two charged forms (pI-6.4-6.6 and 7.4-7.6) which is separable from IL-2 by a series of chromatographic procedures. Efforts to purify it by high pressure liquid chromatography are now in progress. B cells activated with anti-IgM and treated with BSF-p1 will differentiate into Ig synthesizing cells in the presence of two additional factors - B15-TRF (supernatant of B151K12 hybridoma) and EL-TRF (pI 4.5 fraction of supernatant of EL-4 cells induced with PMA). B15-TRF must be added early in culture and must be present continuously whereas EL-TRF addition can be delayed until 24 hrs before assay. Studies of μ mRNA indicate that addition of B15-TRF to B cells cultured with anti-IgM and BSF-p1 causes a ∿4 fold diminution in mRNA capable of hybridizing with a cDNA probe specific for membrane μ (μ_m) without change in the amount of mRNA capable of hybridizing with a cDNA probe for the coding region of μ chain (μ_t), implying that B15-TRF alters the mRNA splicing pattern of cycling B cells. By contrast, addition of EL-TRF to B cells treated with anti-IgM, BSF-p1 and B15-TRF causes an enhancement in μ_t mRNA but μ_m mRNA remains at very low levels. Studies of J chain mRNA in such cells is now in progress. Thus, stimulated B cell responses can be described in terms of distinguishable activation, growth, and differentiation events, each potentially capable of independent regulation.

0244 THE ACTIVATION OF ENRICHED POPULATIONS OF TNP-BINDING B CELLS (TNP-ABC), Ellen S. Vitetta, Randolph Noelle, Charles Snow, Eitan Yefenof, and Jonathan Uhr, Department of Microbiology, University of Texas Health Science Center, Dallas, Texas 75235.

Using a sensitive rosetting technique, we have prepared populations of highly enriched antigen-binding cells from virgin or TNP-KLH primed mice. 80% of the TNP-ABC are specific for the hapten and a large proportion of the cells can be activated by polyclonal stimuli (anti-Ig), thymus-independent (TI) and thymus-dependent (TD) antigens carry the epitope TNP. Both the polyclonal and TI antigens induce proliferation of the TNP-ABC in the absence of T cells when the appropriate sources of cytokines are added. In contrast, the responses to the TD antigen require helper T cells and these responses are most optimal under conditions of linked recognition. Activation occurs in two phases: the first is initiated by cognate recognition and the second is sustained by cytokines. Both virgin and primed TNP-ABC secrete anti-TNP antibody when they are activated by antigen, cytokines, and in the case of the TD antigen, helper T cells. The TNP-ABC from the virgin mice give an IgM response while the TNP-ABC from the primed mice give predominantly an IgG response. The precise requirements for proliferation vs differentiation of the two cell populations will be compared and contrasted and the early events in their activation described.

0245 T CELL-MEDIATED REGULATION OF IMMUNOGLOBULIN EXPRESSION IN A MURINE MYELOMA CLONE. Richard G. Lynch, Gary L. Milburn, Katie R. Williams and Steven B. Binion. Dept. of Pathology, University of Iowa, Iowa City, IA 52242.

Neoplasms of lymphoid cell origin have become useful tools in the study of immunoregulatory mechanisms because many of these tumor cells produce and/or are responsive to immunoregulatory effectors. A well-studied example is MOPC-315 which is a BALB/c plasmacytoma that produces an $IgA\lambda_2$ anti-TNP antibody (M315). MOPC-315 cells differentiate during in vivo growth from small M315-nonsecreting lymphocytoid cells to large M315-secreting plasmacytoid cells. In the presence of TNP-antigen MOPC-315 proliferation and differentiation can be enhanced or antagonized by antigen-specific helper or suppressor T cells, respectively (1). MOPC-315 proliferation and differentiation are also responsive to idiotype (Id^{315})-specific regulatory T cells (2). Separate Id^{315}-specific mechanisms regulate: 1) MOPC-315 proliferation, 2) surface membrane expression of M315, and 3) synthesis and secretion of M315. Id^{315}-specific suppression of M315 synthesis is mediated by θ^+, Lyt 1^-2^+, Id^{315}-binding lymphocytes that selectively inhibit M315 synthesis by down-regulation of λ_2-light chain mRNA expression (3). The cessation of λ_2 light chain synthesis is accompanied by inhibition of α-heavy chain synthesis by a mechanism that operates distal to the occurance of an intact α-heavy chain mRNA. In addition to being responsive to immunoregulatory signals, MOPC-315 is a source of immunoregulatory signals. The polymeric $IgA\lambda_2$ protein secreted by MOPC-315 cells induces large numbers of θ^+, Lyt 1^-2^+, lymphocytes that have surface membrane IgA-Fc receptors ($T\alpha$ cells) (4). The BALB/c T cell lymphoma (BAL-8) expresses IgA-Fc receptors and is being used as a cloned source of $T\alpha$ cells for studies of IgA-isotyope-specific regulation. A large body of evidence from the study of MOPC-315 regulation indicates that functionally distinct regulatory effectors recognize different epitopes on M315 and that surface membrane M315 on MOPC-315 cells functions as a focusing device for a multiplicity of immunoregulatory signals. (Supported by CA-32275, CA-32277, CA-09119, and CA-28848).

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Cellular Basis of Immune Regulation

0246 SYNAPTIC STRUCTURES IN THE IMMUNOLOGICAL CIRCUIT. Tomio Tada, Ryo Abe, Seiji Miyatani, Junji Yagi and Wojciech Uracz, Department of Immunology, Faculty of Medicine, University of Tokyo, Tokyo, Japan.

The question how the genetic restriction is imposed on certain points in the immunological circuit is one of the major concerns in current immunology. To analyse the restricting elements controlled by MHC, we produced a series of monoclonal antibodies that are directed at I-A- and I-J-subregion-controlled determinants uniquely expressed on MHC-restricted functional T cells of $H\text{-}2^k$ haplotype. Anti-$I\text{-}A_T^k$ precipitated a 33K polypeptide from a T hybridoma that does not express any class II determinants. Specificity of anti-I-J antibodies suggested the molecular heterogeneity in I-J products on functionally different T cells, and one of the anti-I-J precipitated a 25K polypeptide from an I-J positive T hybridoma. The effects of these monoclonal antibodies on a variety of T cell responses were studied to find: 1) Some of the anti-$I\text{-}A_T^k$ and anti-$I\text{-}J^k$ could block or kill (with complement) some but not all helper T cells of $H\text{-}2^k$ mice. The target of these antibodies was found to be MHC-restricted, radiation resistant Lyt-1^+ helper T cell. 2) Some of the antibodies also inhibited antigen-induced T cell proliferation which requires the MHC-matched APC. The target was T cell but not APC. 3) The injection of an anti-$I\text{-}A_T^k$ into $H\text{-}2^{kxb}$ F_1 mice induced the suppression of Ir gene-controlled antibody responses associated with $H\text{-}2^k$ haplotype but not with $H\text{-}2^b$ haplotype. The inhibition of the response was due to the elimination of $H\text{-}2^k$-restricted helper T cells. 4) Some monoclonals blocked the autologous and heterologous MLR. The target was always responding T cells but not stimulator cells. 5) These monoclonals were able to stain autoreactive and MHC-restricted antigen-specific T cell lines cultured in the presence of IL-2. These results are best explained, if the epitopes detected by our monoclonals are associated with MHC-restricting elements on T cells. Since the subregion specificities of these antibodies were originally mapped in I-A and I-J subregions, we think that the antibodies react with the receptors for self A and E components, which are utilized as the restricting elements in the immunological circuit. On the above basis we propose the following terms: Self Ia antigens carry lymphocyte-defined (LD) Ia epitopes as internal images to generate sets of self-restricted (and alloreactive) T cells. Such Ia epitopes are called prototopes (epitopes present before T cell repertoire). T cells differentiated adaptively to these prototopes have anti-self receptors that are called antetopes (epitopes produced after the prototopes). Such antetopes may be recognized by the third T cell type which will impose the next restriction point in the circuit. This hypothesis explains both the plasticity of the restriction specificity and the I-J mystery.

0247 CELL INTERACTIONS IN THE REGULATION OF IMMUNE RESPONSE TO LACTATE DEHYDROGENASE B, Zoltan A. Nagy, Zenro Ikezawa, Constantin N. Baxevanis, and Jan Klein, Department of Immunogenetics, Max-Planck-Institute for Biology, 7400 Tübingen, Federal Republic of Germany.

The enzyme lactate dehydrogenase B (LDH-B) induces immune responses of both helper and suppressor T (Th and Ts) lymphocytes. The Th cells are activated by LDH-B presented on antigen presenting cells (APC) in the context of $A_\alpha A_\beta$ (A) molecules controlled by any one of the 15 or so thus far recognized responder A alleles. In contrast, the Ts cells are induced by LDH-B presented in conjunction with $E_\alpha E_\beta$ (E) molecules controlled by one of the two alleles E_β^b or E_β^k (together with E_α; ref. 1). The Ts cell requires also a second activating signal provided by a nonspecific Ts inducer cell (2). The interaction between Th and Ts cells occurs via a soluble factor (TsF) secreted by the Ts cells (3). The factor consists of two covalently bonded chains, one that binds antigen and another that carries serologically detectable determinants of the Aβ chain in addition to determinants specific for TsF and Ts cells. For the suppression to occur, a double bond between Th cell and TsF has to be established: the antigen-binding chain (ABC) of the factor must bind to the antigen receptor of Th cells via an antigen bridge, and the Aβ-like chain of TsF must bind concurrently to the Th-cell receptor that also recognizes Aβ determinants on the APC. In addition to this factor that we term TsF-A, the Ts cells secrete a second factor, TsF-E. The TsF-E possesses the same ABC as the TsF-A, but carries an Eβ-like chain. The latter chain expresses Eβ determinants present also on macrophages or B cells, in addition to the so-called I-J determinants (4). Thus, I-J does not seem to be a separate molecule, but rather a set of antigenic determinants resulting from the modification of Eβ-chain, whatever the nature of this modification may be. The TsF-E inhibits the differentiation of Ts-cell precursors to mature Ts cells. Thus, the production of TsF-E provides a feedback mechanism that regulates the Ts cells, and consequently, the degree of suppression in the response to LDH-B.

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Immunological Dysfunction

0248 IMMUNOLOGIC DYSFUNCTION IN MURINE SLE, Frank J. Dixon, Department of Immunology, Research Institute of Scripps Clinic, La Jolla, CA 92037

Murine SLE like its human counterpart is a complex autoimmune disease mediated for the most part by antibodies directed against various endogenous antigens. By definition, therefore, the ultimate immunologic dysfunction is the abnormal and/or hyperactivity of B cells resulting in increased Ig secretion and autoantibody formation. This disease develops spontaneously in a number of genetically very different kinds of mice which do not share either MHC haplotypes or Ig allotypes.

The immunologic abnormalities leading to the autoimmune dysfunction of the B cells varies in the several kinds of lupus mice. B cell proliferation and differentiation in all lupus prone mice as in normal mice are dependent on accessory signals from both macrophages and T cells. Also, B cells from lupus mice require the same number of signals as B cells from normal murine strains to undergo polyclonal or antigen directed responses. However, the B cells of the BXSB and the (NZBxNZW) F1, but not the MRL/1, differ from normal B cells by their higher sensitivity (or degree of response) to the signals they receive. Thus, these two strains appear to have a primary B cell abnormality resulting in excessive and poorly regulated B cell activation, proliferation and differentiation. The B cells of the MRL/1 mice respond to usual stimuli as do B cells from immunologically normal animals. However, the proliferating T cells in the enlarged nodes and spleens of older MRL/1 mice in the absence of mitogen secrete in vitro abnormally high levels of a B cell differentiation factor(s) that induces terminal differentation of activated B cells to Ig secreting cells. A large number of proliferating T cells which characterize this strain produce large amounts of this BCDF which apparently drives the otherwise normal B cells to pathologic function.

The genetic basis of the various autoimmune responses has been examined in a variety of recombinant inbred strains derived from the NZB mouse or F2 generations derived from various crosses among all the lupus mice. These studies indicate an independent inheritance of most of the specific autoimmune responses. The only two autoimmune responses with a consistent association were those to nuclear antigens and to endogenous gp70. Interestingly, these two associated responses are also highly correlated with severity of disease.

0249 IMMUNE DEFICIENCY DISORDERS: REVERSAL IN VITRO, Henry G. Kunkel and Lloyd F. Mayer, The Rockefeller University, New York, NY 10021.

The immune defects in a number of the immune deficiency disorders are gradually becoming manifest. Perhaps the most useful method to determine these defects is through their reversal through manipulations in vitro with the pure cell types and their products that have now become available. The most widely prevalent disorder is the common variable immune deficiency. It is well recognized that this group is still a mixture of different syndromes. The question of whether B cell or T cell defects are responsible for the poor production of Igs in vitro has been an open question for a number of years. Certainly increased suppressor cell activity has been well demonstrated but this does not appear to be primary in most instances. Recently we have been able to produce great increases in Ig synthesis in certain of these cases by adding human T-T hybridomas and their products, effects that were not produced by pooled T cells or Con-A supernates. Other cases were entirely resistant to these effects. Another defect that has been reversed with specific monoclonal T cells has been the hyper IgM immune deficiency. The "switch" defect in this condition has proven in the past completely resistant to stimulation to produce IgG and IgA. However, a specific clone of T cells has been obtained which causes the switch to occur. Thus evidence of T cell defects in these cases is becoming increasingly clear. The T hybridomas and clones provide large amounts of the missing cell and its products making the defect evident.

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0250 THE INFLUENCE OF Ir GENES ON THE EXPRESSION OF DISEASE, John D. Stobo, Marianne Newton, and B. Matija Peterlin, University of California, San Francisco, CA 94143.

In order to implicate a specific immune response in the expression of disease, several investigators have attempted to show a significant association between the disease and products (HLA-DR molecules) of human Ir genes in the HLA-D locus. In a few instances this association is striking, while in others it is weak. In many situations there does not appear to be a significant association between a single HLA-DR phenotype and disease One explanation for this is that Ir genes do not contribute to the expression of many diseases. An alternative explanation is that Ir genes distinct from those in the HLA-D locus play an important role in the expression of disease. Indeed, two other human Ir gene loci (SB and DC) encoding for two distinct families of Ia molecules (HLA-SB and HLA-DC) have been described. Presently serologic reagents capable of defining polymorphisms associated with HLA-SB and HLA-DC molecules are not readily available and thus analysis of serologically defined HLA-SB and HLA-DC disease associated polymorphisms is not possible. However, another approach to examining the association of Ir genes with disease is to examine the structure of the Ir genes themselves. The availability of molecular probes capable of hybdridizing to SB, DC, and D genes makes this approach feasible. Utilizing the technique of restriction fragment analysis by Southern blots, we have begun to examine Ir gene polymorphisms associated with diseases. Utilizing a cDNA probe for HLA-DC alpha chain genes, initial studies in a patient with Rheumatoid Arthritis and an HLA-DR and MT matched normal control reveal differences in the restriction maps. While the restriction map obtained with the Eco Rl digestion was identical in each case, that obtained with Pst 1 was different. The Pst 1 digest from the patient contained, in addition to shared bands, two low molecular weight bands (less than 3 kb) not present in the normal control.This approach will be useful in searching for Ir gene polymorphisms associated with various diseases.

MHC Restricted Specific Antigen Presentation to Different T Cell Sets (Th, Ts, Tc)

0251 ANTIGEN PROCESSING AND PRESENTATION OF HEN EGG-WHITE LYSOZYME Paul M. Allen, Daniel J. Strydom, and Emil R. Unanue, Harvard Medical School, Boston,MA 02115

To precisely determine the molecular steps involved in antigen processing we have examined the processing and presentation by macrophages of the well-defined protein antigen, hen egg-white lysozyme(HEL). T cell hybridomas, specific for HEL,produced IL-2 upon interaction with an Ia bearing macrophage that had taken up HEL. The processing requirements by macrophages for two of these clones was examined using live macrophages treated with the lysosomotropic agent, chloroquine, and by using macrophages prefixed in 1% paraformaldehyde. Three different forms of HEL were used:native HEL, intact denatured HEL, and a tryptic digest of HEL. From these studies we concluded that 1)both clones required that native HEL be processed by macrophages, 2)neither clone required that a tryptic digest be processed, and 3)one clone required that intact denatured HEL be processed, while the other clone did not.

The precise determinants on the HEL molecule which the two clones recognized were then ascertained by testing for the ability of tryptic fragments of HEL, separated by reverse phase HPLC, to stimulate the clones using prefixed macrophages. Both clones responded to the same 16 amino acid fragment of HEL. The reactivity of the two clones was localized to the N terminus of this fragment by the failure of either clone to react with human lysozyme, which differs from HEL in the 16 residue fragment by 4 amino acids. These results suggest that HEL must be internalized by a macrophage, encounter an acidic compartment, and recycle to the plasma membrane, where it is then presented to a T cell in the context of an Ia molecule. Thus, by using HEL specific T cell hybridomas as functional probes we have begun to define the molecular steps involved in antigen processing.

0252 TOWARD DEFINING THE EPITOPE AND POSSIBLE IA ASSOCIATION SITES OF A MYOGLOBIN PEPTIDE RECOGNIZED BY CLONED T CELLS, Ira Berkower, Gail K. Buckenmeyer and Jay A. Berzofsky, Office of Biologics, FDA and Metabolism Branch, NCI, NIH, Bethesda, Md. 20205

We have found a strict association between the epitope specificity and I-A^d vs I-E^d restriction of 13 T cell clones specific for sperm whale myoglobin and H-2^d, suggesting a strong influence of Ia antigens of the antigen presenting cell in the recognition of different epitopes. Eight clones were specific for an epitope centering on Lys 140 and restricted to I-E^d. In addition, the complete epitope was contained on the myoglobin fragment 132-153.

We have examined whether distinct sites could be found on the fragment for possible interactions with I-E antigens on the antigen presenting cell as well as with the T cell receptor. Fragment 132-153 was selectively degraded by N-Bromosuccinimide cleavage at Tyr 146. The cleavage fragment 132-146 was isolated by HPLC and had full stimulatory activity for the T cell clones. In contrast, after cleavage at Glu 136 by the Glu-specific peptidase from Staph aureus, the purified fragment 137-153 had lost all activity. Therefore, one or more residues between 132 and 136 are required for stimulatory activity in addition to Lys 140, while residues 147-153 do not influence the site. Whether the required residues between 132 and 136 are part of the same epitope as Lys 140 or represent a site for interaction with I-E^d of the presenting cell remains to be determined.

0253 THE ROLE OF UPTAKE AND CATABOLISM IN ANTIGEN PRESENTATION BY DENDRITIC CELLS AND MACROPHAGES, Benjamin M. Chain, Paul Kaye and Marc Feldmann, University College London, Gower Street, London, WC1E 6BT

The role of phagosome/lysozome pathway in antigen presentation is as yet unclear. We have studied the ability of the non phagocytic lymphoid dendritic cells (DC) to stimulate proliferation of hapten-primed (TNP) T cells, in the presence of hapten coupled to antigens of varying size and complexity. Results show that DC are three to four fold as efficient as spleen adherent populations or purified macrophages in presenting keyhole limpet haemocyanin (KLH)-TNP or insulin-TNP, but are also at least as effective with particulate antigens, such as TNP-mycobacterium or TNP-sheep red blood cell conjugates.

In parallel with these functional studies we have carried out biochemical experiments to compare catabolism of radiolabelled antigen by presenting cells of various types. As predicted, in view of their very poorly developed lysosomal system, DC produce and release negligible quantities of TCA soluble (i.e. completely digested) breakdown products. However, PAGE analysis revealed that larger proteolytic fragments are found in cellular extracts of DC, in amounts comparable to those of macrophages. Purified lymphocyte populations do not produce these fragments.

These functional and biochemical data suggest that presentation by dendritic cells does not rely on uptake and lysosomal degradation of antigen. However, limited proteolysis, perhaps by extracellular membrane bound enzymes, may play a role.

0254 B CELL PRESENTATION OF ANTIGEN TO ANTIGEN-SPECIFIC T CELLS VS PRESENTATION OF IA TO ALLOREACTIVE T CELLS, Robert W. Chesnut, Melissa Yanover and Howard M. Grey. National Jewish Hospital, Denver, CO 80206

We have compared the capacity of murine B cells to present conventional antigen to antigen-specific MHC-restricted T cells vs their capacity to serve as stimulator cells in the mixed lymphocyte response (MLR). When B cells were pulsed with soluble protein antigens, these cells were unable to present antigen to antigen-specific T cells. In contrast, when B cells were stimulated by a polyclonal activator such as LPS for 17 hrs these "activated" B cells were able to present antigen. Since activated B cells take up as much as 10 fold more antigen than resting B cells, one explanation for B cells inability to present antigen could result from insufficient antigen uptake. We therefore decided to test the capacity of resting B cells to stimulate a MLR since the Ia molecules required for MLR stimulation are expressed constitutively on the B cell surface. Our results showed that resting B cells were unable to stimulate proliferation of primed alloreactive T cells or IL-2 production by alloreactive T cell hybridomas. In contrast, LPS activated B cells were able to stimulate both T cell proliferation and IL-2 production. We are therefore left with two possible explanations for the failure of resting B cells to serve as APC: 1) the quantity or structure (e.g. sialic acid content) of Ia molecules associated with resting B cells is not adequate for recognition by T cells or 2) resting B cells are incapable of participating in the non-specific cellular interactions that are required before specific ligand-receptor interactions can effect T cell triggering. Experiments to distinguish between these possibilities are in progress.

0255 ACTIVATION OF HUMAN HELPER T LYMPHOCYTES BY SYNTHETIC PEPTIDES OF HERPES SIMPLEX VIRUS GLYCOPROTEIN D, Elaine DeFreitas, Bernard Dietzschold, and Hilary Koprowski, The Wistar Institute, Philadelphia, PA 19104

We have established continuously growing T cell lines from peripheral blood of several human donors by in vitro stimulation with a 23 amino acid synthetic polypeptide (syn pep) corresponding to the NH_2 terminus of native glycoprotein D (gD) of Herpes Simplex virus (HSV). This was accomplished with donors who were either seropositive or seronegative for HSV gD specific antibody. Phenotypic analysis of bulk cultured lines indicated that syn pep induced Leu $2^+,3^-$, Leu $2^-,3^+$ and natural killer cells in all donors. Analysis of the specificity of these T cells by proliferation to syn pep 1-23 or fragments thereof indicated that the recognition of various epitopes differed dramatically between donors. Substitution of a single amino acid at position 7 rendered the syn pep nonstimulatory for two donors. Syn pep fragments of 7 and 11 amino acids from the COOH terminus were either non-stimulatory or markedly suppressive. Syn pep-induced proliferation was blocked by monoclonal antibody against HLA-DR but not by monoclonal antibody against HSV gD. Clonal analysis data will be presented to indicate the major T cell-defined immunodominant portions of this molecule.

Supported in part by NIH grant AI 19987-01

0256 GENETICALLY RESTRICTED RESPONSES BY MONOCLONAL HUMAN T-CELLS SPECIFIC FOR INFLUENZA HEMAGGLUTININ SYNTHETIC PEPTIDES, David D. Eckels and Jonathan R. Lamb, Georgetown University, Washington, D.C. 20007 and University College Hospital, London, England.

Human T-lymphocyte clones (TLC's) were generated against the hemagglutinin (HA) molecules purified from influenza virus A/Texas/1/77. As previously described (Lamb et al. Nature 300:66, 1982) synthethic HA peptides were used to probe the antigenic fine specificity of HA-specific TLC's. Three clones recognized peptide sequences corresponding to amino acids 306-330 (HA1.4, HA1.7 and HA2.43). One TLC failed to respond in the presence any synthetic peptide (HA1.9) although it did recognize A/Texas and A/Japan/305/57. The HA1.4, HA1.7 and HA2.43 TLC's all responded to purified HA from A/Bangkok/1/79 while HA1.9 did not. Thus, the four TLC's recognized three distinct HA determinants with HA1.4 and HA1.7 apparently recognizing the same antigenic determinant. In genetic restriction studies, three distinct restriction patterns were obtained corresponding to the HA determinant recognized: HA1.4 and HA1.7 were restricted identically while HA1.9 and HA2.43 exhibited two completely different patterns of response on a panel of unrelated presenting cells. The fine specificity of TLC genetic restriction was investigated with monoclonal antibodies specific for human Class II antigens; the blocking patterns obtained with these reagents were consistent with the above interpretation. We are currently assessing the possibility that different Class II epitopes might restrict responses to the same antigenic determinant, the frequency of such observations and the effect of differing genetic backgrounds on the specificity of TLC activation.

0257 GENETIC CONTROL OF THE T CELL RESPONSE TO PEPTIDES OF THE GLYCOPROTEIN D(gD) OF HERPES SIMPLEX VIRUS (HSV). Ellen Heber-Katz and Bernhard Dietzschold, The Wistar Institute, Philadelphia, PA 19104

The glycoprotein D of HSV is thought to induce a major part of the immune response which results in protection from a lethal HSV infection in mice. An immunodominant part of this molecule has been mapped to the NH_2 terminus by the use of monoclonal antibodies; peptides have been synthesized which confirm this mapping. Since the use of these peptides as a potential vaccine has been seriously considered and since the protective response to HSV is largely a T cell mediated phenomenon, we began to examine the genetic control of the T cell response to these small peptides of 23 amino acids or less in length.

Using a proliferation assay with various H-2 congenics immunized with the 1-23 peptid and restimulated with the same peptide or smaller peptides, we found different patterns of responsiveness which mapped several immunodominant regions of the 1-23 HSV peptide. The surprising result was seen at the clonal level when we tested for degeneracy in antigen presentation. Thus, all T cell hybrids tested from the B10.A(5R) were able to respond to antigen on B10.A antigen-presenting cells (APC) and most of the B10.A hybrids could respond to antigen on B10.A(5R) APC. Such a result is unusual in terms of previous reports of T cell responsiveness but curiously resembles the T cell response to cytochrome c. We are currently examining the fine specificity of this T cell response as determined by the APC and attempting to map regions of the molecule in terms of T cell and APC sites and are looking for molecular and functional similarities between HSV gD peptides and cytochrome c.

0258 CONFORMATIONAL FLEXIBILITY IN A CLASS I TRANSPLANTATION ANTIGEN. Jonathan C. Howard, Geoffrey W. Butcher, Austin G. Diamond and Alexandra M. Livingstone, ARC Institute of Animal Physiology, Babraham, Cambridge CB2 4AT, U.K.

The surface antigenic structure of the strong rat Class I MHC antigen $RT1A^a$ has been defined by 333 monoclonal antibodies. Nine alloantigenic determinants form a continuous overlapping surface. Antibodies against two of these determinants, called T and X, engage in a positive synergistic interaction: greatly enhanced affinity of binding of one antibody occurs in the presence of the other. The effect is mediated by monovalent Fab fragments as efficiently as by whole antibody molecules, suggesting strongly that a conformational change is involved. Several different pairs of antibodies directed against these two sites appear to engage in synergistic effects, suggesting that these sites represent conformationally labile parts of the molecule.

In a superficially unrelated series of experiments we have shown that a second MHC-linked locus mapping outside RT1A can influence the antigenic structure of $RT1A^a$ in trans. The immunological effects of this modification are as striking at the T cell level as those associated with the "mutations" of $H\text{-}2K^b$. A very small number of monoclonal antibodies can detect this structural modification and they are all directed at the T and X sites. We propose that the T and X sites represent regions of flexibility in the Class I molecule probably associated with sites of glycosylation. In view of the importance of these sites for T cell specificity, we suggest that conformational flexibility is related to the ability of Class I molecules to interact functionally with other molecules in the cell membrane.

0259 T-CELL FINE-SPECIFICITY TOWARDS INFLUENZA HEMAGGLUTININ. Julia L. Hurwitz, Charles J. Hackett, Fritz Melchers, Bernard Dietzschold and Walter Gerhard, Wistar Institute, Philadelphia, PA 19104

Seven T-cell hybridomas and one long-term line from PR8-primed Balb/c mice were analyzed for fine-specificity towards influenza hemagglutinin (HA). Each cell was individually tested for recognition of 51 viruses with known single amino acid substitutions from the PR8 HA. The results revealed three reactivity patterns (R.P.) indicating three sites on the HA molecule preferentially recognized by this group of T-cells. Each R.P. derived from more than one T-cell and more than one mouse. T-cells with the first R.P. were all shown to recognize an 11 amino acid (a.a.) synthetic peptide representing a.a. 109-119 from PR8 HA. The T-cells described here were next compared to a group of more than 200 B-cell hybridomas, also derived from PR8 primed mice. The three R.P.'s for T-cell clones were not seen for a single HA-specific B-cell clone. A difference in T and B-cell recognition of HA may best be explained by considering that T-cells see virus in conjunction with Ia antigens while B-cells see native virus. The processing of HA required for presentation with Ia antigens likely reveals determinants to T-cells which differ in sequence, size, and three-dimensional structure from determinants shown to B-cells on the native molecule.

0260 BIOCHEMICAL CHARACTERIZATION OF THE FUNCTIONAL INTERACTIONS REQUIRED FOR I REGION RESTRICTED, ALLO AND ANTIGEN SPECIFIC T CELL ACTIVATION, David J. McKean, Barbara N. Beck, Margo Stich and Barry Handwerger, Mayo Clinic, Rochester, MN 55905

It is generally believed that T helper/inducer cells are activated by the interaction of allo Ia molecules or antigen plus self Ia molecules and the T cell receptor. Results from other laboratories have indicated that extensive glycosylation of splenocyte Ia antigens may have a negative effect on T cell activation. We have examined the levels of glycosylation on cloned stimulator/antigen presenting cells and the potential role of this glycosylation on T cell activation. A number of different B lymphoma and macrophage cell lines with different levels of Ia glycosylation have been treated with tunicamycin or neuraminidase and tested for their ability to activate a panel of allo and antigen reactive T cells. Our results demonstrate that the level of membrane glycosylation has significant effects only on certain T cells. Neuraminidase treatment of the stimulator/APC significantly increases the activation of some but not all allo- and antigen-reactive T cells. Tunicamycin treatment of the stimulator/APC does not alter the expression of Ia on the surface of the stimulator/APC but does completely inhibit the ability of these cells to activate T cells. Our results also indicate that non-MHC encoded products on the stimulator/APC may have significant effects on T cell activation.

0261 COMPARISON OF T CELL STIMULATION BY CELL- OR LIPOSOME-ASSOCIATED CLASS I ALLO-ANTIGENS, Anne-Marie Schmitt-Verhulst, Françoise Albert, Claude Boyer, Christine Hua and Claire Langlet, Centre d'Immunologie INSERM-CNRS de Marseille-Luminy, Case 906, 13288 Marseille cédex 09, France.

Differences were observed between T cells stimulated with the cell-associated or with the liposome-associated H-2K^b molecule. H-2K^b-specific alloreactive T cell lines and clones maintained in long term cultures with cell-associated H-2K^b all expressed Lyt-2 and were L3T4 negative. They were dependent on the expression of H-2K^b, but not of Ia on the stimulating cells, in addition to exogenous interleukins (Il-1 or Il-2 dependent on a given T cell line) for their stimulation to growth. Their H-2K^b-induced proliferation was inhibited by anti-H-2K^b or by anti-Lyt-2 monoclonal antibodies (mAb), but not by anti-Ia or anti-L3T4 mAb. For most T cell clones tested the integrity of both the first and the second N-terminal domains of the H-2K^b seemed required for "recognition" by T cells. These T cell clones could not be stimulated with immunopurified H-2K^b inserted in liposomes even in the presence of exogenous interleukins. T cell lines initiated in vitro with H-2K^b-liposomes could proliferate in response to H-2K^b-liposomes and feeder cells. Such T cell lines contained T cells bearing the Lyt-2 marker as well as cells bearing the L3T4 marker and their H-2K^b-induced proliferation was inhibited by anti-H-2K^b or by anti-Ia, as well as by anti-Lyt-2 or anti-L3T4 mAb. Characterization of these cells at the clonal level and analyses of the H-2K^b epitopes involved in recognition by these T cells are the under investigation.

0262 PRESENTATION BY FIXED ACCESSORY CELLS OF OVALBUMIN PEPTIDES THAT SUBSTITUTE FOR PROCESSED ANTIGEN TO SPECIFIC T HELPER HYBRIDOMAS. Richard Shimonkevitz, Howard Grey, Philippa Marrack and John Kappler. National Jewish Hospital and Research Center, and the University of Colorado Health Sciences Center, Denver, Colorado 80206.

Recently, we obtained direct evidence that proteolysis is not only necessary but may be sufficient to explain the requirement for an antigen processing step in accessory cells. The experiments that led to that conclusion demonstrated that glutaraldehyde pre-fixed, Ia-positive B lymphoma cells were capable of presenting ovalbumin peptides in an MHC-restricted fashion to specific T hybridomas. In contrast, the intact protein antigen was not presented by pre-fixed cells but could only stimulate the T cells if it were incubated for a suitable period of time in the presence of viable, metabolically-active accessory cells (Shimonkevitz, et al. 1983. J. Exp. Med. 158:303). Our continuing work in this system has led to the isolation by HPLC of a tryptic ovalbumin peptide that is presented by pre-fixed accessory cells to several ovalbumin-specific, I-A-restricted T hybridomas. Knowledge of the amino acid sequence of the P323-339 peptide has allowed us to perform several experiments addressing the manner in which antigen is presented to specific, MHC-restricted T cells. Our experiments show that a) the P323-339 peptide appears to bind with high affinity to fixed accessory cells, b) Ia molecules do not contribute to this high affinity binding, c) a V8 protease-truncated peptide fragment, P323-336, which is stimulatory for 3 of 4 P323-339-specific T hybridomas cannot inhibit accessory cell presentation of the intact P323-339 peptide to the fragment non-reactive T hybridoma, and d) there is no demonstrable affinity for peptide alone by the MHC-restricted antigen receptors expressed by the P323-339-reactive T hybridomas.

0263 DIFFERENTIAL PROCESSING REQUIREMENTS OF NATIVE AND DENATURED PROTEIN AND PEPTIDE FRAGMENT FOR PRESENTATION TO THE SAME T CELL CLONE, Howard Z. Streicher, Ira J. Berkower and Jay A. Berzofsky, Metabolism Branch, NCI, NIH, Bethesda, MD 20205

We have studied the differential requirements for presentation of a single epitope of sperm whale myoglobin to a clone of proliferating T cells when this epitope is expressed on the native protein, 2 denatured forms of the intact protein, or a small antigenic peptide fragment. The B10.D2 T cell clone used, 14.5, was demonstrated to be I-E^d restricted, specific for an epitope centering on Lysine 140, and as responsive to the CNBr fragment 132-153 as to the intact protein. Previous studies had suggested that inhibition of antigen presentation by lysosomatropic agents depended on the size of the antigen. We have found 1) that conditions of chloroquine pre-treatment of splenic presenting cells can be found which completely abrogate the ability to stimulate T cells with native myoglobin but not with the 22-residue fragment; 2) that similar results were found with other agents including the cationic ionophore monensin; but 3) that an unfolded form of myoglobin, produced by methylating the methionine residues to introduce charged groups into the interior of the molecule, behaved like the small peptide fragment even though it is the same size as the native protein; and 4) that apomyoglobin, which is less denatured than S-methyl myoglobin, was partially-inhibited but less so than the native protein. Therefore, 1) the small peptide fragment does not require the same processing as native protein, but 2) conformational change may be as important as size in determining requirements for processing. On the one hand, denaturation may increase susceptibility to proteolysis, but on the other hand, proteolysis of the native protein may merely be one method of inducing denaturation.

0264 SPECIFIC SUPPRESSION OF CLONED T CELL PROLIFERATION BY A RELATED ANTIGEN. Gen Suzuki and Ronald H. Schwartz, NIAID, NIH, Bethesda, MD 20205

B10 and B10.A(3R) mice were originally defined as low responders to the antigen pigeon cytochrome c fragment 81-104, to which the high responder mice, B10.A and 9R, responded well. But it turned out that the LN T cells of B10 or 3R mice proliferated well in response to fragment 1-65, but poorly to whole molecule (WM) although the mice were primed with WM. Interestinglythe proliferation of the LN T cells to fragment 1-65 was suppressed by adding small doses of WM into the culture. The suppression mediated by WM did not require other cell types than proliferation T cells and APC, since the proliferation of T cell clones specific for fragment 1-65 was also suppressed by WM. Some cytochromes c non-stimulatory to the clone, such as human or turtle also effectively suppressed the specific proliferation of the clone. Using one T cell clone (3R.3.11) specific for fragment 1-65 and I-A^b molecule in the H-2 complex, the antigenic epitope (T cell recognizing part) and the agretope (Ia association part) on the fragment 1-65 will be characterized. The suppression mechanism mediated by the low or non-stimulatory antigens will be discussed in relation to the epitope and agretope.

0265 MACROPHAGE PROCESSING OF PEPTIDES: FORMATION OF AN ANTIGENIC COMPLEX, David W. Thomas, Michael D. Hoffman, Maxine Solvay, Mary Spangler, and Roderick Nairn, The University of Michigan, Ann Arbor, Michigan 48109.

To examine macrophage processing of antigen for presentation to T cells, we have determined the mode of uptake and intracellular fate of the octapeptide angiotensin II (AII) by guinea pig macrophages. The cellular uptake of ^{3}H-AII was specific and could be inhibited by non-radiolabelled AII and some AII analogues. The intracellular fate of ^{3}H-AII was determined by solubilization of ^{3}H-AII-pulsed macrophages and resolution by passage over Sephadex G25 columns. Intracellular ^{3}H-AII was contained in four major fractions: peak A-a complex showing an Mr ~50,000; peak B-fragments containing 7-8 AII amino acid residues; peak B_1-containing 5-6 residues; and peak C-containing 4-5 residues. Upon prolonged incubation, peaks B and C were released from the cells while most of peak A and a small amount of peak B_1 were retained. The formation of peak A with ^{3}H-AII could be inhibited by inclusion of nonradiolabelled AII in the incubation mixture and could also be formed by the isolated peaks B and B_1, but not by peak C. Since after overnight culture all of the macrophage-associated AII was in either peak A or B_1, the antigenicity of these peaks was determined by incubation with AII-immune T cells. Peak A stimulated AII-immune T cell proliferation, whereas peak B_1 showed no antigenicity. Taken together, these results suggest that much of AII enters macrophages via a relatively specific process; once in the cell, some AII and its catabolic products are complexed with larger macrophage structures and other products are released from the cell. The complexed form of AII is retained by the macrophages and provides a form of the peptide that is antigenic for T cells.

0266 ANTIGEN RECOGNITION BY Ia MOLECULES, Harley Y. Tse, Sachio Kanamori, William D. Walsh, and Ted H. Hansen, Dept. of Immunology, Merck Sharp & Dohme Research Laboratories, Rahway, NJ 07065 and Dept. of Genetics, Washington University, St. Louis, MO 63110

The idea that the Ia molecule on antigen presenting cells (APC) had antigen binding specificities had not gained popular acceptance since Ia is not clonally distributed. Recent experiments, however, suggest that Ia can be polyspecific in antigen recognition. To explore the possible relationship between Ia and antigen recognition, we prepared antigen specific T cell clones from B6 and the mutant strain bm12. Antigens were presented to these clones on either B6 or bm12 APC. Our experiments showed that the T cell clones could be divided into two groups, one that recognized antigens in association with either B6 or bm12 APC and the other only recognized syngeneic APC. The frequency of clones in each group depended on the antigen used. These experiments suggest that specific Ia epitopes are used to associate with antigenic determinants. Degeneracy exists in the system in that one Ia epitope can associate with more than one antigenic determinant. It is also true that for complex antigens such as PPD more than one Ia epitopes may be involved. We next compared the genetic requirements of these T cell clones in their interactions with macrophages and with B cells. T-MØ interactions were IA restricted for both B6 and bm12 T cell clones as expected. T-B interactions were IA restricted for B6 clones but not for bm12 clones. This raises the possibility that the recognition of Ia by T cells on macrophages and on B cells are genetically different.

Normal and Defective Development (or Interactions) of T Cells and the Role of the Thymus

0267 IMMUNE RESPONSES OF MHC- MISMATCHED EMBRYONIC CHIMERAS IN XENOPUS, Nicholas Cohen, Martin Flajnik, and Louis Du Pasquier, University of Rochester, Rochester, NY

Embryonic head-body chimeras were made in Xenopus at 24 hours post-fertilization, so that the anterior region contained the thymic anlagen and the posterior contained all hematopoietic stem cells. The posterior origin of all lymphocytes in the thymus and spleen was confirmed using a ploidy marker and analyzing lymphocytes for their DNA content with the FACS. We asked whether T cells that had traversed an allogeneic thymus would be educated with cells of their own genotype in vivo to produce an IgG antibody response. The extent of IgG production depended largely on the MHC alleles involved. Although not absolute, a certain degree of restriction was found in all combinations suggesting that thymus education exists in Xenopus. In other experiments, MHC-mismatched chimeras were grafted with skin that was identical to the anterior region at the MHC, but which differed at other histocompatibility loci. Controls in this case were embryos grafted embryonically with an eye to render them tolerant to the allogeneic MHC haplotypes; in this case, both the thymus and stem cells are of the same haplotype. In preliminary experiments, head-body chimeras rejected grafts more quickly than animals made tolerant with the embryonic eye. This result suggests that the cells involved in graft rejection learn to recognize minor histocompatibility antigens in the context of the MHC of the thymus. Experiments are in progess to determine whether suppressor cells are involved in the chronic graft rejection by the animals made tolerant with the embryonic eye.

0268 COMPARISON OF CLASS I AND CLASS II RESTRICTED T CELL PRECURSORS IN ATHYMIC NUDE MICE: A.M. Kruisbeek, M.L. Davis, D.L. Longo, MB, NIH, Bethesda, MD 20205

In nude mice grafted with an allogeneic thymus, splenic CTL with self-recognition specificity for both thymic and extra-thymic (i.e., nude host) MHC determinants are present (1). In vitro generation of CTL specific for nude host class I determinants is dependent on addition of an exogenous source of IL-2, while generation of thymic-MHC restricted CTL is not. It was inferred from these findings that, although nude mice have developed CTL precursors without any thymic influence, they failed to develop precursor T cells specific for class II Ia determinants. The present study directly tests this hypothesis. Chimeras were constructed of irradiated normal parental $H\text{-}2^b$ or $H\text{-}2^d$ mice that received a mixture of $(H2^b \times H\text{-}2^d)$ F_1 nude mouse bone marrow and spleen cells. The donor inoculation was not treated with anti-Thy 1-plus-C, so that any potential precursor T cells are allowed to differentiate in the normal parental recipient. Chimeras were immunized at 8-12 weeks after reconstitution with several protein antigens in CFA in the footpads, and their purified LN T cells tested 1 week later for ability to recognize Ag on APC of either parental haplotype. Also, their splenic and LN CTL responses were tested for ability to recognize TNP on stimulator cells of either parental haplotype. The results indicated that T cell proliferative responses were solely restricted to self-recognizing parental host class II Ia determinants. CTL responses, on the other hand, were generated (in the presence of IL2) with TNP-modified stimulator cells of either parental haplotype. Thus, this study demonstrates that in nude mice self-MHC class I restricted CTL precursors can develop extra-thymically, but class II Ia-restricted T cells are strictly dependent on intra-thymic differentiation. Ref: (1) J.Immunol. 127:2168, 1981.

0269 FUNCTIONAL DIFFERENTIATION OF EARLY EMBRYONIC CELLS OF THE MOUSE YOLK SAC AND OMENTUM, Robert Auerbach, Loya Abel, Louis Kubai, Lawrence W. Morrissey and Amiela Globerson, University of Wisconsin, Madison, WI 53706 and Weizmann Institute of Science, Rehovoth, Israel.

We have previously established that yolk sac cells obtained from early mouse embryos can carry out some of the effector cell functions typically associated with the immune system. Our current work includes analysis of yolk sac cells maintained in vitro to establish long-term cell lines, and comprises both a characterization by flow cytometric analysis of cell surface antigens and a functional analysis of cultured cells as well as sorted subpopulations.

We have continued our studies of the development of prelymphoid stem cells found in the preomental region of the mouse embryo. Our present work includes study of long-term cultures obtained from this region and includes both flow cytometric characterization of cell surface antigens and in vitro tests of immunological effector cell functions.

Work in progress on yolk sac cells which were hybridized by fusion to T-cell lymphomas as well as on the development and analysis of monoclonal antibodies generated against the long-term omentum and yolk sac lines will be discussed.

0270 IL2 PRODUCTION BY MURINE THYMOCYTES: ANALYSIS AT THE CELLULAR AND MOLECULAR LEVEL, Barry Caplan, Rochelle Sailor and Ellen Rothenberg, California Institute of Technology, Pasadena, CA 91125

We have investigated the ability of murine thymocytes to produce the T cell growth factor interleukin-2 (IL2). An important parameter in the mitogen-induced production of IL2 by thymocytes is their costimulation with the phorbol ester TPA. In the presence of TPA, IL2 production by thymocytes is relatively independent of adherent accessory cells. This allows us to compare directly the ability of different subpopulations of thymocytes to produce IL2. Surprisingly, IL2-producing thymocytes are not restricted to populations of small post-mitotic cells. In fact, they are enriched in populations of large, proliferating lymphoblasts. That these IL2-producers are endogenous to the thymus is suggested by our observation that IL2-producing thymocytes are detected in mice as young as four hours old. We have also shown that both Lyt 2^- and Lyt^+ thymocytes can secrete IL2. Recently, we have begun an analysis of the molecular biology of IL2 secretion. Using three synthetic oligonucleotides based on the human IL2 cDNA clone, we can demonstrate specific cross-hybridization to mouse IL2 mRNA. These probes can therefore be used to isolate a mouse IL2 cDNA clone, and subsequently to analyze the expression of IL2 mRNA in different populations of lymphocytes.

0271 ROLE OF PURINE DEOXYNUCLEOSIDE METABOLISM IN T-LYMPHOCYTE DIFFERENTIATION, Amos Cohen, Jerzy Barankiewicz, Howard M. Lederman and Erwin W. Gelfand, Division of Immunology, Research Institute, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario, Canada M5G 1X8.

The association of inborn deficiencies of the purine enzymes adenosine deaminase and purine nucleoside phosphorylase with immunodeficiency indicates a unique role for purine deoxynucleoside metabolism in T lymphocyte development. We have studied the alternative pathways of deoxynucleotide biosynthesis and their regulation in G_1 and S phase human thymocyte subpopulations and compared them with those of differentiated T lymphocytes from peripheral blood. The results of this study can be summarized in a model describing the regulation of deoxynucleotide metabolism in thymocytes. According to this model the regulation of deoxynucleoside triphosphate (dNTP) synthesis in S phase thymocytes is different from that of other cells, the main difference is the relative importance of deoxynucleoside salvage in thymocytes. Thus, in S phase thymocytes pyrimidine dNTP are synthesized primarily by deoxynucleoside salvage whereas purine dNTP are synthesized by ribonucleotide reduction.

These observations may have important implications for the roles of deoxynucleosides in intrathymic T-cell differentiation. They imply that the supply of extracellular pyrimidine deoxynucleosides may enhance DNA synthesis whereas extracellular purine deoxynucleosides are not used for DNA synthesis and are cytotoxic to intrathymic T lymphocytes. Thus, the balance between extracellular purine and pyrimidine deoxynucleosides and the enzymatic levels of activities may determine the fate of intrathymic T cells, and may play a role in clonal selection of T cells. (Cohen, A., et al., (1983). J. Biol. Chem. In press).

0272 TRANSPLANTATION OF LYMPHOID FREE THYMIC EPITHELIUM INTO SYNGENEIC AND ALLOGENENIC IMMUNODEFICIENT HOSTS. D.A. Crouse, G.A. Perry and R.K. Jordan*. Univ. of Nebr. Med. Center, Omaha, NE 68105, and *Univ. of Newcastle-upon-Tyne, Newcastle, England.
Purified thymic epithelium was obtained from low temperature organ culture (LTOC) of fetal thymus from C57Bl/6 and CBA mice as well as their F_1 hybrid. Briefly, thymic lobes from 14d gestational age mice were organ cultured on Nuclepore rafts for 7d at 24°C, and a further 7d at 37°C in a fully humidified atmosphere of 5% CO_2 in air. Such epithelial organ cultures were shown by morphological and marker analysis to be free of lymphoid and Ia^+ adherent cells and were utilized in syngeneic, allogeneic and semiallogeneic thymus transplantation studies with adult thymectomized, lethally irradiated (850 r), fetal liver reconstituted (16d gestation) immunodeficient hosts. At 30d post-grafting : 1) Both CBA and B6CBA F1 LTOC derived lobes were accepted by the C57Bl/6 ATxFL hosts, 2) Although showing significant repopulation in all strain combinations, the allo- and semiallogeneic grafts did not reach the syngeneic control graft size or cellularity, 3) Morophological development of all grafts was not unlike control thymus, 4) Flow cytometric analysis of the intra-graft cells showed a return to relatively normal distributions of thymocyte surface marker profiles and that the repopulating cells were of host origin, 5) Peripheral reconstitution as evaluated by surface marker profiles or functional analysis was not different from the negative ATxFL controls even in the syngeneic grafts. The paradox of intra-graft repopulation without subsequent functional or marker peripheralization to the spleen was speculated to be related to possible effects of LTOC on the endodermally derived component of thymic epithelium while sparing the ectodermally derived epithelial population. (supported by NIH AI15819)

0273 ISOLATION OF A MURINE THYMOCYTE POPULATION WITH CHARACTERISTICS OF AN EARLY PRECURSOR, B. J. Fowlkes, Linette Edison, Thomas M. Chused and Bonnie J. Mathieson, LMI, NIAID, National Institutes of Health, Bethesda, MD 20205
Using many combinations of monoclonal antibodies to lymphocyte surface antigens, we have identified by two-color flow cytometry (FC) a population of murine thymocytes which is Ly1 dull, $Ly2^-$, $T1^-$, H-2 bright, Thy-1 bright and intermediate in PNA binding. This population (designated dLy1) constitutes <5% of adult murine thymus. dLy1 cells have the morphology of immature or dividing cells and contain the largest cells in the thymus. The population is enriched early in repopulated thymus grafts and after treatment with steroids in vivo . Cell cycle analyses reveals a high number of cells in $S+G_2+M$ phases when compared to medullary thymocytes or peripheral T cells. dLy1 cells are lymphoid since they express Ly9, T200, and Ly1. The population can be distinguished from peripheral T cells by its quantity of binding with antibodies: M1/69, 4B9, ThB, Thy1, T200 and Ly1. Two-color FC indicates that these cells have a phenotype identical to the predominant cell type found in day 16 fetal thymus. Isolation of dLy1 has been accomplished by a combination of techniques: (1) enrichment on antibody-coated plates; (2) sequential kills with cytotoxic monoclonal antibodies, and (3) electronic cell sorting. The dLy1 population has been examined for differentiation in vitro and in vivo . Transfer of the isolated population into irradiated congenic mice reveals that these cells are able to home to the thymus. Kinetic studies of the phenotype of repopulated thymuses are in progress. The results suggest that the population may represent an early precursor thymocyte, possibly the earliest T cell, thus far, described. Results will be discussed with respect to lineage relationships and models of thymocyte differentiation.

0274 T-CELL LYMPHOMAS PRODUCE THEIR OWN GROWTH FACTOR, E. Hays, C. Streifinger, D. Goodrum and C. Uittenbogaart.
We report studies of the SL12 T lymphoma from the AKR mouse in which we demonstrate that the cells can grow in a defined medium and produce a factor which stimulates their growth. SL12 is a cell line derived from a spontaneous lymphoma of an AKR mouse. It grows continuously in suspension culture with RPMI medium and 5% FBS. We have tested the line and several cloned lines derived from it in a defined medium (AT-IMDM) containing nutrients plus transferrin and delipidated serum albumin (1). The cells grow exponentially when seeded at 10^5 cells per ml and can be maintained indefinitely in this medium by transferring the cells when they reach confluence. We found that cells seeded at 10^4 per ml do not replicate and decrease in numbers during the first 100 hrs of culture. However, medium harvested from growth phase cultures when added at 20-50% concentration to cultures seeded at 10^4 cells produces consistent exponential growth of the cells. These findings have been repeated in all AKR T lymphoma cell lines which have been tested.
The conditioned medium with growth factor activity of the T lymphoma cell lines does not contain IL-2. It is stable for many weeks at 4°. We have also found that conditioned medium from the mouse myelomonocytic leukemia cell line WEHI-3 which was adapted to the AT-IMDM medium has inhibitory activity on growth of the T lymphoma cells in the serum-free medium. We conclude that AKR T lymphoma cells produce factor(s) which stimulate their own growth. This phenomenon may play an important role in maintaining the malignant phenotype of these lymphoid cells. (1) In Vitro 19:67-72, 1983.

0275 T CELL HYBRIDOMAS AS MODELS FOR INDUCTION AND FUNCTIONAL MATURATION OF CYTOTOXIC T LYMPHOCYTES (CTL). Osami Kanagawa and Jacques Chiller, Lilly Research Laboratories and Scripps Clinic and Res. Fndn., La Jolla, CA 92037.

Functionally inducible CTL hybridomas were constructed by fusing allo-antigen specific T cells (C57Bl/6 α DBA/2) with cells from the rat thymoma line W/FU (C58NT)D. A cloned line was obtained (KSH.4.13.6) which was specifically cytotoxic in the presence of Con A activated rat spleen cell supernatant fluid (rat Con A SN) but which lost activity when transferred to normal medium. However, cytolytic activity could be reinduced by culturing KSH.4.13.6 cells in either rat Con A SN or secondary mixed leukocyte culture SN. Using various sources of SN, it was found that cytolytic induction required two different factors. PMA induced EL-4 SN and SN from antigen activated cloned T cells, neither of which were capable of inducing cytolytic activity alone, were able to synergize in the cytolytic induction of the KSH.4.13.6 hybrid cell line. γ-IFN and IL-1 failed to induce cytolytic activity even in the presence of EL4 SN. Furthermore, this hybridoma was able to produce macrophage activating factor (MAF) when cultured in rat Con A SN but not when exposed to either mitogens or specific antigen. Finally, EL-4 SN which by itself was incapable of inducing cytolytic activity, was able to induce MAF production by KSH.4.13.6 cells. These results suggest that cytolytic induction and MAF producing activity in this cloned hybridoma cell line are regulated by different mechanisms. Such a functionally inducible T cell hybrid may provide a tool for biochemical and molecular analysis of T cell function and regulation.

0276 EXPERIMENTALLY INDUCED T-CELL IMMUNODEFICIENCY ASSOCIATED WITH DECREASED THYMOCYTE RESPONSES TO IL-1 AND MITOGENS, Wayne S. Lapp, Mariza L. Mendes and Thomas A. Seemayer, McGill University, Montreal, Canada.

Previous studies from this laboratory have demonstrated that mice experiencing a graft-versus-host (GVH) reaction, induced across all or part of the H-2 complex, display T-cell immunodeficiency which is associated with an injury to thymic medullary epithelial cells and an arrest of T-cell maturation. Experiments were performed to further characterize the effect of GVH-induced thymic lesions on thymocyte function. Thymocyte function was assessed by in vitro proliferative responses to both IL-1 and the T-cell mitogens PHA and ConA. Thymocytes were fractionated by the use of peanut agglutinin (PNA) into PNA+ve and PNA-ve populations. In addition, thymic epithelial cell supernatants (TES), obtained from normal thymic cultures, were tested for their ability to restore depressed proliferative responses. The results demonstrated that GVH thymocyte populations (obtained 40-50 days after GVH induction) displayed depressed IL-1, and ConA responses in the whole thymocyte preparation and depressed IL-1, ConA and PHA in the PNA-ve fraction. Preincubation of GVH thymocytes in TES enhanced both the ConA and PHA responses but not the IL-1 proliferative response. These results suggest that at least two intrathymic maturation processes are impaired by the GVH-induced thymic injury and that IL-1 induced proliferation may be a useful method to assess normal thymus function.

This work was supported by grants from the MRC and NCI of Canada.

0277 ANALYSIS OF THYMOCYTE DIFFERENTIATION IN SUBPOPULATIONS OF GRAFTED THYMUS, Bonnie J. Mathieson, William M. Leiserson, B.J. Fowlkes, Thomas M. Chused, and Linette Edison, National Cancer Institute, FCRF, Frederick, MD 20217, NIAID, Bethesda, MD 20205, and the Basel Institute for Immunology, Basel, Switzerland

The genesis of thymic lymphoid cells in fetal ontogeny involves large precursor cells with a low level of expression of Lyl antigen before Ly2 antigen is expressed. We have analyzed the regeneration of thymic lymphocytes in young mice engrafted with newborn thymic lobes. An allelic Ly antigenic difference enabled us to monitor both newly generated and older, surviving populations that are replaced with time. In monitoring repopulation of thymus grafts by multiparameter flow cytometry, we have identified 3 basic subsets of murine thymus cells that are defined by presence or absence of Ly2 antigen, quantitative differences in cell surface expression of Lyl antigen, and differences in cell size. We have observed that an Lyl$^{\downarrow}$ (dull), large-sized cell, similar to the fetal "precursor" cell in its phenotype, has a higher than normal proportional representation in the early stages of graft repopulation. This population is reduced in proportion, relative to ungrafted normal thymocytes, in the chronologically older, graft-derived thymus cells and there is an increased proportion of older cells with a more "mature" phenotype for Lyl expression (i.e. Lyl$^{\uparrow}$, bright). The proportion of Lyl+,2+ cells initially is highly reduced in the regenerating population. From the kinetics of the repopulation, the Lyl$^{\downarrow}$ population appears to be the precursor for the Lyl+,2+ cells, as expected from fetal development, and may also be the precursor for the Lyl$^{\uparrow}$ cells. However all 3 subsets were detected when only 3% of the engrafted thymocytes were of host type.

0278 PHAGOCYTIC CELLS OF THE THYMIC RETICULUM : CULTURE, CHARACTERIZATION, FUNCTION AND PARTICIPATION IN THE CONSTITUTION OF THE THYMIC MICRO-ENVIRONMENT, Martine Papiernik, INSERM U 25, Hôpital Necker - 75015 Paris, France.

The non epithelial compartment of the thymic reticulum is an important component of the micro-environment in which thymic lymphocytes develop. We have cultured phagocytic cells of the thymic reticulum (P-TR), which grow on the surface of complex thymic stroma monolayers. From a morphological viewpoint these cells are closer to the interdigitating cells than to the common macrophages. In their mature state, most P-TR are Fc^+ and phagocytic, Ia^+ and $Mac\text{-}1^+$. They display very close contacts with thymic lymphocytes and especially with the cortical type with which they form rosettes. P-TR are able to stimulate the proliferation of thymic lymphocytes in syngeneic co-cultures, and the presence of mature thymic lymphocytes together with immature cortical ones is necessary for this syngeneic stimulation. P-TR are able to produce interleukin 1 (Il-1) together with prostaglandins (PGs) and especially PGE2, which have a well known inhibitory effect on lymphocyte proliferation. Il-1 is revealed by its co-mitogenic effect in the response of thymocytes to Con A. This effect can only be shown if the negative signal given by PGE2 is abrogated, either by inhibiting its production (with indomethacin), or by dyalisis before testing. P-TR which has close contacts with thymic lymphocytes, seems to control their activation by secreting factors which give both positive (Il-1) and negative (PGs) signals.

0279 ONTOGENY OF IMMUNITY IN LARVAL FROGS: DEVELOPMENT OF IN VITRO CON A AND PHA RESPONSIVENESS IN THE SPLEEN PRECEDES RESPONSIVENESS IN THE THYMUS, L. Rollins-Smith, S.C.V. Parsons, and N. Cohen, University of Rochester, Rochester, NY 14642

The in vitro responses of splenocytes and thymocytes from Xenopus laevis-gilli (hybrid clone LG-15) to the T-cell mitogens Con A and PHA were examined at specific stages of larval development and at 2 months postmetamorphosis. The responses of splenic lymphocytes to each mitogen were significant at all stages. Stage-related differences in responses of splencoytes to both mitogens suggest two waves of emergence of proliferative activity divided by a period of diminished responsiveness during the metamorphic crisis. In contrast to splenocyte responses, responses of thymocytes to both mitogens were barely detectable, observed only when thymocytes were cultured at relatively high density (5×10^5 cells/ml), and not improved by increased or decreased concentrations of mitogen or by increased concentrations of fetal calf serum. These findings suggest that PHA- and Con A-reactive cells are present in relatively low numbers in the thymus at all stages of larval development. Early mitogen responsiveness in the spleen at a time when the thymus is unresponsive is contradictory to the pattern observed in mammalian development in which thymocytes become responsive to mitogens in fetal stages and splenocytes become responsive only at birth. The thymocyte inactivity in larval frogs may be necessary to provide a population of cells that can become tolerant to neo-self-antigens that arise during and after metamorphosis. Thus, the larval amphibian thymus may provide a model to study the early events of thymocyte "education" and differentiation in a time framework broader than that possible with fetal mammals.

0280 FUNCTIONAL CAPACITY OF SUBSETS OF THYMOCYTES
Ken Shortman, Roland Scollay, Wei-Feng Chen and Paul Andrews,
The Walter & Eliza Hall Institute of Medical Research, Melbourne, Australia

Multiparameter flow cytometric analysis of thymocytes on the basis of cortical versus medullary markers, on the basis of size and cycle status, and on the basis of functional lineage markers (Ly2 and L3T4) leads to a division into eight distinct subsets. Their function has been assessed in high cloning efficiency non-specific limit-dilution assays for all T cells capable of growth (PTL-p) and for all precursors of cytolytic clones (CTL-p). Cortical type thymocytes seem devoid of function, even in the presence of growth factors. Thymus hormones, four distinct purified preparations, all failed to induce cortical thymocytes to function. In contrast, 70% of all medullary thymocytes are functional. Numerically minor but distinct subsets of blasts with immature features can be identified in both the cortex and the medulla. Those in the medulla appear to be functional. Recent thymus migrants have been identified and isolated using the intra-thymic FITC-injection technique. They are of medullary phenotype and are functional. The pathway of intrathymic T cell development will be considered in terms of these subsets.

0281 LEUKEMIA DERIVED GROWTH FACTOR(S) (LDGF), C.H. Uittenbogaart and J.L. Fahey, UCLA School of Medicine, Los Angeles, California 90024.

In previous work we have shown that the human malignant T leukemia cell line MOLT-4f produces a growth factor to which it also can respond. The growth factor produced by MOLT-4f which we designated Leukemia Derived Growth Factor of MOLT-4f (LDGF-M4) differs from interleukin-2 (IL-2) in biological and physico-chemical properties. In order to determine if other malignant lymhoid cell lines also produce growth factors, the T cell lines CCRF-HSB-2 and CCRF-CEM, the B cell lines BJAB and RAJI and myeloid cell line KG-1 were cultured long-term in serum-free medium (AT-IMDM). Supernatants were collected 2x/week and pooled before purification. Partial purification was obtained with ammonium sulfate precipitation, followed by AcA 54 gel diffusion chromatography. Crude supernatants and fractions were tested for their growth promoting activity on the various cell lines, mentioned above, and on a IL-2 dependent murine cytotoxic T cell line (CTLL-2). The T cell lines produced growth factors which are in the same molecular weight range as LDGF-M4, for their own cells and other malignant T cells. These factors do not stimulate the growth of the malignant B or myeloid cells. A similar growth promoting activity is not found in the supernatants of BJAB, RAJI and KG-1. The LDGFs are new growth factors, which may be important for the independent proliferation of T leukemia cells.

0282 INDEPENDENT REGULATION OF CYTOLYSIS AND PROLIFERATION IN ANTIGEN-DRIVEN CTL CLONES. Michael B. Widmer and Birgitta Clinchy, University of Minnesota, Minneapolis.

Antigen-driven, helper cell-independent cytolytic T lymphocytes (CTL) exhibit both cytolytic activity and exogenous IL-2-independent proliferative characteristics when confronted with appropriate antigen; some cells of this category also secrete IL-2. In an attempt to determine the temporal relationships among different functions of such multifunctional cells, we have measured cytolytic activity and antigen-driven proliferation as a function of time in the culture cycle. In contrast to the invariant cytolytic activity of CTL clones previously reported, we find variation in specific cytolytic activity which correlates with time after previous exposure to antigen and a source of IL-2. Cytolytic activity was greatest on a per cell basis at 3-4 days after subculture initiation and was 20-fold less on day 7. The results with the antigen-driven CTL clones thus parallel those obtained at the population level (with bulk mixed leukocyte cultures) indicating a cyclic nature of cytolytic activity. Examination of the proliferative response of cloned cells to antigen in the absence of IL-2 demonstrated that the cells were refractive to stimulation by antigen at the time when they were most highly cytolytic, but were able to respond proliferatively on day 7 after subculture when cytolytic activity had decreased; con A-induced secretion of IL-2 correlated temporally with the proliferative response. These findings demonstrate that different functions, i.e. cytolytic activity vs. ability to respond proliferatively to antigenic stimulation and secrete IL-2, are manifested (and presumably regulated) independently in clonal populations.

Genetic Mechanisms Controlling Diversity of V Genes and Expression of C Genes

0283 REARRANGEMENT OF ENDOGENOUS AND INTRODUCED V_H GENE SEGMENTS IN PRE-B CELL LINES, Frederick W. Alt, T. Keith Blackwell, George D. Yancopoulos and Michael G. Reth, Columbia University, New York, NY 10032

We have developed Abelson murine leukemia virus transformed pre-B cell lines which in culture undergo all of the gene reorganization events associated with the differentiation of cells of the B-lymphoid (antibody-producing) lineage including heavy and light chain variable region assembly and heavy chain class switching. In several such null, pre-B cell lines, the sequential assembly and expression of functional heavy and light chain genes in culture ultimately leads to the generation of daughter lines which express surface IgM molecules, a property associated with the immunocompetent B-lymphocyte. We have extensively characterized both the assembly of the endogenous V_H and V_L gene segments in these lines as well as site specific recombination events between D_H and J_H segments that were introduced into the cells by DNA transformation precedures. The former studies have elucidated a biased usage of the V_H gene repertoire in pre-B cells while the latter studies have further defined mechanistic and regulatory aspects of the recombinase system.

0284 ANALYSIS OF THE VH GENE(S) CODING FOR AN AUTOIMMUNE IDIOTYPE FAMILY. Kathleen J. Barrett, Tufts University School of Medicine, Boston, MA 02111.

The MRL strain of mice is a model system which closely parallels the human autoimmune disease, SLE. Analysis of anti-DNA binding autoantibodies secreted by hybridomas made from unimmunized MRL/lpr spleen cells showed that one of these hybridomas (H130) defines a high frequency idiotype family (Rauch et al., 1982). Large amounts of this idiotype are found in the sera of all MRL/lpr mice and the amount increases with age. Approximately half of the anti-DNA binding autoantibodies secreted by MRL/lpr derived hybridoma cell lines belong to this idiotype family (Id^+). To determine whether the same VH gene codes for this idiotype family, the fragments containing rearranged JH genes from the prototype hybridoma, H130, were cloned and the sizes of the fragments containing rearranged JH genes in hybridomas that secrete Id^+ anti-DNA binding autoantibodies were determined. Analysis of H130 showed that this hybridoma contains two rearranged JH genes and no parental JH genes. We presume that these two fragments derive from the MRL B cell parent. Both fragments are rearranged to VH genes; the expressed VH gene defined a VH gene family that is polymorphic in different strains of mice. Sequencing of the expressed H130 VH gene will determine the VH gene family to which it belongs. All of the fragments containing the rearranged JH genes in the Id^+ hybridomas were smaller than those found in the H130 prototype. Since both sequences in the H130 DNA have rearranged to JH4, the size difference cannot be explained by rearrangement of the same VH gene to different JH genes. It therefore seems likely that the Id^+ VH genes differ from the expressed H130 VH gene in the 5' flanking region. Subprobes of the cloned expressed VH gene are being used to analyze the relatedness of the coding regions of the VH gene(s) expressed by this idiotype family.

0285 REGULATORY SEQUENCES REQUIRED FOR κ LIGHT CHAIN GENE EXPRESSION, Yehudit Bergman, Douglas Rice, Rudolf Grosschedl and David Baltimore, Massachusetts Institute of Technology, Cambridge, MA 02139

During differentiation of B lymphocytes, the variable and the constant parts of immunoglobulin (Ig) are rearranged to form a complete functional Ig gene. RNA transcripts of the complete gene can be detected only after this translocation occurs. Upon further differentiation of a B cell into a plasma cell, a large increase in the rate of transcription is observed. To study the mechanism of regulation of the κ chain gene, a wild type κ genomic gene and mutant genes containing deletions spanning the entire J_κ-C_κ intron were transfected into B and T cell lines. Deletion mutants were generated by cleavage at a unique restriction site within the intron, followed by digestion with Bal31 exonuclease. The wild type κ gene and the deletion mutants were incorporated into a vector containing the polyoma origin of replication and a modified mouse H4 histone gene as an internal control for transcription. The effect of the different deletion mutants on the transcription of κ chain gene in transiently transfected mouse myeloma cells were measured using the S1 nuclease mapping technique. We find that DNA sequences located 0.4-0.85 kb upstream of C_κ are crucial for accurate and efficient transcription. This region coincides with a DNAse I hypersensitive site. Similar to viral transcriptional enhancer element, these κ intronic sequences will also function when moved 5' to the V_κ promoter, in either orientation. We are studying the level of κ chain gene expression using the deletion mutants by transfecting the genes into B cell lines representing different stages of B cell development.

0286 UNDERMETHYLATION OF THE Cμ GENE CORRELATES WITH CHANGES IN μ CHAIN EXPRESSION, Marcia A. Blackman and Marian E. Koshland, University of California, Berkeley, CA 94720

The methylation status of the murine Cμ gene has been investigated as a function of B cell differentiation. Southern blot analyses of lymphoid cell lines showed there is a progressive undermethylation of specific sites in the Cu gene.

Expression of the membrane form of the μ heavy chain is associated with undermethylation of a single MspI/HpaII site located 5' to the transcriptional enhancer sequence in the J_H-C_μ intron. This site is methylated in non-lymphoid and embryonic tissues and becomes completely undermethylated once transcription of the μ chain gene is initiated.

On the other hand, expression of the secreted form of μ heavy chain correlates with undermethylation of the two MspI/HpaII sites and the one HhaI site that are located closest to the μ_s exon. These sites remain heavily methylated in all developmental stages preceding IgM secretion and become completely hypomethylated in IgM-secreting lines. Thus, undermethylation of the 3' Cμ sequences correlates not with transcription, but rather with the post-transcriptional process responsible for the shift in expression from the membrane to the secreted form.

0287 THE ORGANIZATION AND CONTENT OF MOUSE IGH-V FAMILIES, Peter H. Brodeur and Roy Riblet, The Institute for Cancer Research, Philadelphia, PA 19111

We have used cloned Igh-V genes to study the organization of the Igh-V complex of the mouse. By hybridization (Southern Blot) analysis, we conclude that there are approximately one hundred mouse Vh genes organized in at least six non-overlapping "Vh families." DNA sequence comparisons indicate that genes within a family share $\geq$ 80% sequence homology while the sequence homology between families is $\leq$ 70%.

Analysis of Igh-Recombinant mouse strains with Vh probes from each family indicate that the genes within a family are clustered. We have mapped each family relative to each other, Dh, and the constant region (Igh-C).

From an analysis of 66 Recombinant Inbred strains and over 6000 backcross progeny, we estimate that the overall length of the Igh complex is less than 2 map units.

0288 HEAVY CHAIN VARIABLE REGION GENES OF ANTI-TGAL HYBRIDOMA ANTIBODIES, Condie Carmack*, Seth Pincus*, Peter Brodeur+, and Roy Riblet+, *Department of Pathology, University of Utah Medical Center, Salt Lake City, UT 84132 and +Institute for Cancer Research, Philadelphia, PA 19111

The variable region of the antibody heavy chain is created though the recombination of VDJ genes. Other events, including somatic mutation, increase chain diversity. We have been studing the immune response to the synthetic polypeptide TGAL both at the phenotypic and genotypic levels. We have developed a series of anti-TGAL hybridoma cell lines and have characterized them as to epitope binding and expression of cGAT, Gte, GA-1, and I7 (allotype restricted idiotope) idiotypes. We have more recently begun to characterize the genetic events associated with the antigen recognition and idiotype expression. Southern blot analysis of genomic DNA shows several patterns for the rearranged heavy chain V genes. Bands at 4.9, 4.4 and 3.5 kb all represent productive rearrangements. We have cloned three productive rearrangements as well as two unproductive rearrangements and have used restriction enzyme mapping to characterize these V regions as to the V,D, and J genes selected. The fine structure of the VDJ region is correlated to the phenotype seen in both epitope binding specificity and the idiotype expressed. Antibody molecules recognizing the same epitope can utilize different J genes.

0289 IDIOTYPY AND ANTIGEN-BINDING SPECIFICITY IN THE ID-460 SYSTEM, Elaine A. Dzierzak, Tony Marion, Alfred Bothwell and Charles A. Janeway, Jr., Yale U., New Haven, CT 06510

The murine secondary immune response to dinitrophenol is dominated by antibody expressing Id-460, a private idiotype originally defined on MOPC 460 IgA anti-DNP antibody. It has been shown to map to IgV_H^a and V_{K1}. However, Id-460 is expressed on antibody present in preimmune serum of mice of all strains. It is non-DNP-binding and is not present in the serum of germ-free mice. Some of the preimmune Id-460 antibody has specificity for a determinant associated with environmental antigen, Pasteurella pneumotropica, suggesting that the DNP-binding form detected after immunization may be the non-specific parallel set. We have generated a panel of hybridomas expressing Id-460 of the DNP-binding type as well as the non-DNP-binding type to localize and define on the molecular level the sequences coding for the Id-460 determinant and for antigen binding specificity. The heavy and light chain rearranged genes from MOPC 460 have been isolated and cloned. A 2.3Kb EcoR1 fragment containing the heavy chain coding sequence for the first 83 amino acids of the V region and a 2.1Kb EcoR1 fragment containing the 3' V region plus the rearranged D and J were subcloned in pBr322 and sequenced by the Maxam-Gilbert method. Using the 2.3Kb V gene fragment as a probe we have found 3-4 genes in the germline and will present data on the genes that have undergone rearrangement in the hybridomas which differ in antigen-binding and idiotypic characteristics.

0290 THE USE OF TRANSFECTION VECTORS TO STUDY SOMATIC MUTATION OF ANTIBODY GENES IN LYMPHOCYTES, Patricia J. Gearhart and Daniel F. Bogenhagen, The Johns Hopkins University, Baltimore, MD 21205, and SUNY at Stony Brook, NY 11794

An unusual hypermutational process in B lymphocytes mutates only rearranged immunoglobulin variable genes but not adjacent constant genes. Point mutations are introduced at a very high frequency of 10^{-2} per base pair. We have delimited a region of one kilobase around the rearranged V_{k167} gene where mutation occurs. The specific location of substitutions suggests that there may be a nucleotide sequence in this region that initiates mutation. In order to identify a sequence, we are developing an assay to detect mutation using the V_{k167} gene on a bovine papillomavirus vector. The vector is transfected into SP2/0 cells and integrates into the chromosomal DNA in 10-100 copies per cell. Restriction site and sequencing analyses of the integrated vectors showed that they have not undergone random mutation or rearrangement, and therefore are suitable vectors for further experiments. The rearranged variable and constant genes on the vector are transcribed into cytoplasmic mRNA. Transfected SP2/0 cells are then fused with antigen-stimulated B cells. The variable gene is rescued by cloning it into bacteriophage lambda and then subcloning it into bacteria for selection, or into M13 virus for sequencing. Several approaches are being used to determine if the recovered variable gene has undergone mutation: (1) genetic reversion of an adjacent, defective beta-lactamase gene, that has a nonsense mutation, will permit growth of bacteria on ampicillin, and (2) sequencing of the variable gene will identify mutation.

0291 THE GENERATION OF ANTIBODY DIVERSITY IN THE ANTI-HEMAGGLUTININ INFLUENZA RESPONSE, Konrad E. Huppi, David McKean, Louis Staudt, Walter Gerhard and Martin Weigert, Institute for Cancer Research, Phila., PA 19111

A series of hybridomas have been examined from one BALB/c mouse (H36) originally primed with A/PR8/34 influenza virus. About 22% of these hybridomas were found to express the Vk21 light chain and among these, the predominant gene expressed is Vk21C. We have established clonal relationships among the H36 hybridomas by criteria such as productive and nonproductive light chain DNA rearrangements as well as by direct DNA or RNA sequence comparisons. We conclude that somatic mutation occurs at an extremely high rate (10^{-3}/generation/bp) and is an ongoing process which occurs both before and after class switch.

0292 EVOLUTION OF IMMUNOGLOBULIN GENES, Evelyne Jouvin-Marche, Mary Heller and Stuart Rudikoff. National Institutes of Health, National Cancer Institute, Bethesda, Maryland 20205

This laboratory has established a wild mouse colony containing examples of the genus Mus from throughout the world. The sampling contained in the colony represents a considerable spectrum of the evolution of the species (probably 20-25 million years) and we are employing this genetic source to examine the evolution of particular immunoglobulin genes as a model for multigene families. Initially, a probe corresponding to the V_K region of the M167 light chain has been used to identify genetic polymorphisms. DNA from 17 different species have been analyzed by Southern blot analysis and compared to the inbred BALB/c strain. As might be expected, a high degree of polymorphism was observed and strongly hybridizing bands could be found throughout the colony even at conditions of high stringency.

To begin to analyze the fine structure and evolution of M167-like genes, a partial Embo I library has been constructed from Mus pahari DNA. Pahari is one of the most distant species from the inbred mouse strains and thus probably represents one of the extremes, in terms of evolution, when compared to inbred mice. Using a probe containing the C_K and M167 V_K coding regions a number of positive recombinant phage have been isolated. The characterization of several of these clones is in progress and data on these studies will be presented.

0293 NEW INSIGHTS FROM cDNA SEQUENCES OF RABBIT κ LIGHT CHAINS INTO THE EVOLUTION AND QUANTITATIVE EXPRESSION OF THE CONSTANT REGION ALLOTYPES, Nancy McCartney-Francis, Robert M. Skurla, Kenneth E. Bernstein and R. G. Mage, NIAID, NIH, Bethesda, Md 20205
In $\underline{b^4b^9}$ and $\underline{b^5b^9}$ heterozygous rabbits, pre-B cells with expressed C_K b9, although found in frequencies equal to those with b4 or b5, do not expand into productive Ig-producing cells with frequencies comparable to the other allotypes. Nucleic acid sequences of cDNA clones encoding κ L chains of b9 allotype suggest that in cells differentiating to produce b9 κ L chains, the number and location of cysteines influence immunoglobulin expression. We found a surprising difference in the position of the variable region cysteine that is involved in an interdomain disulfide bond with Cys 171 in the constant region. One b9 cDNA sequence lacks the usual Cys 80 and instead encodes Cys 108, which in three-dimensional models appears capable of forming the interdomain disulfide bond with Cys 171 in the constant region. A second b9 clone encodes both Cys 80 and Cys 108; the translation product of this cloned mRNA could have a free reactive sulfhydryl group that might lead to a non-functional or poorly functional molecule. If assembly of H and L chains signals termination of L chain gene rearrangements and some of the b9 L chains cannot adequately interact with H chains in the pre-B cells, the higher proportions of λ L chains observed in homozygous b9 rabbits can also be explained. A similar explanation for the relatively low expression of the Basilea (K2) isotype can be proposed (See abstract of Lamoyi et al.). Our comparisons of Basilea, b4, b5 and b9 cDNA sequences also suggest that the primordial lagomorph kappa gene had a Basilea-like (K2) sequence.

0294 AMINO ACID SEQUENCE ANALYSIS OF MONOCLONAL ANTIBODIES OF BALB/C MICE EXPRESSING THE MAJOR CROSS-REACTIVE IDIOTYPE OF THE A/J STRAIN, Meek, K., Leo, O., Hiernaux, J., Urbain, J., and Capra, J.D. UTHSCD, Dallas, TX and Universite Libre de Bruxelles. The primary structure of A/J anti-arsonate (anti-Ars) antibodies expressing the major cross-reactive idiotype (CRI) associated with The A strain has been investigated extensively. By chain recombination experiments, the structural differences responsible for variation in expression of the CRI have been localized to the heavy chain. Until recently, this idiotype was thought to be rarely, if ever, expressed in BALB/c mice. In fact, it has been reported that BALB/c mice lack the heavy chain variable segment gene which is utilized by the entire family of anti-Ars antibodies expressing the A/J CRI. However, in the BALB/c strain, it has been possible to ellicit CRI +, Ars binding antibodies by immunizing first with anti-CRI and then antigen. Such CRI + BALB/c antibodies can occasionally be induced with antigen alone. We have determined the heavy chain variable region amino acid sequence of two CRI + hybridoma products derived from BALB/c mice. While their V_H segments (1-98) differ from the V_H segment of A/J CRI +, anti-Ars antibodies in over forty positions, and while rather than the usual J_H2 gene segment used by most CRI + A/J anti-Ars antibodies, one CRI positive BALB/c hybridoma utilizes a J_H1 gene segment while the other uses a J_H4. However, their D segments are strikingly homologous. The amino terminal amino acid sequence of the light chains from these molecules are virtually identical to those of A/J CRI + anti-Ars antibodies. These data imply that BALB/c mice can express the A/J CRI by producing antibodies containing a very similar if not identical light chain and heavy chain D segment, but different V_H and J_H gene segments than their A/J counterparts.

0295 VARIABLE REGION GENES ASSOCIATED WITH THE PREDOMINANT PUBLIC IDIOTYPE (IdXL) FOUND ON MONOCLONAL ANTIBODIES TO HEN EGG-WHITE LYSOZYME (HEL), Alexander Miller, Peter Brodeur Lean-Kuan Ch'ng, Di-Hwei Hsu, and Gerald Leca, UCLA, Los Angeles, CA 90024.

DNA was isolated from murine hybridomas producing anti-HEL antibodies of different nonoverlapping specificities which were IdXL-positive. Southern blot analysis for rearranged V_H genes through use of J_H region probes rule out the use of a single V_H germline gene coding for IdXL-positive antibodies and suggest that many different V_H genes are employed. Antibody chain separation and recombination studies indicate that IdXL is associated solely with the light chain. Preliminary studies using a probe for the intervening sequence between J_K and C_K to detect rearranged V_K on Southern blots, are consistent with the use of a single V_K in generating IdXL-positive anti-HEL. This rearranged V_K is also found in DNA of early, primary hybridomas which produce IdXL-negative anti-HEL. These data suggest a model in which there is a regulatory process which restricts most anti-HEL to a light chain coded for by a single germline V_K. Superimposed is a second selection process for a subset of this anti-HEL which are coded for by somatic mutants of this V_K which also code for IdXL positivity.

0296 SEQUENCES CONTROLLING KAPPA LIGHT CHAIN GENE EXPRESSION, Vernon T. Oi and Sherie L. Morrison, Becton Dickinson Monoclonal Center, Mountain View, CA and Columbia University, New York, NY.

Transcriptional activity of the kappa light chain gene changes dramatically over B cell differentiation. VJ DNA rearrangement generates a complete light chain gene; however, this event by itself appears to be insufficient for gene activation. The amount of light chain gene expression changes during B cell differentiation, from a resting B cell to a plasma cell. Using DNA-mediated transfection to introduce kappa light chain genes into both lymphoma and myeloma (hybridoma) cell lines, we have identified at least two regions in the intron separating the VJ exon and the kappa constant region exon that control kappa light chain gene expression. Since B cell differentiation has several distinct phases, each of which is characterized by the amount and kind of immunoglobulin molecules produced, we feel that these two intronic sequences may represent differentiation state-specific control sequences.

0297 REGULATION OF IMMUNOGLOBULIN GENE EXPRESSION, Brian G. Van Ness, Timothy Schaiff, Janine Davis and Richard Lynch, University of Iowa, Iowa City, IA 52242.

There are many complex cellular interactions and factors which control the production of immunoglobulins (Igs), so that the immune response is precisely regulated. Our approach to studying immunoregulatory controls at the molecular level is to introduce cloned Ig genes into mouse myeloma cell lines which respond to immunoregulatory signals. Two cell lines are being examined, one (MOPC315) in which Ig expression can be suppressed by idiotype specific T cells, and one (70Z/3) in which Ig expression can be induced with LPS. In collaboration with Richard Lynch we have transfected κ genes into MOPC315 cells, and are examining expression and suppression by analysis of protein and RNA. Similarily, we are currently transfecting a variety of constructed light chain vectors into 70Z/3 cells to analyze regulatory sequences necessary to respond to the LPS induction.

We have also recently analyzed (by cloning, DNA sequencing, RNA analysis) several aberrantly rearranged κ genes which have resulted in altered expression of κ mRNA. One of these clones resulted from a recombination event between linked regions of the constant region, involving a V-like flanking sequence and J_1. This recombination, in effect, deleted the J locus and brought the germline promotor closer (by 2.5 Kb) to the constant region gene.

0298 EFFECT OF ANTIGEN STIMULATION ON THE EXPRESSION OF AN IDIOTYPE, Mary E. White-Scharf and Thereza Imanishi-Kari, Massachusetts Institute of Technology, Cambridge, MA 02139

The antibody response to NP has several characteristics which make it particularly useful in the study of antibody diversity. When conjugated with a protein carrier it induces a major idiotypic response (NP^b) in C57BL/6 mice. The antibodies contributing to the idiotype are heterogeneous, but each has one or more idiotypic determinants and all use λ light chains. Heterogeneity within the family of idiotype-bearing antibodies most likely results from heavy chain differences. We have examined the effects of extensive antigen stimulation on the process of diversification. An attempt was made to clone in vivo the B1-8 idiotype according to the methods of B. Askonas (Proc. Natl. Acad. Sci USA 1970. 67:1398). Idiotypic determinants on the monoclonal antibody B1-8 represent a major component of the NP^b idiotype. Furthermore, the heavy chain of B1-8 has an amino acid sequence identical to that encoded by the germline gene 186.2 (Cell 1981.24.625) so that somatic rearrangement or mutation which might occur should be easily detected. Small numbers of immune donor cells were transferred into syngneic irradiated mice. Recipients were immunized with NP-CG and sera were tested for idiotypic determinants cross-reactive with NP^b and B1-8. Mice with significant amounts of B1-8-like antibody were selected as donors for additional transfers. The finding was that despite attemps to enrich for B1-8-like determinants, the amount of both B1-8 and NP^b decreased during transfer. NP^b decreased to a much greater extent than did B1-8 suggesting that enrichment for B1-8-like antibodies was acheived but that extensive mutation had also occurred. B cell hybrids are being constructed at early and late stages of transfer so that variable region sequences can be compared.

Functional Networks vs Circuits: Cellular Dissection

0299 HEN EGG-WHITE LYSOZYME-SPECIFIC MONOCLONAL SUPPRESSOR T CELL FACTOR DIRECTLY SUPPRESSES THE AFFERENT PHASE OF DELAYED-TYPE HYPERSENSITIVITY AND ALSO INDUCES SECOND-ORDER SUPPRESSOR T CELLS. Luciano Adorini+, Vittorio Colizzi°, Gino Doria+ and Paola Ricciardi Castagnoli*, +ENEA-EURATOM Immunogenetics Group, Laboratory of Pathology, C.R.E. Casaccia, Rome, °Institute of Microbiology, University of Pisa; *CNR Center of Cytopharmacology, Milan, Italy.

Culture supernatant obtained from a Radiation Leukemia Virus-transformed hen egg-white lysozyme-specific monoclonal suppressor T cell line (LH8-105) is able, when injected into mice, to specifically suppress the anti-HEL antibody response and the delayed-type hypersensitivity (DTH) to HEL. Injection of I-J purified culture supernatant suppresses the afferent but not the efferent phase of DTH and therefore it contains TsF1-like material. This T cell product not only directly suppresses DTH but also induces second-order suppressor T cells which act in the efferent phase of DTH and require both antigen (HEL) and TsF1 for their induction. Thus, a monoclonal suppressor T cell product able to suppress the antibody response, T cell proliferation, and DTH by acting directly on specific helper T cells can also induce second-order, efferent suppressor T cells. These results will be discussed in the context of current views on suppressor T cell circuits.

0300 T CELL REGULATION OF B CELL ACTIVATION. Yoshihiro Asano* and Richard J. Hodes Immunology Branch, NCI, NIH, Bethesda, MD 20205

T helper (Th) cell dependent B cell activation is regulated by T suppressor (Ts) cells, and the activation and/or effector function of Ts cells is under strict genetic control. Recent studies demonstrated that recognition by T cells of MHC-encoded determinants expressed on accessory (Acc) cells is required for in vitro activation of Ts cells, and this interaction determines the subsequent MHC-restriction of Ts cell effector activity. The further studies identified cloned Ts effector cells which are both antigen-specific and MHC-restricted in their activation requirements. Responses of $(AxB)F_1$ - $parent_A$ (P_A) chimeric Th cells and $(AxB)F_1$ (B + Acc) cells were suppressed by P_A-restricted Ts clones but not by P_B-restricted Ts clones. These findings suggest that the cloned Ts cells function by inhibiting the MHC-restricted interaction between Th cells and B and/or Acc cells. It was further demonstrated that KLH-specific Ts clones suppressed the responses mediated by FGG-specific Th clones in the presence of TNP-FGG which was covalently linked to KLH, (TNP-FGG)=KLH. In contrast, responses mediated by FGG-specific Th clones were not suppressed by KLH-specific Ts clones in the presence of either mixture of TNP-FGG and KLH, TNP-FGG and FGG=KLH, or reduced (TNP-FGG)=KLH. These findings suggested that the MHC-restricted Th cell function is inhibited by cognate interaction of functioning Th cells and Ts cells. Moreover, subsequent studies suggested that T augmenting (T_A) cells function in an MHC-restricted manner to modify MHC-restricted suppression of cloned Ts cells. Thus, MHC-restricted Th cell dependent B cell activation is regulated by cell/cell interactions of Th cells, Ts cells and T_A cells.

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0301 INDUCTION OF A T CELL MEDIATED FEEDBACK SUPPRESSION CIRCUIT BY SPECIFIC ANTIBODY, Catherine E. Calkins, Thomas Jefferson University, Philadelphia, PA 19107

Specific feedback suppression of both primary and secondary antibody responses to sheep erythrocytes (SRBC) can be detected in co-cultures containing PC.1+ B lymphocytes from mice primed with SRBC and Qa-1+ T cells from unprimed mice. Supernatants (24 hr) from antigen stimulated or unstimulated cultures of the primed cells suppress the primary anti-SRBC response, having no direct effect on the responses of primed cells. Depletion of either Lyt 2+ or Qa-1+ cells from the unprimed responding cell population abrogated the suppressive effects of these supernatants, indicating that these cells play an essential role in mediating the suppression. It seems likely that this supernatant factor acts as an 'inducer of feedback suppressor T cells since it is produced early in culture by primed and not by unprimed cells, and since suppression is detected only in the presence of appropriate suppressor cell precursors. Suppression induction with these supernatants is antigen specific and can be eliminated by prior adsorption of the supernatants with SRBC. The active factor in the supernatants also binds to Protein A and anti-mouse immunoglobulin affinity columns, suggesting that the factor is SRBC-specific IgG secreted by the primed B cells. A similar suppression can be detected using a monoclonal IgG anti-SRBC antibody. Suppression induced by the supernatants appears to be dependent upon a prolonged presence of the factor with the suppressor cell precursors. Further studies are now being done to determine the mechanism of induction of the suppressor cells in this system.

0302 PRODUCTION AND FUNCTIONAL CHARACTERIZATION OF ANTI-INTERLEUKIN 2 MONOCLONAL ANTIBODIES. John A. Hare, Douglas Dawson, Denis R. Burger, VA Medical Center Portland, OR 97201

We have previously prepared and characterized a monoclonal antibody to the human interleukin 2 (IL-2) receptor. In order to further characterize the interaction of IL-2 with its receptor, we have now used this anti-IL-2 receptor ab, designated TH5.2, to prepare monoclonal anti TH5.2 idiotype antibodies. Three of these ab have been successfully used to bind IL-2 as demonstrated in an assay with IL-2 dependent HT2 cells. Balb/C mice were immunized with TH5.2 antibody and their spleen cells fused in vitro using standard hybridoma techniques. Culture supernatants were screened and cloned on the basis of their inhibition of Ag specific lymphocyte proliferation, and the inhibition of HT2 cell IL-2 dependent proliferation. Three clones (D3, H3, H5) were selected for expansion and functional studies. Immunoglobulin was removed from culture supernatants with Protein-A sepharose and 200 ug/ml incubated with IL-2. Unbound IL-2 was separated from bound IL-2 with a 50,000 dalton molecular weight cutoff ultrafilter. Assays of the filtrate showed no IL-2 activity in samples incubated with the anti TH5.2 idiotypes D2, H3 and H5. By contrast, IL-2 remained in samples incubated with control antibody preparations. We are using these anti IL-2 antibodies, along with the anti IL-2 receptor ab TH5.2 to further characterize the interaction of IL-2 and its receptor.

0303 A NEW SYMMETRY PRINCIPLE FOR IMMUNOLOGY: A anti-B is anti-(B anti-A), Geoffrey W. Hoffmann, and Anwyl Cooper-Willis, Department of Microbiology, University of British Columbia, Vancouver, B.C., Canada V6T 1W5

Immune system network theory leads to a new symmetry relationship, namely that the antibodies produced in an allogeneic A anti-B immune response (where A and B are, say, two different mouse strains) should have complementary shapes to the antibodies in a B anti-A response. This relationship is due to the existence of two separable populations of antibodies that are present in alloantisera, namely anti-foreign and anti-anti-self antibodies. We present a derivation of the theoretical result together with its experimental validation. The cytotoxic action of an A anti-B serum on B strain cells is specifically inhibited by B anti-A antiserum. More generally, we find that B anti-X inhibits Y anti-B, where X and Y are arbitrary strains. Succinctly stated, B anti inhibits anti-B. We call this relationship the second symmetry principle for immune system networks.

0304 PANCREATIC β CELL SURFACE INSULIN AS A COMPONENT IN AN EXTENDED NETWORK, D.R. Kaplan, J. Colca and M. McDaniel, Washington University, St. Louis, MO 63110

The immune network has traditionally been described as specific interactions among lymphocytes and their products; however, it is possible that antigens on non-lymphocytic cells are also involved. Since the immune network has been demonstrated with soluble insulin as antigen, it was interesting to determine if there exists a surface-bound form of insulin that might extend this network to endocrine cells. Rat pancreatic islets were isolated and then dispersed into a suspension of individual cells. After these cells were incubated for several hours in culture medium, they were stained in the presence of azide and at 4°C with guinea pig antisea specific for insulin and then with a fluoresceinated rabbit anti-guinea pig IgG second antibody. This protocol demonstrated that insulin is a surface molecule for a proportion of the pancreatic islet cells. Surface insulin did not represent insulin bound to receptor or nonspecifically adsorbed to the cell surface since surface fluorescence could not be demonstrated on hepatocytes or tumor cells, since the addition of an inhibitor of insulin receptor formation increased surface insulin, and since incubation of the islets with exogenous insulin, EGTA, or hypertonic saline did not affect the staining. Moreover, insulin on the cell surface must be in some way connected to the cytoskeleton since it capped in the absence of azide and at 37°C. Thus, it seems that insulin is a surface marker for pancreatic β cells, and since it is reactive with immunoglobulin, it thereby constitutes a component in an extended network. This finding suggests that extended network interactions might play a role in physiologic regulation of β cell function or in pathologic β cell destruction.

0305 CO-EXISTENCE OF ANTIGEN-SPECIFIC AND IDIOTYPE-SPECIFIC SUPPRESSOR T CELLS IN MICE INJECTED NEONATALLY WITH A MIXTURE OF ANTIGEN AND ANTI-IDIOTYPE ANTIBODY, Byung S. Kim, Northwestern University Medical School, Chicago, IL 60611

Specific tolerance to phosphorylcholine (PC) can be induced in BALB/c mice by neonatal injection with either pneumococcal C-polysaccharide (PnC) or anti-TEPC15 idiotype (T15id) antibody. Receptors of anti-id-induced suppressor T cells react with the T15id. A brief incubation of anti-id-induced, T15id-specific suppressor T cells with PnC-induced, hapten-specific, and T15id-bearing suppressor T cells resulted in complete cancellation of their suppressor function. However, both types of suppressor T cells were present in mice neonatally injected with mixtures of PnC and anti-id antibody. Based on neutralization experiments using either PnC-induced or anti-id-induced suppressor T cells, only one of the suppressor T cell types is functionally dominant in those mice: most frequently, id-specific suppressor T cells. The suppressor function of the other population is detectable only when one suppressor T cell population has been removed by anti-id or IgM anti-PC (SP45) plus complement. In contrast, both suppressor activities are completely eliminated when one of the suppressor T cell population is removed by adherence to either antigen or id. These results suggest that mice neonatally injected with a mixture of antigen and anti-id antibody possess both types of suppressor T cells. These cells may exist together as a cluster yet display a single suppressor function due to a functional regulation between the suppressor cell types.

0306 GENETICALLY RESTRICTED ANTIGEN PRESENTATION FOR TOLERANCE AND SUPPRESSION. Adam Lowy, J.A.Drebin, J.G.Monroe and M.I.Grenne. Department of Pathology, Harvard Medical School.

Intravenous administration of azobenzenearsonate (ABA) coupled cells to immune animals induces active suppression and phenotypic unresponsiveness. We have demonstrated previously that presentation of hapten by an I-J+ antigen presenting cell (APC) is required for third order suppressor cell activation (Lowy A. et al. J.Exp. Med. 157:353).

We now demonstrate that the I-J+ APC is necessary for the activation of ABA specific first order suppressor cell activation. Presentation of antigen by this I-J+ cell is not needed for the induction of a distinct non-transferrable tolerance mechanism. However, induction of this regulatory mechanism does require genetically restricted recognition of the hapten. The role of differential antigen presentation in the generation of immune and immunoregulatory events will be discussed.

0307 PRESENCE OF V_H a1 ALLOTYPE IMAGES WITHIN AN ANTI-ANTI-a1 (ANTI-IdX) PREPARATION. Dennis W. Metzger and Kenneth H. Roux, St. Jude Children's Research Hospital, Memphis, TN 38101, and Florida State University, Tallahassee, FL 32306.

We have previously shown that injection of rabbits with homologous anti-V_H a1 allotype Ab induces the production of molecules which react in a manner consistent with the presence of either: 1)a highly conserved IdX on all rabbit anti-a1 Ab, or 2)a1-like molecules within the anti-IdX reagent. In an attempt to distinguish between these possibilities, and to assess the complexity of the a1 allotope(s) and the IdX idiotope(s), a mouse monoclonal anti-a1 Ab was prepared. This Ab, termed 3-2F1, reacts with the same percentage of a1 Ig as does polyclonal rabbit anti-a1, and completely inhibits the binding of rabbit anti-a1 to a1 Ig. When tested for the presence of IdX by sequential absorption and inhibition assays, it was found that 3-2 F1 bears the entire set of IdX determinants. This finding would tend to support the concept of a1-like epitopes (latent a1 or, as postulated by Jerne, a1 "internal images") within the anti-IdX reagent. Studies designed to investigate the molecular nature of the putative a1-like epitopes showed that approximately half of the induced anti-IdX molecules lacked the nominal a3 allotype of the rabbit from which they were derived. Analysis of isolated heavy and light chains in conjunction with immunoelectron microscopy indicated that the "anti-IdX" activity of the $a3^-$ fraction is in fact due to latent V_H a1 allotype. Experiments are currently being performed to determine the nature of rabbit $a3^+$, and mouse monoclonal, anti-IdX preparations. (Supported by AI18880, CA 21765, AI 16596, and ALSAC)

0308 ACTIVATION OF CONTRASUPPRESSOR T CELLS (T_{CS}) BY TYPE 3 PNEUMOCOCCAL POLYSACCHARIDE (S3), Helen Braley-Mullen, Univ.of Missouri, Columbia, MO 65212.

S3 coupled spleen cells (S3-SC) can activate S3-specific suppressor T cells (T_S) in cyclophosphamide (Cy) treated mice but not in normal mice (J. Imm., Nov., 1983). Experiments were designed to determine if normal mice given S3-SC had contrasuppressor T cells (T_{CS}) which interfered with the activity of the T_S induced in Cy-treated mice. Spleen cells from mice given S3-SC were fractionated on plates coated with the lectin Vicia villosa (V. villosa). V. villosa adherent cells were able to abrogate (contrasuppress) the activity of T_S when the two cell populations were co-transferred to normal mice. V. villosa nonadherent cells from the same mice as well as V. villosa adherent cells from mice given Mock-SC or from Cy-treated mice given S3-SC had no effect on T_S activity. Contrasuppressor activity of V. villosa adherent cells from mice given S3-SC was abrogated when cells were treated before transfer with anti-Thy 1.2 or anti-Lyt 1.2 and complement. Our results also suggest that T_{CS} act to prevent the expression of T_S activity since V. villosa nonadherent cells from mice given S3-SC are able to suppress the S3 response. That is, removal of T_{CS} on V. villosa uncovers T_S activity in this cell population. These results indicate that S3-SC can activate both T_S and T_{CS} although T_S can only be detected in this system when T_{CS} are eliminated, e.g. with Cy or by removal of V. villosa adherent cells. T_{CS} are Lyt 1+ T cells and appear to be similar to the T_{CS} described by Green et al. in other systems.

0309 CELLULAR COMPETITION PRECEDES CLONAL SELECTION AND THE IDIOTYPE NETWORK, José Quintáns and Zoe S. Quan, La Rabida-University of Chicago Research Institute, University of Chicago, Chicago, IL 60649

It is not known how the immune system develops during ontogeny to become a connected idiotype-network. We postulate the existence of a pre-network period in which developing clones are not under network regulatory constraints. Because the resources allocated for lymphocyte differentiation during ontogeny cannot be unlimited we argue that cellular competition for a finite number of differentiation slots is a fundamental process determining clonal profiles. The complement of B cell clones which saturates this maturational capacity is the functional immune system, from which network interactions can be selected, i.e. cellular competition precedes clonal selection and the idiotype network. This hypothesis provides the framework for alternative interpretations of different experimental findings and is particularly applicable to the case of T15 idiotypic dominance in murine responses to phosphorylcholine antigens.

0310 IDENTIFICATION OF A Ts DIFFERENTIATION FACTOR, S. Rich, M. Carpino and S.C. Arhelger, Baylor College of Medicine, Houston, TX 77030

To identify soluble Ts growth and differentiation factors we have developed a Ts costimulator assay and have characterized an interleukin distinct from IL2 that promotes primed Ts function. Glutaraldehyde-fixed, in contrast to irradiated stimulator cells, fail to trigger TsF production from alloantigen primed Ts (MLR-Ts). However MLR-TsF production is largely reconstituted by addition of supernates of 48 h primary MLR. This Ts costimulator is neither alloantigen-specific nor genetically restricted and fails to induce Ts from unprimed precursors. Three approaches were taken to determine if Ts costimulator was associated with or distinct from IL2. First, HT2 adsorption of MLR supernates removed IL2 but failed to diminish Ts costimulator function. Second, inclusion of cyclosporin A into MLR cultures prohibited IL2 production while Ts costimulator activity was unaffected. Finally G100 fractionated MLR supernates expressed Ts costimulator activity in peaks of ∿20K and ∿40K mol wt that were distinct from the peak containing IL2. Thus the Ts costimulator activity in MLR supernates is distinct from IL2. EL4 supernates also promoted MLR-Ts activation and this function was retained in IL2-depleted EL4 preparations. To preliminarily examine the Ts costimultor function, i.e. differentiative or proliferative, the reconstituting activity of MLR supernates was compared in Ts costimulator assays containing untreated or irradiated MLR-Ts. Ts costimulator triggered TsF release in the absence of proliferation and thus appears to promote a differentiative rather than proliferative process required for primed MLR-Ts function (NIH grants AI13810 and AI17048).

0311 DEPRESSED ACTIVITY OF A SUPPRESSOR CELL CIRCUIT IN PRIMED SPLEEN CELL POPULATIONS. Annette L. Rothermel and Catherine E. Calkins. Thomas Jefferson Univ., Phila., PA 19107

The relevance of feedback suppression as a regulatory mechanism in vivo depends upon the ability of cell populations within a primed host to generate suppression independent of the added unprimed T cells used to detect this activity in vitro. Specific feedback suppression activated by primed B cells in co-cultures of primed and unprimed cells is dependent upon the presence of Lyt 123+, Qa-1+ T cells from unprimed mice. We therefore tested T cell populations from primed mice for the presence of a counterpart to the feedback suppressor cell precursors found in the unprimed spleen. Early experiments indicated that unselected primed T cell populations lack the ability to mediate feedback suppression. The loss of this activity is antigen specific and occurs as early as 24 hr after priming. Recent experiments show that suppressor T cells are present in the Lyt 2+ subset of primed T cells but their activity can only be demonstrated after depletion of the Lyt 2- subset, including helper/inducer and contrasuppressor cells. Primed and unprimed Lyt 2+ T cells have a similar capacity to suppress in the presence of the feedback signal from primed B cells. They differ, however, in their requirement for activation by this signal. Whereas unprimed T cells require an inducing signal in order to become suppressive, primed T cells suppress in the absence of this signal. These studies indicate that the primed spleen does contain Lyt 2+ suppressor T cells but their activity is masked in the unselected T cell population. At least part of the suppression by this population is due to suppressor cells activated in vivo that do not require a feedback induction signal in vitro. Studies are underway to determine if feedback suppressor cell precursors are also active in the primed T cell population.

0312 CROSSREACTIVE IDIOTYPES ON HETEROLOGOUS ANTI-ALLOTYPE ANTIBODY. Kenneth H. Roux and Dennis W. Metzger, Florida State University, Tallahassee, FL 32306, and St. Jude Children's Research Hospital, Memphis, TN 38101.

Several recent studies have revealed an exceptionally high degree of IdX on rabbit anti-allotype Ab. It has been inferred that this IdX sharing represents an unusually high level of (anti-allotype) gene conservation within the rabbit and, by implication, an important regulatory function for anti-allotype and anti-IdX Ab. In this report, we demonstrate that rabbit anti-IdX (anti-anti-a1) reacts with anti-a1 Ab preparation from mice, guinea pigs, chickens and a goat. Specifically, each of these preparations was able to inhibit the reaction between a1 IgG and rabbit anti-a1 Ab as well as the reaction between rabbit IdX (anti-a1 Ab) and rabbit anti-IdX (anti-anti-a1 Ab). Although the relative binding efficiencies of each preparation varied widely, there was a general positive correlation between the ability of an anti-allotype Ab reagent to bind to a1 IgG and to anti-IdX Ab. Each of the heterologous anti-a1 Ab samples was able to form precipitin bands with rabbit anti-IdX Ab. These bands fused with each other and with rabbit anti-a1 Ab. Our results weaken the interpretation that the ubiquotous expression of IdX, previously observed in the rabbit, reflects shared conserved genes. We suggest that either (1) the anti-IdX Ab represents high fidelity internal images of the a1 epitopes or (2) latent a1 allotype Ig is present in the anti-IdX preparation. In either case, any anti-a1 Ab would be potentially reactive with the anti-IdX preparation.

0313 REGULATION OF THE IMMUNE RESPONSE TO FERREDOXIN BY A T CELL IDIOTYPIC NETWORK. Rakesh Singhai, Michael Weaver*, Julia G. Levy, Department of Microbiology, University of British Columbia, Vancouver, B.C. V6T 1W5, *Department of Microbiology and Immunology, UCLA, Los Angeles, CA 90024.

The role of an idiotype defined by a monoclonal anitbody (Fd-B2) directed against the carboxy-terminal of ferredoxin (Fd) was investigated in non-responder B10.D2 ($H\text{-}2^d$) mice. Previously, we have shown that a xenogeneic anti-idiotypic antiserum (α -id) specific for the idiotype Fd-B2, as well as the idiotype Fd-B2 play a major role in anti-Fd response at the T cell level. Linkage studies indicated that T cell idiotype-anti-idiotype interactions were involved and that idiotype bearing molecules were neither H-2 nor IgH linked. In the present study we show that non-responder B10.D2 mice, using the adoptive transfer system, receiving enriched syngeneic T cell populations treated with either the idiotype (id) or α-id plus complement, do respond. B cell enriched populations treated analogously had no influence. Furthermore, using the panning technique, we show that the anti-id recognizes an Lyt 1^+ T cell population(s). The Lyt phenotype of the id-recognizing population is being investigated. Control mice remain non-responders. Irradiated mice reconstituted with both the id and the anti-id-treated T cell respond to Fd. These results indicate the existence of a T cell-T cell network in regulation of the response to Fd. The uncoupling of this network by elimination of either T cell population(s) leads to responsiveness.

0314 **Regulation of Hapten-Specific T Cell Responses**

Man-Sun Sy, Charles F. Scott, Stanley J. Naides, Makoto Tsurufuji and Baruj Benacerraf
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In the murine system, subcutaneous immunization with either self-reactive haptens (DNFB, TNCB) or with hapten conjugated spleen cells (ABA-SC, TNP-SC) usually results in the activation of various T cell subpopulations. These include DTH mediating T cells (T_{DH}) helper T cells (T_H) and cytotoxic T cells (CTL). In contrast, intravenous injection of the identical antigen prior to immunization results in complete abrogation in some of the T cell mediated responses but not in others. This depends critically on the hapten used in the study and the form of T cell mediated responses being investigated. The down regulation of these responses has been shown to be mediated by a complex suppressor T cell pathway, involving multiple T cell subsets and their soluble factors. Attempts will be made to summarize and compare all the suppressor T cell pathways which have been shown to down regulate hapten-specific T cell mediated immune responses.

0315 T CELL ACTIVATION AND DIFFERENTIATION : ANALYSIS OF TH CELL CLONES GENETIC VARIANTS, Jacques Thèze, Georges Bismuth, Marie-Lise Gougeon, Jean-Louis Moreau and Maryse Duphot, Institut Pasteur, Paris, France.

Three examples will be given that illustrate the information that can be gained from the analysis of the properties of different T helper cell clones and lines and of their genetic variants. A) Clone IGC-52.3 mimics in vitro normal T activation. It can exist in two reversible differentiation states : (i) in the absence of filler cells and antigen (GAT) it resembles a small resting lymphocyte being insensitive to IL-2, not expressing the IL-2 receptor and incapable of acting on B cells ; (ii) after exposure to syngeneic filler cells and antigen it becomes blastic, expresses the IL-2 receptor as measured by IL-2 adsorption and reaction with m.Ab. 7D4 (E. Shevach et al.) and secretes high amounts of BRF (B Cell Replicating Factor) and BCDF (B Cell Differentiation Factor). LPS activated filler cells (in the absence of specific antigen) can provide enough information to promote transition from state (i) to state (ii). B) Clone IGC-14.1.6 is a genetic variant of line L14 (Fathman et al.) expressing constitutively the IL-2 receptor. It can grow in presence of purified IL-2 alone and stay specific. This line secretes constitutively BRF and BMF and appears as a variant blocked in the activated state. C) Clone IGC-1.6.12 is a genetic variant that does not express antigen L3T4 defined by m.Ab. GK1.5 (F. Fitch et al.). This variant interacts perfectly and specifically with antigen presenting cells (APC) but does not interact with primed B lymphocytes or provide help in a hapten carrier system. However, after interacting with APC and antigen it secretes large amounts of BRF and BCDF.

T Cell Regulation of IgE Production and Allergic Reactions

0316 FORMATION OF IgE BINDING FACTORS BY HUMAN T CELL HYBRIDOMAS, Thomas F. Huff and Kimishige Ishizaka, The Johns Hopkins University, Baltimore, MD 21239.

IgE binding factors are isotype specific regulatory lymphokines released by FcR(+) cells. They have affinity for IgE and, depending upon their carbohydrate moieties, can either suppress or potentiate in vitro IgE antibody responses. The induction and glycosylation of IgE binding factors have been previously characterized using rat-mouse T cell hybridomas. We have extended these studies into the human system. Normal human T cells which proliferated in the presence of interleukin-2 (IL-2) formed IgE-binding factors when incubated with human IgE. These cells were then fused with a HAT-sensitive mutant of the human T cell line, CEM, to yield 70 HAT-resistant hybridomas. Incubation of 5 of these hybridomas with human IgE or culture of the cells in IgE-coated wells resulted in the formation of IgE-binding factors. One hr incubation with 10 µg/ml human IgE was sufficient to induce the hybridomas to form IgE-binding factors. Polymerized IgE was much more efficient than monomeric IgE for the induction of the factor formation. As little as 10 ng/ml of IgE dimer was sufficient to induce factor formation. The IgE-binding factors produced by the hybridomas bound to human IgE-coated Sepharose and were recovered from the beads by elution at acid pH. The factors had low affinity for rat IgE but failed to bind to human IgG. The IgE-binding factors formed by 4 hybridomas had a molecular weight between 26,000 to 30,000 daltons, while one hybridoma formed IgE-binding factors of 30,000 daltons and 15,000 daltons. The IgE-binding factors formed by all of the hybridomas had affinity for concanavalin A, indicating that the factors are glycoproteins. Culture of the hybridomas in IgE coated wells induced a small number of Fc_ε receptors on the cell surface as detected by binding of radioactive human IgE dimers.

0317 **HUMAN T CELL REGULATION OF ON-GOING IgE SYNTHESIS IN VITRO, Susan Kanowith-Klein, Ann Fischer and Andrew Saxon, University of California, Los Angeles, CA 90024**

IgE synthesis in atopic individuals may result, in part, from inappropriate T cell control of IgE-secreting B cells. Therefore, to study T cell regulatory control of on-going IgE synthesis, we have developed an in vitro isotype-specific culture system by taking advantage of human Ig-secreting lymphoblastoid B cell lines. In control wells of a microtiter plate, we have cultured together the following B cell lines at 5,000 cells/ 0.2 ml/ cell type: AF-10 (a mycoplasma-free clone derived from the IgE-secreting U266 cell line from Dr. K. Nilsson), GM 1500 (IgG-secreting) and GM1056 (IgA-secreting). After 4 days the culture medium from each well was assayed for IgE, IgG, and IgA synthesis by isotype-specific radioimmunoassays. In preliminary experiments, the following ranges of Ig were measured: 5-30 ng/ml IgE, 50-100 ng/ml IgG, and 200-400 ng/ml IgA. When T cell supernatants (Tsn), obtained from 24 hour cultures of PBL-T cells from atopic donors (serum IgE levels greater than 1,000 ng/ml), were added to the mixed-B cell lines, a 2-4 fold enhancement of all Ig isotypes was observed. However, when control Tsn from PBL-T cells of nonatopic donors (serum IgE levels less than 100 ng/ml) were added to the mixed-B cell lines, the amount of IgE, IgG, and IgA synthesized was equivalent to that when Tsn from the Molt-4 line was added to the B cells. These preliminary results suggest that the Ig synthesized by mixed-B cell lines can be altered and that Tsn from the atopic donors is capable of enhancing Ig synthesis. Experiments are now in progress to enrich for Fc epsilon-expressing T cells, which may specifically alter IgE synthesis.

0318 HEAT-LABILE PLASMA FACTOR IN IMMUNE PLASMA WHICH CURES MURINE TRYPANOSOMIASIS. Patricia A.L. Kongshavn and Daniel Wechsler, Dept. of Physiology, McGill University, Montreal, Quebec.

Trypanosoma musculi, a natural parasite of mice, produces a characteristic, self-limiting infection which lasts for approximately three weeks and comprises a growth phase, a plateau phase and an elimination phase. The host defense mechanism responsible for elimination of parasitaemia and cure of the mice is not well understood. Both T-cell and B-cell function must be intact for this process, suggesting a role for a T-cell dependent antibody. Yet passive transfer of serum from immune (cured) hosts reportedly fails to cure infected mice (accordingly to several groups of investigators). Recent findings in our laboratory have demonstrated a thermolabile factor in immune plasma which brings about rapid and complete elimination of the parasitaemia, is effective when administered i.v. but not s.c., and which appears to act in conjunction with an effector cell of unknown origin. These findings are consistent with the notion that cure is mediated by an IgE-dependent ADCC (antibody-dependent cellular cytotoxic) mechanism. If so, this underscores the importance of IgE in host defense not only against metazoan, but also against protozoan, parasites. Striking strain differences are found in the curative effect of immune plasma. Positive identification of the antibody class mediating cure and an investigation of the factors regulating its production and mode of action are being explored in this natural host-parasite system.

0319 T SUPPRESSOR FACTOR INDUCTION OF ANTIGEN-SPECIFIC T SUPPRESSOR CELLS THAT REGULATE IGE FORMATION, Arthur Malley, Stanley M. Shiigi, and Linda M. Bradley, Oregon Regional Primate Research Center, Beaverton, OR 97006

Normal spleen cells cultured with timothy grass pollen antigen-B-specific T suppressor factors (T_{SF_1} and T_{SF_2}) in mini-Marbrook chambers for 4 days produce significant levels of T suppressor (T_S) cells. The cells harvested from these cultures were injected intravenously into mice primed 20 days earlier with 10 µg of WST adsorbed on 1 mg of alum. Recipients were given a secondary antigen challenge of the same dose of WST adsorbed on alum within 24 hr after the cell transfer, and the anti-timothy IgG and IgE responses were measured by enzyme-linked immunoassay. The T_S cells produced can be enriched on anti-Ig-coated dishes, are sensitive to anti-Thy 1 and complement treatment, and give a significant (50-100%) suppression of a secondary antigen-B-specific IgE response, but do not affect the IgG response to antigen B. Thus, the T_S cells produced are not only antigen-specific, but also isotype-specific. Attempts to induce T_S cells by culturing T cells enriched on anti-Ig-coated petri dishes with T_{SF} were unsuccessful. However, the addition of 10^3 macrophages (bone marrow cells grown in culture with colony-stimulating factor for 7-10 days) to cultures of enriched T cells and T_{SF} fully reconstituted the ability of T_{SF} to induce T_S cells. The role of $I\text{-}J^+$ macrophages and a macrophage Fc receptor in the induction of T_S cells will be discussed.

0320 ISOTYPE-SPECIFIC REGULATION OF IgE SYNTHESIS AND ITS PERTURBATION DURING IRRADIATION-ENHANCED IgE SYNTHESIS, John F. Marcelletti and David H. Katz, Medical Biology Institute, La Jolla, CA 92037.

Appropriate levels of IgE initiate a cascade of cellular and molecular interactions which function as a network to control IgE synthesis. There are two major opposing components to this isotype-specific regulatory network, a suppressive arm and a contrasuppressive arm, both of which are mediated through a family of soluble factors termed IgE-induced regulants (EIR). Thus, when elevated levels of IgE are encountered, a subpopulation of B cells respond with the release of the regulant EIR_B. EIR_B induces other B cells to express Fc receptors for IgE (FcRε) and also induces Lyt-1 cells to make suppressive factor of allergy (SFA). SFA in turn acts on other Lyt-1 cells causing the release of a suppressive effector molecule (SEM), which functions to directly inhibit continued IgE synthesis. However, the increased expression of FcRε in B cells induced by EIR_B is the stimulus for a subset of Lyt-2 cells to make the T cell-selective EIR, EIR_T. EIR_T induces other Lyt-2 cells to express FcRε and also induces Lyt-2 cells to elaborate an inhibitor (EIR_T) of EIR_B synthesis. EIR_T is thus an effector molecule of the contrasuppressive arm, since inhibition of EIR_B release results in the inhibition of SFA induction. Finally, other Lyt-1 cells function in this network by regulating net EIR_T activity. Low IgE responder SJL mice are converted to high IgE responder status when given low dose irradiation (250R). Others have shown that transfer of syngeneic Lyt-1 cells can restore low responder status, implying a lesion in the Lyt-1 cells involved in isotype-specific regulation. Evidence will be presented which suggests that low dose irradiation induces a lesion in those Lyt-1 cells which regulate net EIR_T activity.

0321 MURINE MAST CELL-INDEPENDENT, T CELL-DEPENDENT DELAYED REACTIONS TO DNFB INITIATED BY IgE ANTI-DNP ANTIBODY, Robert E. Tigelaar, M.C. Ray, M.D. Tharp and T.J. Sullivan, University of Texas Health Science Center at Dallas, Dallas, Texas 75235

We have reported that epicutaneous challenge with DNFB of Balb/c mice passively sensitized (p.s.) i.v. 48 hr before with monoclonal IgE anti-DNP produces ear swelling which peaks at 24-48 hr (J. Immunol. 131:1096, 1983). The following studies were undertaken to more fully characterize the pathogenesis of these reactions. We first compared the responses to DNFB challenge 48 hr after p.s. with IgE anti-DNP of mast cell (m.c.)-deficient mice (W/W^v and Sl/Sl^d) and their m.c.-replete (+/+) littermates. Such p.s. m.c.-deficient mice exhibited reactions greater than nonsensitized controls (21±2 vs $7\pm1\times10^{-4}$ in., $p<.001$) and equal to reactions in p.s. +/+ mice (18±1). Nu/nu mice have normal numbers of skin m.c. and normal immediate (PCA) reactions after p.s. with intradermal IgE followed 48 hr later by i.v. antigen. However, nu/nu mice challenged with DNFB 48 hr after p.s. i.v. with IgE anti-DNP failed to exhibit delayed swelling above that of nonsensitized controls ($p>.5$). Finally, we have adoptively transferred delayed reactions to DNFB challenge to naive mice with thoroughly washed spleen and lymph node cells from donors p.s. 48 hr before with IgE anti-DNP (23±2, vs 10±1 in control mice). We conclude that these IgE-initiated delayed reactions are mast cell-independent, but T cell-dependent: furthermore, a critical role for cells with IgE F_c receptors is suggested. The possibility that similar mechanisms may be involved in the as yet poorly understood dermatitis seen in individuals with the atopic diathesis or other hyper-IgE syndromes must be considered.

Organization of the MHC Complex: Analysis at the DNA Level

0322 REGULATED EXPRESSION OF PORCINE MAJOR HISTOCOMPATIBILITY DNA SEQUENCES INTRODUCED INTO MOUSE L CELLS. Dinah S. Singer and M. Leonardo Satz, Immunology Branch, NCI, NIH, Bethesda, MD 20205

Mouse L cells have been transformed with a swine genomic clone, which encodes a major histocompatibility (MHC) antigen SLA^d. The regulation of expression of this heterologous genomic DNA segment and its chromatin structure in mouse L cells have been investigated. The transformed L cells, which contain about 2 copies of the 17.8 Kb pig DNA insert per haploid genome, stably and uniformly express SLA antigen on their surface. This expression is the result of differential transcription of the 3.4 Kb long SLA gene; the other 14 Kb of pig DNA sequences flanking the coding suquence are not transcribed. Although the entire pig DNA segment is packaged into nucleosomes, only the transcriptionally active DNA sequences are packaged in a DNase I sensitive conformation. Treatment of the transformed cells with mouse interferon (IFN) markedly enhances the expression of the heterologous SLA^d as well an endogenous $H\text{-}2^k$ antigens, but does not affect the expression of an unrelated surface antigen, gp 70. Flanking swine DNA sequences which are not transcribed in the transformant in the absence of IFN are also not transcribed in the presence of IFN. The elevated expression of SLA^d antigens results from an increased rate of transcription of SLA coding sequences in IFN-treated cells. Taken together, these results suggest that the expression of the foreign DNA segment is actively regulated in L cells.

0323 POLYMORPHISM OF RAT CLASS I MHC GENES. Melanie Palmer*, Peter J. Wettstein+, and Jeffrey A. Frelinger*. *University of North Carolina at Chapel Hill, N.C. 27514 and +Wistar Institute, Philadelphia, Pennsylvania 19104.

The major histocompatibility complex of the rat (*RT1*) has been poorly characterized with respect to the number, linkage, and polymorphism, of Class I genes. In order to estimate the number of Class I *RT1* genes and the relative extent of their polymorphism, we performed Southern blot analysis with liver DNA from rat strains expressing eight *RT1* haplotypes. Following digestion with *Eco*RI or *Bam*HI, the DNA was separated on agarose gels, blotted onto nitrocellulose paper and hybridized with mouse H-2 cDNA probes, pH-2III or pH-2IIa. Ten-20 *Eco*RI and 13-20 *Bam*HI bands hybridized with pH-2III or pH-2IIa. The restriction fragment length patterns were observed to be highly polymorphic. The restriction fragments associated with different *RT1* haplotypes differed by 17 to 70%; this range is similar to the differences observed between mouse *H-2* haplotypes. The same restriction fragment pattern was observed in DNA from congenic rat strains sharing the same *RT1* allele, confirming that the patterns were *RT1* associated. Further, the $RT1^l$ and $RT1^{lv1}$ haplotypes which differ at a single, previously identified *RT1*-linked locus were associated with *Eco*RI restriction pattern differences of 39 to 50%, confirming the supposition that *RT1* Class I genes identified by previous serological and T cell-mediated assays have only identified a minority of the actual number of *RT1*-linked Class I genes. In summary, the results reported in this communication demonstrate that the *RT1* complex encompasses a large family of highly polymorphic Class I sequences similar to the *H-2* and *HLA* complexes of mouse and man.

0324 MOLECULAR AND BIOCHEMICAL ANALYSIS OF THE $E_\alpha^+E_\beta^-$ RECOMBINANT STRAIN A.TFR5, Ann B. Begovich and Patricia P. Jones, Stanford University, Stanford, CA 94305

The A.TFR5 strain, a recombinant between A.CA and A.TL, is the one known example of an H-2 haplotype that is phenotypically $E_\alpha^+E_\beta^-$. Serological and 2-D PAGE analyses have localized the recombination event between the I-A and I-E subregions giving A.TFR5 the proposed haplotype: $K^fA^fJ^fE^kD^d$. This recombinant is $I\text{-}A^f$ and therefore should not synthesize E_β chains. Since it is $I\text{-}E^k$ it synthesizes E_α chains; however, the levels of E_α expressed and found intracellularly are very low. In order to localize more precisely the recombination event and determine its effect on the level of expression of the E_α chain, we have used single copy probes for DNA segments within the I region to compare the restriction maps of DNA from A.TFR5 to those of the parental strains. Current results place the recombination between the 5' region of E_β and the $E_{\beta 2}$ pseudogene, consistent with it being in the recombination "hot spot" within the E_β gene. Preliminary Northern blot analysis indicates comparable levels of E_α^k mRNA in the A.TFR5 and A.TL parental strain, suggesting that the low level of E_α chains might be due to instability of E_α chains in the absence of E_β chains. Experiments are currently underway to determine the stability of newly synthesized E_α chains in A.TFR5 and to further pinpoint the site of recombination.

0325 MOLECULAR MAP OF THE MURINE S REGION, David D. Chaplin, Alexander S. Whitehead, Derek E. Woods, Gabriel Goldberger, Harvey R. Colten, and J. G. Seidman, Harvard Medical School, Boston, MA 02115.

cDNAs encoding portions of human factor B, the second component of complement (C2), and the fourth component of complement (C4) were used as probes to isolate cosmid clones from a murine H-2^d genomic library. The clones were linked by chromosomal walking procedures, yielding overlapping clones spanning 275 kb. The cluster of clones contains two regions with sequences homologous to the C4 cDNA, both in the same orientation, representing a direct duplication of at least 55 kb of chromosomal DNA, separated by a less than 25 kb segment of non-duplicated DNA. Restriction fragment length polymorphism (RFLP) seen with the C4 probe maps these sequences to the S region of the MHC. 5' to the C4-like sequences is an 80 kb long region of chromosomal DNA with little RFLP, containing sequences homologous to the factor B and C2 cDNAs. These data show that the structural genes for murine C2 and factor B are located in the S region of the MHC, and that this region contains a large duplication encoding the structural genes for C4 and, we presume, for the sex-limited protein variant, Slp. In contrast to the surrounding regions which encode class I and class II genes, the S region shows very little RFLP amongst inbred strains. Northern blots of total liver RNA from H-2^b, H-2^d, and H-2^k mice showed that steady state levels of C2 and factor B-hybridizing RNA were similar amongst these strains. In contrast, steady state levels of C4-hybridizing RNA were much greater in high C4 than low C4 strains. This suggests that regulation of circulating C4 concentrations is at least partially at the level of mRNA transcription, processing, or degradation.

0326 Characterization of the HLA B locus specific probe
Hélène Coppin, Sherman M. Weissman, Carl Grumet and Hugh O. McDevitt

The remarkable degree of homology between the different Class I HLA genes has made it difficult to correlate bands with known loci or haplotypes on genomic Southern blots hybridized with the available HLA Class I probes. Using a computer dot plot analysis we have compared the nucleic and sequence of different genomic clones coding for HLA Class I genes. The comparison between the genomic clones JY150 encoding for HLA B7 antigen and different Class I pseudogenes has allowed us to find a region showing little homologous sequence between JY150 and all the other clones. From this 3' noncoding region, we have isolated a fragment of about 500 base pairs. This fragment, when used for Sourthern blot analysis of genomic DNA obtained from homozygous typed cell lines (cut with BamHI and BgLII restriction enzymes), allowed us to observe a pattern of one or two bands. This fragment specifically hybridizes only with JY150 and not with P3.2 (encoding for HLA-A2 antigen), nor the other Class I genomic clones tested. Studies have been initiated to identify restriction fragment polymorphism with this probe in the DNA of ankylosing spondylitis patients.

0327 EXPRESSION OF HUMAN HLA CLASS I HYBRID GENES BY MOUSE LMTK$^-$ CELL TRANSFORMANTS, Terry L. Delovitch, Bertrand R. Jordan and Francois A. Lemonnier, University of Toronto, Toronto, Canada M5G 1L6 and Centre d'Immunologie de Marseille-Luminy, Marseille, France 13288

Hybrid genes containing exons of different human HLA class I genes were constructed to study the structure-function relationship of class I human transplantation antigens. The serologic reactivity of hybrid human class I molecules expressed after cotransformation of LMTK$^-$ mouse fibroblasts with TK genes was examined using HLA-specific monoclonal antibodies and allo-antisera. The reactivities of the expressed hybrid molecules were found to be determined by the first three 5' exons of the genes used, i.e. by the first and second extracellular domains of the class I heavy (H) chain. Although β2-microglobulin is believed to be associated with the third extracellular domain of a class I H-chain, our results indicate this association may also occur with, or otherwise be influenced by the conformation of, the first and/or second domains of the H-chain. The latter H-chain domains express most of the allospecificities of such molecules. Analyses of the expression of deletion mutants of class I genes and of hybrid class I genes carrying an altered exon encoding either the first or second extracellular H-chain domain will be presented.

0328 CLASS I-LIKE GENES OF THE C57BL/10 MOUSE, James J. Devlin, Andrew Mellor, Elizabeth Weiss, Karen Farhner, and Richard A. Flavell, Biogen Research Corporation, Cambridge, Massachusetts 02142

We have cloned 26 Class I like genes from the C57Bl/10 mouse. Three of these genes map to the H-2 complex, two in the K region and one in the D region. Clusters of 10 and 13 linked genes have been mapped to the Qa2,3 and TL regions respectively. Sequence analysis of the bm-1 mutant of the K^b gene has shown that a 13-51 nucleotide segment has been altered, perhaps by gene conversion. An oligonucleotide probe, homologous to the altered nucleotide sequence in the bm-1 gene, has identified a potential donor of this sequence in the Qa2,3 region. The nucleotide sequence of the putative donor gene has shown that the exon that normally encodes the transmembrane segment of Class I like genes is both shorter than usual and contains two termination codons, indicating that the product of this gene may be secreted. We are currently analyzing the expression of this gene by DNA medicated gene transfer.

0329 POLYMORPHIC RESTRICTION ENDONUCLEASE SITES LINKED TO THE HLA-DR α CHAIN GENE: LOCALIZATION AND USE AS GENETIC MARKERS, Henry A. Erlich and Deborah Stetler, Cetus Corporation, Emeryville, CA 94608

A cDNA clone for the HLA-DR α chain has been used to detect restriction endonuclease site polymorphisms within and closely linked to the HLA-DR α chain gene. Hybridization of BglII-digested human genomic DNA with pDRα-1 has revealed three allelic restriction fragment lengths: 3.8 kb, 4.2 kb, and 4.5 kb. Hybridization of EcoRV-digested human genomic DNA with the same probe has revealed two allelic polymorphic restriction fragment lengths: 9.2 kb and 13.0 kb. Using double digests of genomic DNA from individuals homozygous for each of the allelic variants, the polymorphic restriction sites have been localized precisely with respect to the HLA-DR α gene and are clustered near the 3' end. These polymorphic restriction sites can be used as precisely defined HLA-DR α genetic markers for the analysis of genetic predisposition to HLA associated diseases like IDDM. The distribution of the HLA-DR α allelic restriction fragment variants has been determined in control and IDDM populations. In addition, the correlation between individual restriction fragments and serologically determined DR specificities has been examined in families by segregation analysis, in homozygous typing cells, and in hemizygous deletion variants. The 3.8 kb BglII fragment is associated with the HLA-DR1 specificity and the 4.2 kb BglII fragment, with the HLA-DR3 and the DR6 specificities, suggesting linkage disequilibrium between these markers and the sequences determining the DR specificities. The frequency of the 4.2 kb BglII fragment is increased in the IDDM probands relative to the control population. In IDDM family analyses, the 4.2 kb fragment segregated with HLA-DR3 but not with HLA-DR4 chromosomes. This fragment therefore may represent a marker for DR3 but not DR4 associated susceptibility to IDDM.

0330 STRUCTURAL ANALYSIS OF MURINE CLASS II BETA CHAIN GENES, Pila Estess, Jonathan B. Rothbard and Hugh O. McDevitt, Stanford University, Stanford, CA 94305.

A synthetic DR beta-chain specific oligonucleotide was used to select DR and DC-1 beta chains from a human cDNA library. The DC-1 clone was in turn used as probe for murine I-A beta chain cDNA clones. I-A beta chains have been isolated from several different murine haplotypes. These are being sequenced in an effort to localize epitopes responsible for serologically detectable alloptyic differences and structural determinants involved in the ability or inability to mount an immune response to a particular antigen. Based on these as well as published A_β sequences, synthetic peptides have been made in an effort to delineate which structural variations contribute to serological differences and haplotype associated immune responsiveness. Regions in which sequence differences are likely to be reflected as profound alterations in the topological nature of beta chains have been selected as initial candidates. Monoclonal antibodies and rabbit and mouse antisera which react with both BSA-congugated peptide and splenocytes of the appropriate haplotypes have been generated. These will be tested for their ability to inhibit particular haplotype restricted immune responses in vitro and in vivo (i.e., blockage of antigen specific, I-A restricted helper T-cells). In this fashion, we hope to delineated the role(s) of particular epitopes in antigen presentation and MHC (Ir) restriction.

0331 Expression of HLA-DR heavy chain by transmissible retrovirus vectors.
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The study of the mechanism of immune response genes requires the expression and manipulation of the DR/Ia antigens in several cell types. We have approached this problem through the development of retrovirus vectors. We have constructed a Moloney Leukemia Virus (MLV)-derived vector which contains a selectable marker (G418 ^{r} in eukaryotic cells and Kan ^{r} in bacteria) whose expression is independent of the MLV long terminal repeats (LTRs). cDNA and genomic clones of the HLA-DR heavy chain have been inserted into these vectors under the control of the MLV LTRs and transfected into ψ 2, a 3T3 line which packages defective viral genomes in trans (Mann et. al.(1983) Cell, 33, 153). Supernatants from transient harvests of these transfections were used to infect 3T3 cells, which were then selected for growth in G418. G418 resistant colonies were shown to express the HLA-DR heavy chain by immunoprecipitation of metabolically labelled cells. The structures of the transmitted genomes has been analyzed by Southern blotting.

0332 NUCLEOTIDE SEQUENCE OF THE MUTANT I-A BETA GENE FROM THE B6.C-H-2^{bm12} MOUSE,
Katherine R. McIntyre and J.G. Seidman, Harvard Medical School, Boston, MA 02115

Nucleotide sequence analysis of the coding regions of a mutant immune response (Ir) gene, the I-Aβ gene of the B6.C-H-2^{bm12} (bm12) mouse, has revealed three nucleotide substitutions relative to the previously sequenced wild-type I-Aβ^{b} gene of C57BL/6 (B6) mice. All three nucleotide substitutions occur within a stretch of 14 nucleotides in the exon encoding the first extracellular (β_1) domain. Each nucleotide substitution leads to an amino acid replacement, two of which are nonconservative. Clustered mutations of this type that occur in a single event are generally thought to result from a gene conversion event or from the reciprocal exchange of genetic information between closely related sequences. Amino acid sequence comparisons reveal that two out of the three amino acid substitutions found in the bm12 I-Aβ chain relative to I-Aβ^{b} are found at the same positions in several human class II antigen β chains. These sequence comparisons suggest the possibility that the bm12 mutation in the I-Aβ gene arose from a gene conversion-like event in which another class II β chain gene acted as the donor sequence. Nucleotide sequence analysis is in progress to determine whether the I-Eβ^{b} gene could have served as this donor sequence. While gene conversion-like events may play a role in the generation of polymorphism in class II antigens, we suggest that the high level of this polymorphism is primarily due to selection.

0333 MOLECULAR GENETICS OF THE HUMAN CLASS II ANTIGENS, Bernard Mach, Jack Gorski, Michel Strubin, Eric O. Long and Claude de Préval, Department of Microbiology, University of Geneva Medical School, Geneva, Switzerland

Studies on the organization and number of human class II β chain genes have established the existence of 3 non-allelic HLA-DR β chain genes (2 of which have been found linked 12 kb apart), 2 DC β chain genes, and 2 genes for SB β chain, one of which is flanked by 2 α chain genes. Thus, 7 β chain genes have been identified and cloned. Sequence comparisons show that the variation among DR β chains are clustered within the first domain. The genes of DR, DC and SB have diverged equally and have maintained conspicuous segments of totally conserved DNA sequence in the first domain. Comparison of human and mouse class II genes confirms the DR/I-E and DC/I-A correlation and reveals in the mouse I region a gene called E*β2 as the murine equivalent of SB β. The human D region is clearly more complex than the mouse I region. Studies on the expression of the different class II genes indicates a coordinate regulation : induction in response to γ interferon of all three subregion DR, DC and SB, and surprisingly also of the gene for HLA-associated invariant chain gene, which is located on another chromosome.

0334 GENE TRANSFER OF H-2 CLASS II GENES: ANTIGEN PRESENTATION BY MOUSE FIBROBLASTS AND HAMSTER B CELLS, B. Malissen, M. Price, J. Goverman, M. McMillan, J. White, P. Marrack, A. Pierres and L. Hood, California Institute of Technology, Pasadena, CA 91125; University of Southern California, Los Angeles, CA 90033; National Jewish Hospital, Denver, CO 80206; Centre d'Immunologie de Marseille-Luminy, France.

We have transferred the mouse A^k_α and A^k_β genes, which encode the class II I-A^k molecule, into mouse L cell fibroblasts and hamster B cells. I-A^k molecules are expressed on the surface of both cell types. The class II genes require retrovirus long terminal repeats to be expressed in L cells, a tissue type which does not ordinarily express class II molecules. The L cell I-A^k molecules appear normal by serological analyses and two-dimensional gel electrophoresis. Furthermore, the I-A^k molecules on L cells can act as targets for the allogenic T-cell killing of the transformed L cells. The I-A^k molecules in both mouse fibroblasts and hamster B cells can present certain antigens to T-cell helper hybridomas. Thus it will be possible to dissect the structure-function relationships existing between Ia molecules, foreign antigen and T-cell receptor molecules by in vitro mutagenesis and gene transfers.

0335 MOLECULAR ANALYSIS OF THE Ia AND COMPLEMENT GENES FROM THE B10.WR7 (H-2^{WR7}) WILD MOUSE STRAIN, Marie Malissen, Elizabeth Gibb, Michael Steinmetz* and Lee Hood, California Institute of Technology, Pasadena, CA 91125; and *Basel Institute for Immunology, Basel, Switzerland

We have cloned from a liver DNA cosmid library the immune response (I) region of the major histocompatibility complex of the B10.WR7 (H-2^{WR7}) wild mouse strain. Our analysis has identified all of the four serologically defined Ia antigens. In addition, two more Ia genes or pseudogenes designated $A\beta_2$ and $E\beta_2$ have been cloned. Chromosomal walking procedures have established the linkage relationships of these genes on chromosome 17. Comparison of the restriction maps of the I regions from B10.WR7, AKR and BALB/c revealed that their right halves are virtually identical whereas their left halves differ extensively (Steinmetz et al., manuscript in preparation). We have recently cloned complement genes of the B10.WR7 mouse. We will presently attempt to express cloned complement genes through gene transfer experiments.

0336 COEXPRESSION AFTER GENE TRANSFER OF HUMAN HLA HEAVY CHAIN AND HUMAN β2-MICROGLOBULIN IN MOUSE L CELLS, Richard T. Maziarz, Carmelo Bernabeu, Cox P. Terhorst, and Steven J. Burakoff, Dana-Farber Cancer Institute, Boston, MA 02115

Mouse L cells have been demonstrated to synthesize and express HLA-A2 and HLA-B7 gene products after DNA mediated gene transfer of cloned copies of A2 and B7 genes. The preliminary DNA sequence of these clones is consistent with the published protein sequence of the A2 and B7 gene products isolated from the same cell line (P.A. Biro, personal communication). However, functional recognition was not found by bulk mouse anti-human xenogeneic cytolytic T lymphocytes (CTL) directed to the A2 or B7 gene products or by HLA specific human CTL clones. Since these transfectants did not express the human β2-microglobulin, we tested whether the lack of CTL recognition was due to the absence of the human β2-microglobulin on the cell surface. Specifically, we used a mouse L cell line (J27)[1] that was previously transfected with large human genomic fragments, and selected for its expression of the human β2-microglobulin and then re-transfected with the cloned copies of the A2 and B7 genes. Several clones were obtained that demonstrated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and isoelectric focussing(IEF) the expression of HLA molecules associated with the human β2-microglobulin. Again, neither human CTL clones nor bulk mouse xenogeneic CTLs were found to specifically lyse these transfected target cells. Due to the failure to lyse these cells, further experiments to elucidate the role of other species specific molecules needed for the lytic mechanism are in progress.

1. Kavathas and Herzenberg, PNAS 80:524, 1983.

0337 ISOLATION AND CHARACTERIZATION OF cDNA CLONES FOR THREE MURINE I-E_β ALLELES, Laurel Mengle-Gaw and Hugh O. McDevitt, Stanford University Medical Center, Stanford, CA.
I region restriction and genetic control of specific immune responsiveness implies a certain degree of polymorphism in the structure of Ia antigens in mice of different haplotypes, since the levels of immune responsiveness to certain antigens can vary substantially among haplotypes. We have isolated and characterized cDNA clones for the I-E_β polypeptide chain from k-, b-, and u-haplotype mice. Comparison of the predicted amino acid sequences of these clones and a recently published E_β^d genomic clone shows most allelic variability to be clustered in short stretches of peptide sequence in the first external domain. This clustering suggests that Ia-antigen interactions may occur at defined sites near the N-terminus of Ia molecules. In fact, the length and spacing of variable regions in the alleles characterized so far are reminiscent of those in the immunoglobulins. This observation leads to the prediction that, like immunoglobulins, the folding of Ia polypeptide chains may result in the juxtaposition of all 3 or 4 variable regions to form an antigen binding site.

We have compared E_β^k vs A_β^k amino acid substitutions with allelic E_β substitutions. Conserved sequence between E_β and A_β and their alleles may direct proper protein folding. The E_β vs A_β nonhomology regions in the first domain are generally the same regions where E_β alleles differ, and also where A_β alleles differ from one another. There are E_β vs A_β nonhomology regions in the second domain where there is no E_β nor A_β allelic variation. These regions may represent peptide sequences required for association of α and β chains--regions conserved among alleles which direct association with the proper class IIα chain

0338 FUNCTIONAL EXPRESSION OF MURINE CLASS II MHC GENES, Michael A. Norcross, David H. Margulies and Ronald N. Germain. NIAID-LI, NIH, Bethesda, Md 20205
To study the structural basis for the class II (Ia) restricted recognition of antigen by T lymphocytes, we have isolated cloned A_β and A_α genes from genomic libraries and introduced these genes into a variety of cell lines. Spheroplast fusion was used to introduce a covalent construct of A_β^k and pSV2-gpt into the BALB/c (H-2^d) B cell lymphoma M12.4.1. Clones surviving selection in mycophenolic acid and xanthine containing medium were found to have stably incorporated the A_β^k gene and to express A_β^k on the surface as detected with 10.2.16 (anti I-A_β^k antibody). These transfectants stimulate allogeneic MLR and permit antigen stimulation of an I-A^k restricted GAT specific T cell clone. They do no stimulate, however, IL-2 release by antigen specific T cell hybridomas restricted to I-A^k. To determine if this is related to involvement of the A_α^k product in the restriction of these hybridomas, the A_α^k gene is being introduced together with A_β^k into M12.4.1. Transfectants were also established using the calcium phosphate precipitation method with LTK$^-$ cell (C_3H fibroblast) targets. Co-transformation with A_β^d and TK led to transfectants with substantial cytoplasmic A_β mRNA, but no surface expression. Addition of the A_α^d gene to such cells together with pSV2-gpt DNA, followed by selection in HAT plus mycophenolic acid and xanthine resulted in isolation of clones expressing low amounts of I-A^d on the cell surface as detected by staining with MKD6 monoclonal antibody. Preliminary studies indicate that these Ia expressing fibroblasts can present complex antigens to T cell hybridomas.

0339 TRANSFORMATION AND EXPRESSION OF β_2-MICROGLOBULIN-κ HYBRID GENES: INTERACTIONS OF CONTROL ELEMENTS, Jane R. Parnes, Kurt C. Sizer and Vernon T. Oi, Stanford University Medical Center, Stanford, CA 94305
We have been studying the control regions responsible for murine β_2-microglobulin and immunoglobulin κ light chain gene expression to determine the mechanism responsible for their differntial expression. The rearranged κ light chain gene has an intronic control element (I.C.E.) just 5' of the constant region exon which is required for transcription of the gene in long term transformants of B lymphoid cells. The I.C.E. does not act as an "enhancer" of transformation efficiencies using gpt selection. We have constructed hybrid genes in which we have replaced the 5' end of the S107 κ gene (promoter, leader, V, J and part of the following IVS). We have transfected these hybrid genes (in pSV2gpt or pSV2neo vectors) into both lymphoid cells and fibroblasts. The hybrid genes are transcribed and spliced normally to yield an mRNA consistent with the β_2-microglobulin leader exon joined to the κ constant region exon. We have found that the frequency of stable gpt- or neo-positive transformants is extremely low when the β_2-microglobulin 5' end replaces the normal κ 5' end in the presence of I.C.E., but that the efficiencies increase from 5-50 fold in both lymphoid cells and fibroblasts when the I.C.E. is deleted. The results suggest that the 5' end of the β_2-microglobulin gene contains an "enhancer" of transformation (possibly acting by increasing gpt or neo transcription) which is inhibited by the κ I.C.E., and that different control elements can interact with each other in unexpected ways.

0340 AT LEAST SEVEN GENES ENCODE β CHAINS OF HUMAN CLASS II HISTOCOMPATIBILITY ANTIGENS, Cecile Tonnelle and Eric O. Long, Laboratory of Immunogenetics, NIAID, National Institutes of Health, Bethesda, MD 20205

The HLA-D region of the human major histocompatibility complex encodes multiple class II antigens. These highly polymorphic antigens consist of two subunits, the α and the β chains. At least seven β chain genes exist in the D region. The nucleotide sequence homology between the β genes clearly shows that the SB, DC and DR subregions are evolutionary distinct. Multiple β genes within the subregions share a much higher nucleotide sequence homology and must have arisen from more recent gene duplications than those which generated the SB, DC and DR subregions. Comparisons with β genes of the murine I region show that the SB, DC and DR divergence preceded mammalian radiation. The murine analogue of the SB β genes appears to be the Eβ2 gene. In contrast to the human D region, recent gene duplications are not apparent in the murine I region. We are cloning chain genes from a hemizygous mutant cell line in order to analyze further the multiple nonallelic β genes. The functional role of these various β chains will be tested by transfection experiments in B cells.

0341 ORGANIZATION OF THE H-2 I REGION OF THE B10 MOUSE., Claire T. Wake, Georg Widera, Torben Lund, and Richard A. Flavell, Biogen Research Corp., Cambridge, MA 02142

More than 150 kilobases from the I region of the B10 mouse ($H\text{-}2^b$) has been analyzed by a series of overlapping cosmid clones. The overall organization of the $H\text{-}2^b$ I region is similar to the published I region map of the Balb/c mouse ($H\text{-}2^d$) in that it contains the class II genes Aα, Aβ, Eα, and Eβ as well as the crosshybridizing sequences Aβ2 and Eβ2. In addition we have isolated two smaller cosmid clusters containing sequences that crosshybridize with class II β genes. One of these smaller clusters was mapped within I-E by a restriction endonuclease polymorphism. The other small cluster was linked to the class I K region by chromosomal walking.Studies on the structure and expression of these two class II β-like sequences will be presented.

Chemistry and Serology of Antigen and Idiotype Specific T Cell Secreted Factors

0342 CLONED T CELLS EXPRESS CELL SURFACE DETERMINANTS SHARED WITH THEIR SECRETED ANTIGEN-SPECIFIC FACTORS, Eric J Culbert, Sirkka Kontiainen*, Roger F L James and Marc Feldman, ICRF Tumour Immunology Unit, Dept. of Zoology, Unversity College, Gower St., London. WC1E 6BT, and Dept. of Immunology, University of Helsinki, Haartmaninkatu 3, 00290 Helsinki 29, Finland.

Conventional and monoclonal antibodies raised against antigen-specific T cell factors were used for serological analysis of regulatory factors produced by cloned helper and suppressor T cell lines. Monoclonal helper factors from keyhole limpet haemocyanin (KLH)-specific helper clones had distinct constant regions (c_t) determinants from those found on monoclonal KLH-specific suppressor factors, although these factors shared variable region (V_t) determinants. FACS analysis of cell surface determinants on these lines showed the presence of C_t and V_t determinants shared with their secreted factors, suggesting that T cells may utilise similar C_t and V_t determinants for cell surface and secreted antigen-binding molecules

0343 A NOVEL ANTIGEN-SPECIFIC SUPPRESSOR T CELL WITH CYTOTOXIC ACTIVITY, Günter J. Hämmerling and Carolyn A. Blanckmeister, Deutsches Krebsforschungszentrum, D-6900 Heidelberg, W-Germany.

A series of T cell hybridomas obtained from CBA mice immunosuppressed with low dozes of bovine serum albumin (BSA) shows two distinct functional activities, namely suppression and cytotoxicity. The T hybridomas and their culture supernatants suppress efficiently in an antigen-specific manner a proliferative response of BSA immunized lymph node T cells, whereas an ovalbumin response is not suppressed. The suppressive activity is $I\text{-}A^k$ restricted. The suppressor factor in the supernatant cannot be absorbed with sepharose conjugated BSA_k rabbit anti-v_H, anti-H-2 and most monoclonal anti-$I\text{-}A^k$. However, one particular anti-$I\text{-}A_k^k$ mAb (K25-137) which was raised against B cell Ia absorbs the factor. The same anti-$I\text{-}A^k$ also stains the cells, although faintly. These findings suggest that the Ia determinants on the factor are different from those on B cell derived Ia.
In addition to the suppressive activity the T hybridomas also display a strong NK-like activity because they lyse a series of tumors, such as YAC. The relationship between the cytotoxic and suppressive activity is not yet clear.

0344 PURIFICATION OF T CELL SUPPRESSOR FACTOR FROM HYPERIMMUNE SERUM USING MONOCLONAL ANTIBODIES. Thomas A. Ferguson, Kenneth D. Beaman and G. Michael Iverson, Howard Hughes Medical Institute, Yale University, New Haven, CT 06510

We have developed two rat monclonal antibodies (mAbs) to the T suppressor effector factor (TSF) isolated from hyperimmune mouse serum. One of the mAbs has been used to purify the TSF from hyperimmune serum to SRBC. The purified TSF is antigen specific, MHC restricted and requires a non-antigen binding chain for its biological activity. The purified TSF gives a single band of 68,000 daltons on an SDS-PAGE, under reducing conditions. 2D gel analysis reveals that some of the 68,000 mw material is reduced from a band that has an apparent molecular weight of 135,000 daltons. ELIZA analysis shows that the two monoclonal antibodies bind different antigenic determinants on the TSF molecule. Interestingly, one of them binds the TSF only when the TSF is bound to plastic or when it is bound by the other monoclonal antibody. However both monoclonal antibodies can be used to: 1)stain suppressor T cells and 2)to inhibit the *in vitro* suppressor activity of the TSF. In addition the monoclonal antibodies bind some but not all TSF's from other systems differing in both antigen specificity and MHC restrictions, suggesting that the monoclonal antibodies recognize "isotypic" determinants on TSF's. These include TSF's that have been produced by cell free *in vitro* translation of mRNA derived from T cell hybrids and splenic T cells from mice hyperimmune to SRBC. These monoclonal antibodies are being used as serological probes in experiments designed to understand the nature of T suppressor factors.

0345 STUDIES ON SUPPRESSOR FACTOR(S) PRODUCED BY T-HYBRIDOMAS, Young Tai Kim, Tony DeBlasio, and Gregory W. Siskind, Cornell University Medical College, New York, NY 10021

Antigen specific and non-specific suppressor T-cells were generated when spleen cells were incubated with trinitrophenylated polyacramide beads (TNP-PAA) *in vitro*. T-hybridomas were prepared by fusion between spleen cells cultured with TNP-PAA for 4 days and the thymoma cell-line BW5147. More than 100 hybridomas were generated, and 20 of them suppressed the anti-TNP PFC response of fresh spleen cells cultured with TNP-PAA. The suppression was antigen specific with 5 of these 20 hybridomas. Those hybridomas which cause antigen specific suppression typically depressed the anti-TNP PFC response by 90% while depressing the total number of immunoglobulin secreting cells (reverse PFC) by only 10%. Supernatants from hybridomas that caused antigen specific suppression contained a factor(s) which brought about suppression of the anti-TNP PFC response by spleen cells cultured with TNP-PAA. By ELISA the specific suppressive supernates contained material which bound TNP-BSA but which lacked antigenic determinants of mouse immunoglobulin.

0346 INDUCTION OF CYTOTOXIC LYMPHOCYTE RESPONSES BY ANTIGEN-SPECIFIC HELPER FACTORS. John F. Krowka*†, Chiaki Shiozawa†°, Erwin Diener†°, Verner Paetkau†‡, and Linda M. Pilarski†. Departments of Immunology† and Biochemistry‡ and MRC Group on Immunoregulation°, University of Alberta, Edmonton, Alberta, CANADA T6G 2H7,

Previous reports from this laboratory and others have demonstrated that antigen-specific helper factors (ASHF) derived from "helper" T lymphocytes (T_h) are able to induce cytotoxic T lymphocyte (CTL) responses from thymocyte CTL precursors (CTL_p) *in vitro*. Our recent experiments have characterized the antigen-specificity and H-2 restriction properties of ASHF. We have used EGTA, which chelates Ca^{++}, in order to dissociate the molecular subunits of ASHF. Our studies demonstrate that after EGTA treatment, the ASHF is still able to bind nominal antigen but (in contrast to untreated ASHF) now requires antigen and major histocompatibility complex (MHC)-encoded products from adherent cells syngeneic to the ASHF-producing T_h line in order to trigger CTL_p. The results of our experiments suggest an H-2 restricted molecular interaction model in CTL_p induction by ASHF. An antigen-specific T_h-derived receptor appears to require association with self MHC-encoded molecules in order to form a "complete" ASHF that is able to trigger CTL_p in an H-2 unrestricted manner. We suggest that ASHF may be required as a primary signal for CTL induction prior to the effects of non-specific lymphokines that mediate maturation and clonal expansion.

Funded by Alberta Heritage Foundation for Medical Research, Medical Research Council of Canada and National Cancer Institute of Canada.

0347 SPECIFIC SUPPRESSION OF RESPONSES TO LEISHMANIA TROPICA BY A CLONED T CELL LINE AND ITS CULTURE SUPERNATANT, F.Y. Liew, The Wellcome Research Laboratories, Beckenham, UK.

BALB/c mice are exceptionally susceptible to L. tropica infection. The disease can be induced with minimum infecting doses and is inexorably progressive, accompanied by specific suppression of delayed-type hypersensitivity (DTH). This potentially curative cell-mediated immunity is abrogated by the generation of a specific Lyt-1$^+$2$^-$, I-J$^-$ population of suppressor T (Ts) cells. A cloned T cell line (LTC5) derived from this Ts cell population has been established and maintained in long term culture. LTC5 and its culture supernatant specifically suppressed the induction of DTH to L. tropica. They also inhibited the in vitro proliferation of T cells to L. tropica antigens, but not T cells specific for alloantigens. LTC5, but not the culture supernatant, was able to significantly enhance lesion development in infected animals.

0348 INDUCTION OF A NONSPECIFIC T SUPPRESSOR FACTOR FROM DNFB-PRIMED Ts-AUXILIARY CELLS FOLLOWING ACTIVATION WITH A MONOCLONAL DNP-SPECIFIC T SUPPRESSOR FACTOR. Stephen D. Miller, Northwestern Univ. Med. School, Chicago, IL 60611

26.10.2 is a T cell hybrid clone producing a monoclonal, efferent-acting factor (mDNP-TsF) which suppresses passive tranfer of DNFB contact sensitivity after incubation with DNFB-immune T cells (T_{DH}) for 1 hour at 37°C. mDNP-TsF is antigen-specific (DNFB-, but not TNCB- or oxazalone-immune T_{DH} are suppressed) and its activity is restricted by H-2D gene products. The factor is Ig$^-$, bears H-2 $\perp$ region determinants and a receptor(s) for DNP. Direct, MHC-restricted suppression of DNFB-immune T_{DH} is independent of antigen-primed Ts-auxiliary (Ts-aux) cell activity as T_{DH} from cyclophosphamide (CY) treated mice are suppressed. In addition, mDNP-TsF induces a secondary, nonspecific TsF upon incubation with syngeneic, DNFB-primed (not TNCB-primed) T_{DH} from untreated mice. CY-treatment of the DNFB T_{DH} donors resulted in no secondary factor production indicating the requirment for Ts-aux cells. The secondary factor producing cells are Thy 1$^+$, Lyt 1$^+$2$^-$, I-J$^+$, a phenotype similar to our previously described Ts-aux cell. Both H-2 and non-H-2 restrictions are required for induction of the nonspecific secondary Ts-aux factor by mDNP-TsF (mDNP-TsF activated BALB/c, but not B6.C-H-2^d or C.B6-H-2^b DNFB-immune cells to produce factor). The non-H-2 restricting element appears to be an IgV_H product. Once induced, the ability of the Ts-aux factor to suppress DTH shows no apparent antigen, H-2, or IgH restrictions. Thus, a monoclonal TsF can act directly on relevant DNP-specific T cell targets or indirectly via activation of a secondary, nonspecific Ts-aux factor. (Supported by USPHS NIH Grant AI-18755)

0349 SUPPRESSOR T CELL CLONE SPECIFIC MONOCLONAL ANTIBODIES, P. Ricciardi-Castagnoli, F.Robiati,F.Sinigaglia,C.Pini°,G.Doria°°,L.Adorini°°,CNR Center of Cytopharmacology,Dept. of Pharmacol.,Milano;°Ist.Sup.Sanità,Roma; °°Lab. Immunogenetica, ENEA, Roma.

Two suppressor T cell clones, LH_8-105 and LA_{41}, obtained by RadLV induced transformation of T lymphocytes has been demonstrated to constitutively release products able to specifically suppress the antibody and T cell mediated response to hen-egg white lysozyme (HEL) and Torpedo nicotinic acetylcholine receptor (AChR) respectively. Monoclonal antibodies specifically recognizing surface structures of these T cell clones have been identified by solid phase radioimmunoassay and flow cytometry.

Surface molecules immunoprecipitated from these clones may represent the clonotypic receptor of suppressor T cells. Structural and functional analysis of T cell products recognized by these monoclonal antibodies will be presented.

0350 RECEPTORS AND FACTORS FROM HUMAN T LYMPHOCYTE CLONES, Ed. D. Zanders, Jonathon Lamb and Marc Feldmann, Human Tumour Immunology Unit, University College Hospital, University Street, London WC1E 6JJ.

A series of T cell clones specific for influenza virus have been generated and maintained in IL-2 containing media. One of these, HA1.7 was shown to proliferate in response to a synthetic peptide corresponding to the C terminal portion of HA1, p20. Direct interactions between HA1.7 and this peptide, p20, were shown in binding studies with labelled cells or antigen. This clone was also shown to bear receptor like molecules by surface iodination and ~~non-cellular antigen~~ non-reducing ~~producing~~ electrophoresis. We have previously shown that the generation of non responsiveness in HA1.7 using superoptimal doses of p20 was the result of a down-regulation of the receptor associated T3 antigen. To explore this further, the relationship between receptor subunits and T3 was investigated using chemical cross linking techniques.

HA 1.7 was also shown to function on a helper cell in specific B cell antibody responses. In addition, supernatants derived from these clones either by culture alone, or with presenting cells and antigen, contained a helper factor activity which appeared to be genetically restricted.

The Development and Diversity of B Cells

0351 CHARACTERISTICS OF COLONY-FORMING CELLS MAINTAINED IN DEXTER CULTURE AND POSSIBLE REGULATORS INVOLVED IN THEIR MAINTENANCE, Thomas A. Alberico, James M. Ihle, Helen E. McGrath, Richard J. Gualtieri, Lisa A. Rexrode and Peter J. Quesenberry, University of Virginia School of Medicine, Charlottesville, Virginia and NCI Frederick Cancer Research Facility, LBI Basic Research Program, Frederick, Maryland.

Interleukin-III (IL-3) induces Thy-1 in marrow cells, stimulates granulocyte, macrophage and megakaryocyte colonies, and maintains murine marrow cell lines such as FCD-P1. The major T cell derived hemopoietic colony stimulating activity (CSA) is GM-CSA-II, which is biochemically distinct from IL-3. Stem cells responsive to purified IL-3 and GM-CSA-II were assayed in soft agar-McCoy's cultures from the supernatant and stromal phases over 6 weeks of long term murine liquid marrow culture. IL-3 responsive stem cells were maintained in supernatant and stroma (at 6 weeks 38,000/flask and 50,610/flask, respectively) while GM-CSA-II responsive stem cells rapidly fell to near 0 in supernatant and low levels in stroma (at 6 weeks 10,020/flask). With an agar overlay system, hemopoietic colony formation induced by irradiated Dexter stroma is similar to that seen with IL-3 or GM-CSA-II. However, when 2-7 week old normal murine (BDF1 or C57B1) Dexter stroma was overlayed with FDC-P1 cells in McCoy's-agar, clonal proliferation was seen and this was markedly increased with irradiated (1100R) stroma at 3-4 weeks of culture. Peak growth at 5 days of culture was 1,249 clones (3-50 cells) and 2,500 clones (over 50 cells)/10^5 cells. IL-3 stimulated high levels of FDC-P1 clonal growth in agar but GM-CSA-II had virtually no activity. These data suggest that the Dexter stroma either produces low levels of IL-3 which act on IL-3 responsive stem cells or, alternatively, produces a unique factor active at a pre IL-3 stem cell level.

0352 Pre-B Lymphoma X B Lymphoma Hybrids Have a Pre-B Phenotype. Timothy P. Bender, Marilyn Lightfoote, David McKean and W. Michael Kuehl. National Cancer Institute Bethesda, MD and The Mayo Clinic, Rochester, MN.

Many published studies report fusion of myeloma cells to normal or transformed B cells from different developmental stages. In all cases it was found that the hybrid cells codominantly express immunoglobulin (Ig) molecules but have the phenotype of the more differentiated myeloma cell. We have tested the generality of this result by constructing somatic cell hybrids between a mouse pre-B cell line and a surface Ig positive B lymphoma cell line. The mouse pre-B cell line, 70Z/3B, expresses cytoplasmic μ heavy (H) chain but does not express surface Ia antigen, J chain, or light (L) chain. The mouse B lymphoma cell line, A202J, expresses both membrane and secreted forms of IgG_{2a} (γ_{2a},K), as well as large amounts of surface Ia antigen and cytoplasmic J chain. We have analyzed 20 independent 70Z/3B X A202J somatic cell hybrids. None of these hybrids express detectable J chain mRNA or secreted Ig. Eighteen hybrids express no detectable surface Ia antigen, and two hybrids express markedly reduced levels of surface Ia antigen. All hybrids express μ H chain, but only four hybrids express markedly reduced levels of H chain (selective chromosome segregation may account for lack of H chain expression in some cases). Eighty percent of hybrids express low levels of one or both of the parental K L-chains, and 20% express little, if any, of either K chain. These results suggest that the hybrid cell phenotype resembles the pre-B cell parent and not that of the more differentiated B cell parent, contradicting the generalization that the more differentiated B cell parent determines the phenotype of somatic cell hybrids between B cells.

0353 AL-2: A NEW MARKER OF ACTIVATED LYMPHOCYTES

Judith Cebra-Thomas and Joseph M. Davie. A xenogeneic monoclonal antibody has been produced which defines a novel lymphocyte differentiation antigen. Rats were immunized with the murine myeloma P3 and fused to the nonsecreting myeloma SP2/0. One of the monoclonals produced detects an antigen present on cells from spleen, lymph node and Peyer's patch, but not thymus or bone marrow. Separation of spleen cells reveals that resting B cells, but not T cells express the antigen. Both activated T and B lymphocytes express the antigen at high levels after mitogenic stimulation. LPS-stimulated B cells show an increased expression of AL-2 beginning about 36 hours after stimulation and reaching a plateau between 48 and 60 hrs. Low doses of anti-IgM (5 μg/ml) was not sufficient to induce increased expression. This suggests that AL-2 is not required for the initial stages of activation, but instead is required either for the maintainance of the activated state or for cell division. Supported by AI-18440.

0354 DEVELOPMENTAL STAGE SPECIFIC REGULATION OF TRANSFECTED IMMUNOGLOBULIN GENES Robert J. Deans, Ronald Law, and Randolph Wall, Dept. of Microbiology and Immunology, UCLA Los Angeles, CA 90024

The developmental stage specific expression of immunoglobulin (Ig) light and heavy chain genes is regulated in part at the level of transcriptional initiation or alternate RNA processing. The re-introduction, into murine lymphocytes, of Ig genes with specific domain or control element alterations is a strong approach to identifying the molecular basis for this control.

The enhancer elements of both mu heavy chain and kappa light chain genes function differently in pre-B cells when assayed for rescue of polyoma T-Ag expression in an appropriate shuttle virus vector. This pattern reflects the stage specific expression of heavy chain genes prior to light chain gene expression in B lymphocyte maturation. These data will be presented in combination with in vitro transcription data from purified light chain minichromatin in attempts to define and/or isolate light chain specific transcription factors.

In vitro generated mutations in mu heavy chain gene sequences involved in the regulated switch from membrane to secreted forms of IgM have been constructed. The expression of these mutants in developmentally staged lymphoctye lines will be presented, as well as their response to maturation factor stimulus.

0355 IN VITRO CULTURE OF MURINE B LYMPHOCYTES FROM FETAL LIVER. Kathleen A. Denis, Joy I. St. Claire and Owen N. Witte. UCLA, Los Angeles, CA 90024.

The earliest identifiable B lymphocytes are found in the fetal liver of prenatal mice. It is here that early progenitors of lymphocytes and other hematopoeitic cells reside and differentiate. Unfortunately, non-transformed populations of these cells have been difficult to obtain for study. Using an adaptation of our long term bone marrow culture conditions (Proc. Natl. Acad. Sci. USA 79:3608, 1982), liver cells from mid-gestational fetuses of Balb/c mice have been successfully cultured in vitro. These cells proliferate in culture when placed over an established adherent feeder layer derived from bone marrow cells and have also been cloned in a similar manner. These cultures have from 5-20% immunoglobulin positive cells and thus are a source of B lymphocyte lineage cells for study. The cultured fetal liver cells are being compared at both the protein and nucleic acid levels to fresh fetal liver and Abelson MuLV transformants of fresh fetal liver and transformants of various stages of the cultured cells to further our understanding of immunoglobulin expression in early B lymphocytes.

0356 FOUR COLOR IMMUNOCYTOFLUOROMETRIC DEFINITION OF B CELL SUBPOPULATIONS, Richard R. Hardy, Kyoko Hayakawa, David R. Parks and Leonard A. Herzenberg, Stanford University, Stanford, CA 94305

Subpopulations of mouse B cells express different amounts of two antigens (BLA-1 and BLA-2) recognized by rat monoclonal antibodies (53-10.1 and 30-E2). Immunofluorescence analysis on the FACS shows that the 10.1 monoclonal antibody reacts with a similar proportion of spleen cells from normal and CBA/N (xid) mice, whereas E2 reacts with most CBA/N B cells but only with a fraction of normal B cells. In order to investigate the distribution and relationship of these B cell subset antigens more thoroughly, we developed four color immunofluorescence techniques using algal-derived fluorescent proteins that permit simultaneous quantitative measurements of the levels of four surface antigens along with forward and right angle scatter. We excite fluorescein and phycoerythrin fluorescence by the 488 nm argon ion laser and excite phycocyanin and allophycocyanin fluorescence by the 600 nm dye laser. Data from four color immunofluorescence analyses with xid, nude and normal mice suggest that the order in which BLA-1 and BLA-2 are lost during B cell differentiation distinguishes two B cell lineages: immature B cells express both antigens; intermediate stage B cells of one lineage express only BLA-1 and of the other, only BLA-2; and mature resting B cells express neither. CBA/N mice lack one of the putative intermediate populations ($BLA\text{-}1^{+}/2^{-}$); the other putative intermediate population ($BLA\text{-}1^{-}/2^{+}$) is decreased (but not completely absent) in nude mice. Surprisingly, both BLA-1 and BLA-2 antigens rapidly reappear after specific (antigen) or nonspecific (LPS) triggering. IgM PFC derived from such triggered cells continue to express both antigens while IgG PFC express only BLA-1.

0357 ONTOGENY OF B CELL MARKERS IN HUMAN FETAL LIVER, F.N. Hofman, J. Danilovs, L.A. Husmann, C.R. Taylor, University of Southern California, Los Angeles, CA 90033

Fetal liver has been considered the site of B cell development in mammalian systems. In the past, stages of B cell maturation have been recognized using immunoglobulin isotype markers. In this study we have used monoclonal antibodies to B cell antigens: BA-1 and B-1, as well as heavy chain markers, to identify B cell lineage in human fetal liver. We examined frozen sections of fetal liver specimens from 12 to 22 weeks gestation, using single and double immunoperoxidase staining methods. The results demonstrate that both immunoglobulin markers and B cell antigens appear sequentially and increase in frequency with age. BA-1-positive cells are present at the earliest time period (12 to 13 weeks). A subpopulation of these BA-1-positive cells are μ positive. At 15 to 16 weeks gestation, δ-positive cells are visible. Double staining studies demonstrate that the δ-bearing cells also express the B-1 antigen. By 20 to 22 weeks gestation, three distinct B cell populations can be recognized: BA-1-positive; BA-1, μ-positive; BA-1, μ/δ, B-1-positive. Thus the expression of specific B cell antigens as well as immunoglobulin isotypes reflect different stages of B cell maturation.

0358 PLEIOTROPHIC EFFECT OF THE xid GENE ON THE IMMUNE RESPONSE TO PHOSPHOCHOLINE (PC) IN CBA/N X CHROMOSOME CONGENIC MICE. James J. Kenny, Gretchen Guelde, Carl Hansen and James J. Mond. USUHS and NIH, Bethesda, MD 20814.

The immune response to PC-KLH has been analyzed in 6 strains of mice made congenic for the CBA/N X chromosome. When the PFC responses of these xid congenic strains were compared to both the CBA/N and the backcross parental strains, two strains, C.CBA/N and C_3.CBA/N, exhibited responses which were only 5 to 10% of those observed in either the parental or the CBA/N strain. This altered immune responsiveness is exhibited not only in the in vivo immune responses to PC-KLH but also in the in vitro responses of the spleen cells from these strains to B cell mitogens. Thus, the response of C_3.CBA/N and C.CBA/N spleen cells to LPS, and Nocardia water soluble mitogen is less than 25% of that seen in CBA/N spleen cells, whereas, their responses to T cell mitogens remains unaltered. Although we have previously shown that CBA/N and CBA/N x BALB/c F_1 male mice produce anti-PC-KLH antibodies which bear little or no T15 idiotype, 3 of the 6 strains of xid congenic mice tested produce a T15 dominant IgM response and significant levels of $T15^+$, IgG antibody. These data show that the background (non xid) genes of a given strain can significantly alter the expression of the xid genetic defect.

0359 DIFFERENTIATION ANTIGENS EXPRESSED BY B CELL PRECURSORS, John P. McKearn, Basel Institute for Immunology, Basel, Switzerland.

Two monoclonal antibodies (MAbs) have been selected which recognize cell surface antigens expressed preferentially by early B cell populations. Several independent lines of evidence support the conclusion that MAb clones AA4 and GF1 recognize different antigens displayed on B cell precursors and a subset of immature surface IgM-positive ($s\mu^+$) cells. MAb AA4 binds to all cytoplasmic IgM-positive ($c\mu^+$) cells found in adult bone marrow or among a panel of Abelson virus-transformed cell lines derived from fetal liver and adult bone marrow. MAb GF1 reacts with approximately 50% of all cells expressing $c\mu$. Both MAbs detect subsets of $s\mu^+$ cells. Bone marrow has the highest relative frequency since roughly half of all $s\mu^+$ cells co-express AA4 and/or GF1. These antigens are not expressed at high frequency among cells from peripheral lymphoid organs, while they are present on pre-B cells and B cells in the fetal liver. Fifty to 65% of all $s\mu^+$ LPS-inducible B cells in the bone marrow express one or both of these markers, whereas small percentages of LPS-responsive $s\mu^+$ spleen cells bear AA4 or GF1. Ontogenetic studies, as well as induction experiments using the LPS-responsive pre-B cell lymphoma 70Z/3, indicate that AA4 precedes the appearance of GF1. These MAbs detect cell surface molecules preferentially expressed by B cells during early stages of differentiation. These determinants are distinguished from those defined previously (B220, ThB, Lyb2) by ontogenetic differences and by their selective recognition of immature B cells. Results will be presented which define the functional role of these antigens in B cell differentiation.

0360 THE MURINE B CELL REPERTOIRE RESPONSIVE TO SALMONELLA TYPHIMURIUM, Eleanor S. Metcalf and Lise W. Duran, Uniformed Services University, Bethesda, MD 20814

Previous studies have analyzed the specific B cell repertoires for a variety of synthetic antigens. However, to date, no studies of the B cell repertoire specific for a bacterial infectious disease agent have been undertaken. Therefore, we have modified the splenic focus system to analyze the B cell repertoire specific for S. typhimurium strain TML (TML). This system permits the identification and characterization of individual B cells and their products. Our studies define the conditions for obtaining monoclonal responses to acetone-killed and dried TML in CBA/Ca mice. The frequency of primary TML-specific splenic B cells is approximately $1/10^5$. Only 20% of these B cells are specific for the major Salmonella surface antigenic determinant, the LPS molecule. In contrast, the frequency of secondary TML-specific B cells is approximately $1/10^3$ and the majority of these clones are specific for LPS. The fine specificity of the secondary anti-LPS clones is being analyzed using a panel of bacterial immunoadsorbents which selectively express O antigens. Taken together, these results suggest that the frequency of primary TML-specific B cells is extremely low but expands approximately 100-fold after antigen exposure and that the LPS-specific B cell clones apparently expand preferentially. Preliminary studies suggest that Salmonella-susceptible, CBA/N mice have a reduced number of TML-specific cells, even after priming. These studies and the studies with normal B cells should elucidate the extent of the B cell repertoire for an infectious disease agent, and provide insights into the regulation of repertoire expression since Salmonella typhimurium antigens cross-react with environmental agents.

0361 Regulated expression of cloned immunoglobulin genes. A. Ochi, T. Hawley, R.G. Hawley, R. Tisch, and N. Hozumi. The Ontario Cancer Institute and Department of Medical Biophysics, University of Toronto, Toronto, M4X 1K9, Canada.

We have established a gene transfer system using the immunoglobulin (Ig) genes (μ_{TNP} and k_{TNP}) specific for hapten 2,4,6-trinitrophenyl(TNP). Functional pentameric IgM production was observed in several representative lymphoid cells after gene transfer (Ochi. et al. Nature 302:340 (1983), Ochi et al. Proc. Natl. Acad. Sci. USA, in press). Since Ig expression is a specialized function of the B cell lineage, we are also investigating whether expression of Ig genes can occur in heterologous cells. Our results indicate that the Ig genes are aberrantly transcribed in cells of non-B cell lineage and the k chain gene is more rigorously regulated than the μ chain gene. The expression of the transferred μ_{TNP} and k_{TNP} genes are differentially regulated in pre-B cells as well. Furthermore, we have constructed a plasmid carrying both μ_{TNP} and δ genes and this plasmid has been transfected into several lymphoid cell lines in order to study the differential expression of Ig heavy chain genes. These results will be discussed in relation to B cell specific differentiation factor(s) and enhancer elements. A.O. was supported by a Terry Fox Cancer Research Fellowship.

0362 FUNCTIONAL CHARACTERISTICS OF BONE MARROW DERIVED PRE B CELL LINES. Ryuji Nagasawa, Osami Kanagawa and Jacques Chiller, Lilly Research Laboratories and Scripps Clinic and Res. Fndn., La Jolla, CA 92037

Long term culture of a pre-B cell line was established from Balb/c bone marrow by the method described by Whitlock, et al. Propagation of such non-adherent pre-B cells was dependent on adherent filler cells, also derived from bone marrow. The non-adherent cells possessed the following cell surface characteristics (FACS analysis): μ^-, k^-, Ia^-, 14.8^+ (B cell marker). Cytoplasmic immunofluorescence demonstrated the presence of μ but absence of k chain. Such pre-B cells did not respond in vitro to mitogens such as LPS or $DxSO_4$ and were not stimulated by specific helper T cells. Furthermore, various supernatant fluids (SN) containing IL-2, IL-3, GM-CSF or M-CSF were found to be incapable of maintaining pre-B cell growth in the absence of adherent marrow cells. When such pre-B cells (Balb/c origin) were transferred to heavily irradiated $B_6D_2F_1$ mice, it was possible to recover splenic cells which were able to respond to LPS in vitro. These in vivo passaged pre-B cells expressed μ chain as well as the 14.8 marker. Furthermore, the response observed was abrogated by anti $H\text{-}2^d$ serum and complement (C) but not anti $H\text{-}2^b$ and C, demonstrating that the responder cells were of donor rather than host origin. Since the pre-B cell population did not give rise to granulocytes, macrophages and other cell types in an in vitro colony assay, it would appear that cells recovered from the in vivo passage were derived from the transferred pre-B cells rather than from contaminating stem cells. The mechanisms of in vivo and in vitro conversion from pre-B cells to mature B cells are being currently investigated.

0363 CYTOFLUOROMETRIC ANALYSIS OF T-CELL DEPENDENT, ANTIGEN-SPECIFIC B-CELL ACTIVATION, Randolph J. Noelle, E. Charles Snow, J.W. Uhr and E.S. Vitetta, University of Texas Health Science Center, Dallas, Texas 75235

We have studied the activation of enriched populations of trinitrophenyl (TNP)-binding B cells (TNP-ABC). Carrier-primed T cells and the TNP-carrier have been shown to induce antigen-specific proliferation and differentiation of TNP-ABC. Subsequent to their activation by T cells and TNP-carrier, B cells undergo alterations in the expression of membrane antigens, enter the cell cycle, increase in size and differentiate into cytoplasmic immunoglobulin-positive (cIg^+) cells. The functional status (cytokine responsiveness, antibody producing capacity, etc.) of the TNP-specific B cells has also been assessed. After activation by T cells and antigen, the expression of sIgD is decreased and the expression of Ia is increased 2-4-fold. This expression of Ia was maximal by 48 hours after activation, and returns to normal levels by day 5. Acridine orange staining has been used to establish that both T-cells and TNP-carrier are required to induce resting G_0 B cells to enter cycle. Cells that go through cycle, become cIg^+ and remain in G_1.

0364 DIFFERENTIATION OF B CELL PRECURSORS IN AGAR CULTURE. C.J. Paige, R.H. Gisler, and J.P. McKearn. Basel Inst. for Immunol. Basel, Switzerland.

We have established a semi-solid in vitro assay in which B cell precursors can be detected, quantitated, and clonally analyzed. Murine fetal liver cells, dispersed in supportive medium containing 0.3% agar and plated over a previously established adherent layer of fetal liver cells gave rise to numerous colonies, many of which were morphologically identifiable as myeloid colonies. By the 8th day of in vitro culture colonies containing antibody secreting cells were also found. That the cell which gave rise to the plaque forming colonies was a B cell precursor is suggested by a delayed onset of antibody secretion, delayed reactivity to LPS, large size as assessed by sedimentation rate, the failure to adhere to anti-μ antibodies and their presence in 12-16 day fetal liver. On day 14 the precursor cells were positive for antigens detected by 14.8 and AA4 monoclonal antibodies. The slope of the log/log relationship between input cell number and the number of plaque forming colonies detected (0.99) suggested that only one cell type is limiting under the conditions used.

Similar results were obtained when the fetal liver adherent layer was replaced by fetal liver cells adherent to cytodex beads. In contrast, adult bone marrow cells adherent to cytodex beads only stimulated when fetal liver cells were present in high density and regression analysis of titration curves revealed slopes of 2-3. The colony stimulating factors CSF-1 and BPA also stimulated B cell development but also were found to be cell density dependent.

0365 LONG TERM CULTURE OF FACTOR DEPENDENT MOUSE B CELL PRECURSORS, Ronald Palacios, Geoffrey Henson, Michael Steinmetz and John P. McKearn. Basel Institute For Immunology, Basel.

We have been able to maintain for several months mouse lymphocytes derived from bone marrow and spleen of normal (CBA/J, BALB/c, C57BL/6, SJL, B10.T(6R)), nu/nu BALB/c, and autoimmune (MRL/lpr and NZB/W F1) mice. The cell lines have been classified as B cell precursors on the following basis: First, they have surface antigens characteristic of the B cell lineage (B-220, GF1.2, AA4.1) and they do not carry μ or kappa chains on their cell membrane. Second, the Ig genes coding for μ or kappa chains are rearranged in the cell lines tested. Third, immunoprecipitations of the cell lysates with anti-μ antibody show that all the cell lines studied express small amounts of μ chains in their cytoplasm. Fourth, the cell lines can be induced in vitro to mature into IgM secreting cells. The factor responsible for the growth of the pre-B cell lines is IL 3, as homogeneous IL 3 support their growth and they bind purified IL 3, suggesting the presence of receptors for IL 3 on their cell membrane. The growth of all these pre-B cell lines is totally dependent on IL 3, since in its absence these cells die in 24 h. We have not been able to establish cell lines of surface Ig positive B cells with IL 3. Together with the findings that previously described growth factors for mature B cells (BRF/BCGF) do not support proliferation of the pre-B cell lines reported here, indicate that distinct growth factors act on B cell precursors and on mature B lymphocytes. Our findings mark IL 3 as a growth factor for a subpopulation of mouse B cell precursors.

0366 HUMAN B CELL ACTIVATION BY POLYSACCHARIDE ANTIGENS, Ger T. Rijkers and Donald E. Mosier, Fox Chase Cancer Center, Philadelphia, PA 19111

Several lines of indirect evidence suggest that the murine antibody response to T-independent polysaccharide antigens is generated by a distinct subpopulation of B lymphocytes (e.g., the subset missing in the CBA/N xid mutation). No clear evidene for functional B cell populations in humans has yet emerged although some monoclonal antibodies react with only a fraction of B cells. In order to generate a functional assay of human B cells that might address a distinct subpopulation, we have established conditions for the induction of an antibody response in vitro to the 14 pneumococcal polysaccharides contained in Pneumovax®.

The antibody response could be detected both at the level of plaque forming cells (with either polysaccharide coated or prot A indicator cells) and secreted antibody in culture supernatants. The dose dependent response was optimal in cultures stimulated with 10^{-8} µg polysaccharide/ml. Using this assay (and PWM and T-dependent antigens for comparison) we will study the effects of a panel of monoclonal antibodies directed against human B lymphocytes.

0367 CLONAL CELL LINES DERIVED FROM MOUSE BONE MARROW SUPPORT THE CONTINUOUS GROWTH OF B LYMPHOCYTES IN VITRO, Debra Robertson and Owen Witte, UCLA, Los Angeles, CA 90024

The in vitro B cell culture system established by Whitlock and Witte (1) supports the continuous growth of bone marrow derived B lymphocytes. The in vitro proliferation of mass B lymphocytes is dependent on the establishment of an adherent cell layer derived from the stroma of the mouse bone marrow. The media conditoned by these adherent cells shows a soluble growth promoting activity. This activity stimulates ^{3}H-thymidine incorporation by the mass population of the bone marrow derived B lymphocytes. Clonal cell lines have been derived from these adherent cells by pausi-cloning and limiting dilution. The established cell lines produce a similar B cell growth activity. The cell type producing this activity has been analyzed by histochemical and functional analysis. The cells appear to be similar to a dendritic cell type. The clonal adherent cell lines can be maintained in serum free conditions. This has allowed preliminary biochemical analysis of the growth activity. The growth activity is heat stable and trypsin sensitive.

1. Whitlock, C.A., Robertson, D., Witte, O.N. (in press)

0368 THE MAJORITY OF PHOSPHOCHOLINE (PC) SPECIFIC MEMORY B CELLS LACKS THE COMPLEMENT RECEPTOR (CR). Tullia Lindsten, Craig Thompson, Gretchen Guelde, Lyn Yaffe, Irwin Scher and James Kenny. Uniformed Services University of the Health Sciences, Bethesda, MD 20814.

The CR is present on 80-90% of splenic B cells of normal mice. The CR and Lyb5 surface antigen seem to delineate similar functional B cell subsets, as both Lyb5$^+$ and CR$^+$ B cells are responsive to TI-2 antigens. The T15 idiotype in the primary anti-PC antibody response is restricted to the Lyb5$^+$ subset. Thus, Lyb5$^+$ B cells are responsible for the synthesis of T15$^+$ IgM, IgG3 and IgA anti-PC antibodies. In contrast, Lyb5$^-$ B cells give rise to most of the T15$^-$ IgG1 and IgG2 antibodies. Since CR$^+$ and CR$^-$ B cells of normal mice are functionally similar to the Lyb5$^+$ and Lyb5$^-$ B cells respectively, we investigated the primary and secondary antibody response of these cell populations to a TD PC antigen using an adoptive transfer system. CR$^+$ and CR$^-$ small B cells were purified by counterflow centrifugation and EAC-rosette sedimentation. Both CR$^+$ and CR$^-$ B cells give rise to T15$^+$ IgM antibodies in a primary response. However, in the secondary response the majority of both the T15$^+$ (IgM, IgG3 and IgA) and T15$^-$ (IgG1 and IgG2) plaque forming cells arose from the CR$^-$ subset. The memory response to PC in xid mice also arose mainly from the CR$^-$ subset. Although the antibody response to TNP-Ficoll was restricted to the CR$^-$ subset, the majority of the PC specific B cells responsive to the R36a strain of S. pneumoniae, a TI-2 PC containing antigen, were found in the CR$^-$ B cell subset. This could indicate that environmental antigenic stimulation results in the loss of the CR. In conclusion; 1) both CR$^+$ and CR$^-$ B cells display the T15 idiotype in anti-PC responses, 2) the CR$^-$ subset of both normal and xid mice are largely responsible for the anti-PC memory response and 3) environmental priming leads to responsiveness to TI-2 antigens in the CR$^-$ subset.

0369 THE USE OF HAPTENATED IMMUNOGLOBULIN MOLECULES TO INDUCE TOLERANCE IN ADULT AND NEONATAL B CELLS, Thomas Waldschmidt and Ellen S. Vitetta, University of Texas Health Science Center, Dallas, Texas 75235

Haptenated immunoglobulin molecules are more effective tolerogens than haptenated non-immunoglobulin proteins, thereby implicating a role for the carrier in the induction of tolerance. The role of the carrier was further examined by tolerizing B cells in vitro with a variety of haptenated immunoglobulin molecules which differ in their ability to bind to Fc receptors. Hapten-specific tolerance was induced in adult and neonatal B cells in vitro and the degree of tolerance was assessed by challenging the tolerized B cells with a thymus-independent antigen. Using B cells from adult mice, it was found that only lightly haptenated immunoglobulin molecules which bind to Fc receptors (HGG, murine IgG_1 and IgG_{2a}) were tolerogenic. Lightly haptenated non-cytophilic molecules ($F(ab')_2$ of HGG and murine IgG_3) were ineffective tolerogens. The requirement for Fc receptor binding was shown to be independent of T cells and macrophages. Using B cells from neonatal mice, it was found that lightly haptenated HGG was an effective tolerogen and that $F(ab')_2$ fragments of lightly haptenated HGG were also effective when cells from mice 6 days of age or younger were used. It appears, therefore, that Fc receptor binding can augment tolerance induced in both adult and neonatal B cells but that neonatal B cells are more sensitive to tolerance induction by the TNP-epitope per se.

0370 DISTINCT UNRESPONSIVE STATES IN IMMATURE MICE ARE MEDIATED BY CARRIER RECOGNITION. Cory A. Waters and Erwin Diener, University of Alberta, Edmonton, Alberta, T6G 2H7

In order to examine the universality of the clonal abortion model for self tolerance, neonatal CBA/CaJ mice were made unresponsive to the haptenated protein conjugates $TNP_{10}HGG$ and $TNP_{10}BSA$ by twice-weekly i.p. injections from birth. As adults, these mice display a profound antigen-specific reduction of the IgG T-dependent (T-D) humoral response. Only $TNP_{10}HGG$, however, is able to render unresponsive IgM T-D B cells as well as both IgG and IgM T-independent B cells. It is not clear whether this reflects an intrinsic sensitivity of IgG T-D B cells to negative signals from antigen or whether the sensitivity is mediated by a susceptibility to active suppression. Evidence favoring the latter interpretation comes from data showing an antigen-specific suppressor activity in $TNP_{10}BSA$, but not in $TNP_{10}HGG$, treated mice. These results would not be anticipated if clonal abortion were of general applicability as a mechanism of B cell self tolerance. T cells in both groups of tolerogen-treated mice, on the other hand, were completely and specifically deficient in their proliferative response to the tolerated antigen, as measured by the incorporation of ^{3}HTdR. Unlike hapten-specific tolerance induced by polymerized flagellin, (a) antigen-pulsed APC from normal mice do not break the unresponsive state in T cells of treated mice, and (b) antigen-pulsed APC from unresponsive mice effectively present the antigen to normal T cells in both proliferative and humoral responses. Preliminary evidence has been obtained for a titratable suppressor activity in each treated group which inhibits the proliferative response of normal T cells to the tolerated antigen. [1]Diener, E. *et al* 1976. J. Exp. Med. 143:805.

0371 CELL DIFFERENTIATION IN NUDE/X-LINKED IMMUNE-DEFICIENT MICE. Henry H. Wortis, Domna Karagogeos and Naomi Rosenberg. Tufts University School of Medicine, Boston, MA 02111

Mice expressing the nude mutation lack mature T cells and have impaired antibody responses. Mice with x-linked immune dificiency (xid) have a partial defect of immune responsiveness expressed by a failure of $Lyb3^+b5^+$ B cell differentiation and a lack of response to type II thymus independent antigens. Mice expressing both nude and xid have a severe deficit of both mature T cells and B cells. They have normal numbers of haemato-poietic colony forming units (CFU-S) but lack an early pre-B cell marker (susceptibility to transformation by Abelson tumor virus). Thus there is a defect in lymphocyte development prior to pre-B cell differentiation but after haematopoietic stem cell formation. The possibility emerges that early pre-B cell differentiation of $Lyb3^+Lyb5^+$ B cells is blocked by a mutation at the xid locus while development of mature $Lyb3^-Lyb5^-$ B cells is thymus dependent and thus blocked in homozygous nude mice.

0372 BLOCKAGE OF DIFFERENTIATION AND MATURATION PROCESSES OF B CELLS OBTAINED FROM MATURATION IMPAIRED MICE. Israel Zan-Bar, Department of Cell Biology, The Weizmann Institute of Science, Rehovot 76100, ISRAEL.

Activation of normal B cells in vitro by LPS or anti-Ig antibodies causes the appearance of new receptors specific to B cell growth and differentiation factors (BCGF, BCDF). These factors drive the activated cells to proliferate and to differentiate. In this study splenic B cells obtained from mice treated with total lymphoid irradiation (TLI) were examined for their capability to be activated and to proliferate in response to LPS or anti-Ig antibodies. Normal or TLI-treated splenic B cells of different strains of mice were incubated in vitro in microtiter plates with LPS, anti-Ig antibodies and with BCGF or BCDF and their proliferative response was measured. Rate of synthesis of BCGF receptors on the activated cells was assessed by measuring the difference in BCGF in a given medium before and after a short term incubation of BCGF with TLI or normal activated cells. The results revealed that peripheral B cells of TLI-conditioned mice were incapable of proliferating in response to LPS and anti-Ig antibodies although activation processes of the cells did take place. This was indicated by the capacity of the activated TLI-conditioned cells to absorb BCGF at a rate which was indistinguishable from that of normal activated B cells. Thus immature B cells originating from TLI-treated mice can be activated, but are blocked in their proliferative response to polyclonal activators.

Evidence for Receptor Specific Regulatory Cells

0373 REGULATION OF EXPRESSION OF IGκ AND IGλ, Linda C. Burkly and Henry H. Wortis, Tufts University School of Medicine, Boston, MA 02111

One generally held explanation for the differential expression of the two light chain isotypes is that their production simply reflects the frequency of Vκ and Vλ genes. Thus an affinity based selection process may result in the preferential stimulation of κ clones in the mouse. However, additional possibilities are that B cells bearing λ and κ belong to functionally distinct sets with different activation requirements and/or these B cells are regulated differently. Our work is directed at understanding the control of expression of κ and λ bearing lymphocytes at the cellular level. This investigation has lead to three independent observations: 1) in the immune response to DNP, the κ/λ ratio expressed is much high for a TD than for a TI-2 type antigne, 2) the $X^{\underline{xid}}$ gene preferentially reduces the level of Igλ found in nonimmune sera and 3) the *nude* gene reduces the level of Igκ while Igλ is unaltered. These findings are consistent with an hypothesis in which B cells bearing Ig κ and Igλ differ in their ability to respond to or in their requirement for T cell signals. In order to distinguish between the possibility and the affinity based explanation we have measured the affinity of the Igκ and Igλ anti-DNP antibodies made following TD and TI-2 immunizations. We find that affinity does not account for the differences in κ/λ antibody production in the early response to TD and TI-2 antigens. Data consistent with the possibility that the quantity/quality of T_H signals influences the κ/λ expressed have been obtained in an in vitro system for generating polyclonal PFC. We observe a clear difference in the κ/λ elicited by anti-μ + Con A SN versus help provided by individual T hybridomas and clones.

0374 IMMUNE REGULATION BY AUTOLOGOUS ANTI-IDIOTOPIC (ANTI-ID) T CELLS: RAPID CHANGES OF THE PHENOTYPE OF ANTI-ID T CELLS DURING A PRIMARY ANTIBODY RESPONSE. Jan Cerny, The University of Texas Medical Branch, Galveston, Texas 77550.

We have observed periodic changes in the idiotopic (Id) profile of the population of antibody plaque-forming cells (PFC) during a primary response of mice to pneumococcus (Pn). C57BL/6 mice were immunized with Pn and the specific splenic PFC were tested for expression of two distinct Id (defined by monoclonal anti-Id) of T15 family, daily for 2 weeks. Neither Id was detectable on PFC during the log phase of the repsonse (d.2 to d.4), but both Id were dominantly expressed (>70% of PFC were Id+) at the peak of the response (d.5 to d.7). There was a decrease of Id expression further on, and very little Id if any was present on the PFC between d.10 and d.14. The possibility that the apparent clonal switches may be regulated by anti-Id T cells was tested *in vitro*. Normal B cells were stimulated with Pn, either alone or in the presence of T cells isolated from the spleen of mice at the appropriate intervals after Pn immunization (see above): d.2, d.5 and d.12. B cells alone expressed a moderate level of the Id. Addition of d.2-T cells abolished the Id expression; these T cells were Lyt 1^+2^+. D.5-T cells (Lyt 1^+2^-) enhanced the Id expression, dramatically whereas the d.12-T cells (Ly 1^-2^-) suppresed the Id. All these T cells were Id-specific because (a) they influenced the Id expression but not the total PFC responses of B cells, and (b) they adhered to T15-coated plates. These data suggest that immunization with an antigen leads to a periodic activation of clone-specific suppressor and helper T cells.

0375 REGULATORY T CELLS DO NOT APPEAR TO PLAY A ROLE IN THE T15 DOMINANT RESPONSE TO PHOSPHOCHOLINE, Ann J. Feeney and D. E. Mosier, Fox Chase Cancer Center, Phila., PA

Our studies address the postulated role of regulatory T cells in the control of the murine antibody response to phosphocholine (PC). The response to PC is comprised of 3 antibody families (T15, 603, and 511/167) which can be distinguished by anti-idiotypic sera. The response to PC is dominated by the T15 family under most circumstances. The question we addressed was whether T cells promote this T15 dominance in BALB/c mice. Two approaches to this question were taken. In the first, the anti-PC response of T cell deficient nude BALB/c mice was evaluated. After immunization with S. pneumoniae R36a, both nu/nu and +/nu mice responded with a T15 dominant response. In the second set of experiments, we re-evaluated the ability of T cells from mice with the CBA/N xid defect to support a T15-dominant anti-PC response in an adoptive transfer secondary response to PC-KLH. We found no difference in the response of normal B cells supported by xid T cells as compared to normal T cells with regard to not only $T15^+$ anti-PC production, but also for 511^+ and 603^+ anti-PC antibodies. Mice were analyzed both for splenic plaque-forming cells, and for serum levels of circulating $T15^+$, 511^+, and 603^+ antibodies. In a related set of experiments, we generated long-term T cell lines specific for KLH from CBA/N x BALB ♀ (phenotypically normal) and ♂ (xid defective) mice. Both sets of T cell lines provided help for a T15-dominant IgM anti-PC-KLH response in vitro. Thus, in all of these experiments we were unable to find support for the hypothesis that regulatory T cells promote T15 dominance.

0376 IDIOTYPE CONNECTANCE IN THE IMMUNE SYSTEM: SHARED IDIOTYPY BETWEEN ANTI-AZOBENZENE-ARSONATE (ABA^+) AND ANTI-2, 4, 6 -TRINITROPHENYL (TNP^+) RESPONSES, Peter V. Hornbeck, and George K. Lewis, UCSF, San Francisco, CA 94143.

In an earlier report (J. Exp. Med. 157:1116, 1983), idiotype(id) suppression in ABA^+ responses was correlated with the prior stimulation of idiotypically related responses not specific for ABA (ABA^- responses). The id studied was id-AD8 defined by a rat monoclonal anti-id (AD8) which recognizes a determinant found on VH5 encoded heavy chains. To further define the relationships between ABA^+ and ABA^- responses, the specificity of the latter was sought. Immunization with TNP conjugated hemocyanin (TNP-KLH) reproducibly elicited TNP^+ $id\text{-}AD8^+$ antibodies. The existence of TNP^+ $id\text{-}AD8^+$ antibodies was confirmed by hybridoma production. LPS stimulated spleen cells which had been enriched on TNP coated plates prior to stimulation were fused with SP2/0, and two TNP^+ $id\text{-}AD8^+$ hybrids were identified. One was lost, and the other, TL1-N5, was shown to be a u,λ antibody which bound to immunoabsorbents conjugated with either TNP or AD8. Specific id suppression could be induced in anti-TNP responses by pretreating mice with AD8 prior to immunization with TNP-KLH. Possible regulatory influences of TNP^+ $id\text{-}AD8^+$ responses on ABA^+ $id\text{-}AD8^+$ responses will be discussed.

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0377 REGULATION OF THE EXPRESSION OT T-CELL IDIOTYPES. C.Martinez-A., P.Pereira, A.Bandeira, A. de la Hera, E.L.Larsson, P.A.Cazenave and A.Coutinho, Dep.Of Immunology, Clinica Puerta de Hierro, Madrid and Dep. of Immunobiology, Inst.Pasteur, Paris.

We have investigated the expression of anti-hapten antibody idiotypes by helper T cells with matching nominal specificities, using functional helper assay to study inhibition by monoclonal anti-idiotypic antibodies. Anti-TNP BALB/c helper cells were specifically inhibited by a monoclonal anti MOPC 460 idiotype (F6 (51) and the T-cell expression of this idiotope was qualitatively controlled by Ig-allotype linked genes and quantitatively regulated by MHC-linked genes. The expression of this antibody idiotope, however was not an intrisic property of anti-TNP T cells as it was absent in helper cell population of identical specificity but derived from anti-μ suppresed BALB/c mice.
This putative regulation of T-cell idiotypes by idiotypic interactions with the antibody and B cell comportment appears particularly complex. Thus, anti-TNP -C57 Bl/6 helper cells do not share fine specificity or idiotopes with a predominant anti-NP antibody of C57Bl/6 mice. In contrast, such helper cells are specifically inhibited by the F6(51) antibody. Furthermore, idiotypic manipulation of BALB/c mice which result in profund alterations in the B cell and antibody repertoires, have little or no effect on the expression of the relevant idiotopes on the helper cell compartment. Our results are compatible with a thymic or ontogenically restricted selection of helper cell repertoires for mimicry and/or complementarities with available antibody repertoires.

0378 THE ACTIVATION OF HAPTEN-SPECIFIC B CELLS BY RECEPTOR SPECIFIC T HELPER CELLS, P.S. Pillai and David W. Scott, Duke Medical Center, Durham, N.C. and Immunology Unit, University of Rochester Cancer Center, Rochester, N.Y. 14642

The induction of B lymphocyte responses by regulatory T cells specific for idiotype determinants has received much recent experimental support. The availability of idiotype-recognizing T cells would help to understand the mechanism of transmission of T helper signals to the appropriate antigen-specific B cells. We have induced T helper cells specific for the idiotype expressed on a monoclonal anti-fluorescein (FL) antibody, designated F9-4. These T helper cells, expanded in vitro in IL2-containing medium, proliferate in a dose-dependent manner when stimulated with irradiated F9.4 hybridoma cells. Interestingly, receptor specific T helper cell lines also induce B cells or highly purified FL-specific B cells to secrete anti-fluorescein antibody in the absence of added antigens. As few as 10^3 T helper cells were sufficient to induce significant numbers of anti-FL plaque forming cells. This response was specific for the FL hapten since no anti-TNP response was observed. Further data on the idiotype specificity and restrictions of these T helper cell lines will be presented. (Supported by AI-10716 and AI-10757)

0379 AN IMMUNOREGULATORY B CELL-B CELL INTERACTION AFFECTED BY VARIATION IN THE SITE OF D-J_H GENE REARRANGEMENT, Brian Pollok, Meenal Vakil, John Kearney and Robert Perry Institute for Cancer Research, Philadelphia and Univ. of Alabama in Birmingham

Spleen cells from BALB/c mice actively responding to phosphocholine (PC) were fused with the Ag8.653 line to form a B cell hybridoma (MH-60) which by serological and nucleic acid sequence analyses has been shown to secrete a monoclonal auto-anti-(anti-T15 idiotype) antibody. In vivo administration of MH-60 antibody suppressed $T15^+$ anti-PC antibody production in an idiotope-specific manner by activating a restricted set of anti-T15 B cells. These T15-specific B cells appear to express germline-encoded variable region gene products, develop independently from $T15^+$ B cells and suppress the target B cells in a T cell-independent fashion. Idiotypically-variant $T15^+$ anti-PC B cells which lack the idiotope recognized by the anti-T15 B cells were predictably unaffected by the in vivo treatment of MH-60 (Ab_3) antibody. A panel of BALB/c hybridomas representative of this variant $T15^+$ B cell set produce anti-PC IgM antibody of germline T15 V_H, J_H1, and T15 V_L sequence, but which possessed an altered CDR3 region structure due to an unusual site of D-J joining. Consistent with the idea that these idiotope-negative B cells which can escape this regulation are generated upon H chain rearrangement and not due to somatic mutation was the ability to detect these variants in the neonatal anti-PC B cell repertoire using the splenic focus assay. This study shows that expression of certain "regulatory" idiotopes on immunoglobulin can be influenced by variation in D-J_H gene joining and suggests that the conserved length of the CDR3 region of the heavy chain which occurs within a specific B cell clonotype may be necessitated by regulatory mechanisms rather than the conventional constraints of antigen binding or light chain pairing requirements.

0380 REGULATION OF B CELL SECRETORY DIFFERENTIATION AND CLONE GROWTH ARE MEDIATED BY DISTINCT T CELL SUBSETS WHICH RECOGNIZE DIFFERENT RESTRICTION ELEMENTS. J.W. Rohrer, C.A Janeway,Jr., and J.D. Kemp. Univ. of S. Alabama Coll. of Med.,Mobile,AL 36688, Yale Univ. Sch. of Med., New Haven,CT 06510, and Univ. of Iowa Coll. of Med., Iowa City, IA 52242.

By using the anti-TNP IgA-secreting BALB/c mouse myeloma MOPC-315, I have found that antigen-specific enhancement of B cell clonal proliferation can occur independently of secretory differentiation enhancement and that each activity is modulated by a distinct T cell subset. Immunization of BALB/c, CAF_1, or $CB6F_1$ mice i.p. with 4 x 10^8 SRBC induces 2 T_H cell subsets. One is an Ly $1^+,2^-,Qa\text{-}1^-$ T cell which selectively promotes MOPC-315 proliferation (growth) via secretion of an SRBC-specific soluble factor and requires a hapten-carrier bridge and histocompatible MØ to effect help. No T_{Hg} cell:315 cell or 315 cell:MØ histocompatibility is required. The second T_H cell is an Ly $1^+,2^-,Qa\text{-}1^+$ T cell which recognizes not only SRBC epitopes but also binds λ_2^{315} idiotopes and selectively promotes secretory differentiation of MOPC-315 non-secretory, lymphocytoid cells. T_{Hd} cells require no TNP-SRBC bridge or histocompatible MØ to work, but must see SRBC in close proximity to the targe 315 cells and are V_H restricted. Unlike the T_{Hg} cells, the T_{Hd} cells do not develop in appropriately immunized, anti-μ-suppressed mice. Thus, markedly different T_H cell:MØ:B cell interactions are required for clone growth enhancement than for enhancement of secretory differentiation. This work was supported by NIH research grant CA-28708 (J.W.R.).

0381 IDIOTYPE SPECIFIC, Lyt-1$^+$ B HELPER CELLS, David H. Sherr and Martin E. Dorf, Harvard Medical School, Boston, Massachusetts 02115

Most helper or suppressor populations which specifically regulate immune responses are composed of T lymphocytes specific for determinants on conventional antigens, allogeneic cells, antigen presented in the context of MHC determinants or immunoglobulin molecules. It is therefore generally accepted that T lymphocytes perform a major role in the regulation of immune responses. In contrast, the role of B cells as regulators of the immune response has been less well established. In this report we describe a regulatory B cell population in the NP-specific B cell response of cultures of NP-primed C57BL/6 spleen cells. This Ig$^+$, Lyt 1.2$^+$, Lyb-3$^+$, Thy 1.2$^-$, Lyt 2.2$^-$ B helper population, termed B_H, is present in normal mice and preferentially augments the PFC response of NP^b idiotype bearing B cells in the absence of T helper populations. The B_H population functions in an Igh restricted fashion and specifically binds to NP^b idiotypic determinants suggesting that its regulatory function is dependent on idiotype-anti-idiotype complementation.

0382 CTL PRECURSOR AND SUPPRESSOR CELLS IN NEONATAL TOLERANCE. B. Stockinger, ARC Institute of Animal Physiology, Babraham, Cambridge, England.

Induction of neonatal tolerance leads to profound reduction in CTL-precursor frequencies against the tolerogen as evaluated in limiting dilution analysis. The limiting dilution curves were linear and thus did not yield any evidence for the presence of a regulatory cell population in the tolerant cell inocula. This result would have been predicted in a clonal deletion model. However it could be shown that in some cases adsorption of tolerant spleen cells prior to limiting dilution culture on monolayers of syngeneic irradiated blasts bearing receptors for the tolerogen drastically increased their CTL-P frequency against the tolerogen. Adsorption on blasts with receptors for third party antigens did not result in an increase in CTL-P against the tolerogen. Frequencies of normal spleen cells and responses against unrelated antigens were not influenced by adsorption.

This finding implies that CTL-P against the tolerogen are not generally deleted in tolerant animals, but rather suppressed by a regulatory cell population which seems to be present in high frequency (as judged by the failure to dissociate it from cytotoxic precursors by mere limiting dilution) and act in an anti-idiotypic fashion. Cells adherent to the monolayers could be recovered and were shown to specifically suppress the generation of CTL in limiting dilution cultures of normal cells.

0383 REGULATION OF IMMUNE RESPONSES VIA GENETICALLY-RESTRICTED CELLULAR INTERACTIONS : AUGMENTATION OF ANTIBODY RESPONSES BY IDIOTYPE-SPECIFIC ENHANCING B LYMPHOCYTES, Hiroshi Yamamoto, Soji Bitoh and Shigeyoshi Fujimoto, Kochi medical School, Kochi 781-51, Japan.

We previously reported that the B lymphocytes obtained from BALB/c mice which have been immunized with M104E myeloma protein have an idiotype specific enhancing activity for anti-dextran B1355S antibody responses in vivo(J.Immunol.129:2069,1982). The enhancing cells were Thy-1$^-$, Lyt-1$^-$,2$^-$, nylon wool adherent and RAMG-dish adherent. Furthermore, the cells were specifically enriched by M104E myeloma protein coated dish. The significance of this B-B cell interactions were futher analyzed in vitro using various Igh-1 and H-2 congenic strains of mice. The idiotype immune B lymphocytes obtained from BALB/c and BAB-14 could enhance the anti-dextran antibody responses in idiotype specific manner. On the other hand, C.AL-20 and CB-20 strains could not induce the idiotype specific enhancing B cell activity for dextran immune BALB/c B cells. The results indicate that the capability of enhancing B cell induction is determined by the producibility of the respective idiotype in that strain of mice. Moreover, the B cells obtained from BALB.K mice immune to the idiotype cooperated well with the dextran immune B cells of BALB.K but not of BALB/c and vice versa. The cellular mechanisms of those genetically-restricted cellular interactions will be discussed.

New Approaches to Immunotherapies of Cancer

0384 Human Leulla Positive Natural Cytolytic Cells are Potentiated By Human Purified IL-2, Edwin W. Ades, Ann Hinson, Phil Marder, and Larry D. Butler, Lilly Research Laboratories, Indianapolis, IN. 46285

Considerable interest and information regarding the natural cytotoxic cell population associated with normal lymphoid tissue and its cellular lineage as well as its potentiation has recently been reported. A monoclonal antibody reactive with human natural killer cells (anti-Leulla) was utilized to sort NK functional and non-functional sub-populations of human lymphocytes. Human interleukin-2 was examined for its effect on potentiation of Leulla positive or Leulla negative NK cell function. IL-2 was found to enhance NK activity of PBL two-to-three fold. When IL-2 was added to either Leulla positive or 11a negative cells, the enhancing activity was found to be primarily for the Leulla positive population (at a 3:1 E:T, killing was enhanced four-fold for the Leulla positive population and not significantly for the Leulla negative cells).

0385 EVALUATION OF HUMAN INTERFERON ACTIVITIES IN A HAMSTER MODEL SYSTEM. Bruce W. Altrock, *Eleanor N. Fish, Helen Hockman, Linda Miller and Nowell Stebbing. Amgen, Thousand Oaks, CA 91320, *Hospital for Sick Children, Toronto, Ontario M5G 1X8

Human interferons (huIFN) exhibit immunomodulatory activities which may contribute to their antiproliferative and antiviral effects. IFN alters the function of B cells, T cells and macrophages. IFN stimulation of antibody production by sorted human peripheral blood cells has been observed. IFNs also modulate expression of the major histocompatibility complex. Translation of in vitro observations to the in vivo situation has been difficult in the absence of suitable animal test systems. In the present work we describe a hamster model system for the evaluation of huIFN therapy of tumor cell challenge and viral infections. Human IFNα and huIFNγ and their recombinant DNA derived analogs were evaluated for antiproliferative and antiviral effects on cell lines of a variety of species and were notably effective on hamster cells. Also, the IFN's were therapeutic in hamsters challenged ip with the TBD932 lymphosarcoma. The extent of protection against the tumor was generally IFN dose related though short courses of treatment were more effective than prolonged therapy. Immunological parameters related to these observations are under study. Additionally, a novel leukocyte huIFN exhibited significant antiviral activity in animals challenged with either EMC or HSV. Studies using analogs of huIFNs and monoclonal antibodies (neutralizing and non-neutralizing) against prescribed regions of these molecules have facilitated the definition of biologically significant domains within huIFN's. Work correlating this data with cell receptor binding is in progress.

0386 DMSO EFFECTS ON TUMOR HISTOCOMPATIBILITY ANTIGEN EXPRESSION, David W. Bahler and Edith M. Lord, University of Rochester Medical Center, Rochester, NY 14642

Tumor specific T cells recognize tumor associated antigens in conjunction with antigens on the tumor cell surface coded for by the major histocompatibility gene complex (MHC). Thus, it follows that tumor cells deficient in expression of cell surface MHC antigen would be offered some degree of protection against immune system mediated tumor defense mechanisms. Although histocompatibility antigens are ubiquitously distributed on normal somatic tissue, a variety of murine as well as human tumors have been reported to lack cell surface class 1 MHC products. We show in this study that the spontaneous BALB/c lung carcinoma, Line 1, normally expresses H-2D,K, & L coded class 1 antigens at marginal levels just detectable using flow cytometry. More importantly we demonstrate that dimethyl sulfoxide (DMSO), a substance capable of terminally differentiating certain leukemias, can increase, in a dose dependent temporal fashion, the expression of cell surface class 1 antigen in Line 1 to levels seen in high class 1 antigen expressing BALB/c tumor lines. The class 1 antigen deficiency exhibited by Line 1 then is clearly not genetically based. DMSO induced class 1 antigen expression in Line 1 is also shown to be accompanied by an increased susceptibility to lysis mediated by C57BL/6 anti-BALB/c cytotoxic T cells. Our results suggest therefore that DMSO could make MHC antigen deficient tumors more sensitive to host generated immune system destruction.

0387 2',5'-OLIGOADENYLATE AND ANALOGS INCREASE THE TUMORICIDAL ACTIVITY OF NATURAL KILLER CELLS, Paul L. Black, Marie Sabo, Earl E. Henderson and Robert J. Suhadolnik, Temple University School of Medicine, Philadelphia, PA 19140

Interferon (IFN) augments the lytic activity of natural killer (NK) cells, inhibits the transformation of human peripheral blood lymphocytes (PBL) by Epstein-Barr virus (EBV) and induces a 2',5'-oligoadenylate (2',5'-A_n) synthetase. Exogenous 2',5'-A_3 and 2',5'-A_4, in a dose-dependent manner and in the absence of IFN, can inhibit the transformation of human PBL by EBV. 2',5'-A_n and its analogs also increase the tumoricidal activity of human NK cells. Incubation of nylon wool-passed PBL for 1-2 hr with the 5'-dephosphorylated core trimer of 2',5'-A_n boosted natural killing of tumor target cells modestly, but consistently. The cordycepin analog (3'-deoxyadenylate) also augmented NK activity. The optimal concentration both of 2',5'-A_3 core and of 2',5'-3'dA_3 core was 50 μM, and the optimal time for this effect was 2 hr of treatment. On the other hand, 3',5'-A_3 core did not increase NK activity even at concentrations up to 300 μM, at which point it became toxic. Kinetic analysis revealed that 2',5'-A_3 core increased the lytic rate of NK cells by about a third. This increase was due to an even greater increase (about 50%) in the lytic activity of individual NK cells, because the number of actual NK effector cells decreased slightly after treatment with 2',5'-A_3 core. In addition to their inhibition of EBV-induced transformation, these oligonucleotides' augmentation of NK activity represents another IFN-like action of 2',5'-A_3 core and its analog. In order to determine if 2',5'-A_n molecules are taken up by and act directly on NK cells, we have generated Interleukin-2 dependent, long-term cultured and cloned lines of human NK cells. Studies on the effect of 2',5'-A_n on the lytic activity of cloned NK lines are in progress.

0388 MONOCLONAL ANTIBODIES TO TISSUE-SPECIFIC ANTIGENS: BINDING, MEMBRANE REDISTRIBUTION AND INTERNALIZATION OF ANTI-PROSTATE ANTIBODY, Ann M. Carroll, Michael Zalutsky, Linda L. Perry, Baruj Benacerraf and Mark I. Greene, Harvard Medical School, 25 Shattuck St., Boston, MA 02115.

Monoclonal antibodies which identify a human prostate tissue antigen have been developed. One monoclonal antibody, F77.129, binds to the PC 3 human prostate carcinoma cell line with an affinity of $1.6 \times 10^9\ M^{-1}$; it identifies a mean of 165,000 binding sites per cell. By surface immunofluorescence and radioimmunoassay, binding of F77.129 to PC 3 cells at 37° C results in antigen patching, capping and ultimate internalization of the antigen-antibody complex. The F77.129 antibody has also been iodinated and administered i.v. to tumor-bearing nude mice; specific localization to PC 3 prostate carcinoma but not to antigen-irrelevant tumor results. The in vivo localization and surface modulation properties of this tissue-specific antibody suggest that it may be useful in therapy.

0389 FURTHER STUDIES ON THE SYNERGY BETWEEN RICIN-A AND RICIN-B-CONTAINING IMMUNOTOXINS, R. Jerrold Fulton, Jonathan W. Uhr, and Ellen S. Vitetta, University of Texas Health Science Center, Dallas, Texas 75235

Recent studies have demonstrated that antibodies coupled to the A chain of ricin can be used to kill cells bearing the relevant cell surface determinants. The specificity of A chain conjugates is much greater than antibody-ricin conjugates; however, the specific toxicity is much lower, possibly due to the lack of a "transport" function contributed by the B chain. We have recently demonstrated that immunotoxins containing either A chain alone or B chain alone, but directed toward the same cell surface determinants, can synergize in vitro to give greater toxicity while retaining the specificity observed using A chain conjugates alone. In this report, we describe the use of a "piggyback" system in which the primary antibody (conjugated to ricin A chain) is specific for cell surface determinants and the secondary antibody (conjugated to ricin B chain) is specific for the primary antibody. Rabbit anti-human Ig antibodies (either IgG or Fab' fragments) conjugated to ricin A chain (RAHIg-A or RAHIgFab'-A) were found to synergize with goat anti-rabbit Ig antibodies, conjugated to ricin B chain (GAR-B) in in vitro killing assays with a human B lymphoma (Daudi) which expresses cell surface Ig. Likewise, RAMIg-A conjugates synergized with GAR-B in the in vitro killing of a murine B cell leukemia (a subclone of BCL_1). Both systems demonstrated an absolute requirement for specific antibody, both primary and secondary, and for the relevant chain of ricin. Reversing the order of chain delivery, i.e., RAHIg-B + GAR-A did not result in synergistic killing. The separate and sequential delivery of the two ricin chains may result in an increased therapeutic index both in vitro and in vivo.

0390 IMMUNOLOGIC INHIBITION OF UVR-INDUCED TUMOR-SPECIFIC SUPPRESSOR CELL ACTIVITY. Richard D. Granstein, John A. Parrish, and Mark I. Greene*, Department of Dermatology, Massachusetts General Hospital, Boston, MA 02114, and *Department of Pathology, Harvard Medical School, Boston, MA 02115

C3H mice exposed chronically to ultraviolet radiation (UVR) develop suppressor T cells that recognize most UVR-induced regressor skin cancers as a class (Ts_{uv}). These Ts_{uv} are defined by their ability to inhibit rejection of transplanted UVR-regressor tumors upon adoptive transfer to syngeneic recipients. This study demonstrates that administration of monoclonal anti-IJ^k antibody or cyclophosphamide (CY) inhibits the activation of the Ts_{uv} in chronically irradiated mice. C3H mice were divided into 4 groups. Group 1 was exposed to 39.6 KJ/m^2 of FS-40 sunlamp radiation to the shaved dorsum twice weekly for 12 weeks, group 2 mice were similarly treated with UVR but additionally received 20 mg/kg CY i.p. twice weekly, group 3 received UVR and additionally 500 ng anti-IJ^k monoclonal antibody (WFC 12.8) i.p. twice weekly, group 4 received UVR and media i.p. twice weekly and group 5 was untreated. One week after the last irradiation the mice were sacrificed and spleens harvested. 10^8 nucleated splenocytes were administered i.v. to each of several recipients. Shortly thereafter all recipients were given $2x10^6$ cells of UVR-regressor tumor 2240 s.c. and tumor growth was scored as a measure of Ts_{uv} activity. Groups 2 (UVR+CY), 3 (UVR+anti-IJ), and 5 (untreated) had significantly less Ts_{uv} activity compared to group 1. We conclude that IJ^+ /CY-sensitive elements are involved in Ts_{uv} activation. These results may suggest novel therapeutic approaches.

0391 IN VITRO AND IN VIVO STUDIES WITH LYMPHOKINE ACTIVATED KILLER CELLS (LAK). Elizabeth A. Grimm, Amitabha Mazumder, Maury Rosenstein, Suyu Shu, Jim Mule and Steven A. Rosenberg, Surgery Branch, NCI - NIH, Bethesda, Maryland 20205.

In both mouse and man, incubation of fresh lymphocytes in purified IL-2 for 2 to 3 days results in the development of lytic activity against noncultured autologous and allogeneic tumor cells, including those which are NK resistant. These lymphokine-activated killer cells (LAK) represent a class of cytolytic effectors distinct from CTL or NK cells, based on a number of criteria including kinetics of activation, stimulating signal, target specificity, and cell phenotype. While the LAK effector is identical to CTL (OKT 3^+ and OKT 8^+ in the human, and Thy 1^+, and Lyt 2^+ in the mouse) the precursor cell does not necessarily express a T cell phenotype: in the human the precursor is OKT 3^-, Leu 1^-, and OKT 11^-, and in the mouse is found in the spleen of nude and B mice. In order to test a possible therapeutic role for LAK cells, adoptive immunotherapy studies of a murine tumor have been initiated. LAK prepared by in vitro activation of either normal or tumor bearing mouse splenocytes with IL-2, were transferred by iv injection into syngeneic mice bearing pulmonary metastases from the B16 melanoma. Significant reduction in numbers of metastases and a significant improvement in survival was observed after adoptive transfer of LAK cells.

0392 TUMOR IMMUNOTHERAPY BY ANTI-IDIOTYPIC ANTIBODIES, Joseph Haimovich, Daphna B. Burowski and Esther Hurwitz, Dept. Human Microbiology, Sackler Faculty of Medicine, Tel-Aviv University, Ramat-Aviv 69978, and the Weizmann Institute of Science, Rehovot 76100, Israel.

Idiotypic determinants of immunoblogulins on B-lymphocyte tumor cells are considered as tumor associated antigens and as convenient targets for immunotherapy by anti-idiotypic antibodies. In our studies, the murine B-cell lymphoma 38C-13, propagated in C3H/eB mice, is used as a model for human lymphomas and for human tumors in general. Anti-38C-13 idiotypes were obtained upon immunization of LOU/c rats with the Fab of 38C-13 IgM (prepared by trypsin digestion). Spleen cells from the immunized rats were fused with the cells of murine plasmacytoma cell-lines. One hybridoma, producing monoclonal anti-38C-13 idiotype antibodies of the IgG1 subclass was selected for further studies. Affinity-purified antibodies from the growth medium of this hybridoma, administered at 10 µg/mouse i.p., 2 days after a challenge of 10^4 38C-13 cells i.p., had a significant protective effect (causing a marked delay in tumor growth and lowering tumor incidence). At 1 µg/mouse, no protective effect was observed. Affinity-purified goat anti-38C-13 idiotype antibodies and their $F(ab')_2$ fragments, when administered at high doses (1 mg/mouse), were also effective in either preventing or delaying death due to tumor development. The influence of $F(ab')_2$ fragments on tumor development suggests their indirect effect on tumor growth through the induction of the host's immune system. Studies are underway to select for hybridomas producing anti-38C-13 idiotype antibodies of different subclasses. This is being done in order to investigate the mechanisms of their action on the tumor cells by either a direct cytotoxic effect or regulatory effects implied by the "Immune network" theory.

0393 REGRESSION OF FRIEND VIRUS LEUKEMIA BY THE TRANSFER OF SPECIFICALLY REACTIVE T CELLS, Candace S. Johnson and Philip Furmanski, AMC Cancer Research Center, Lakewood, CO 80214.

Friend virus (FV) induces an erythroleukemia characterized by massive splenomegaly, severe immunosuppression and death. A variant strain of Friend virus (RFV) induces an identical disease except that it spontaneously regresses in 30-70% of the leukemic animals. Regression is immunologically mediated with T cell and macrophage function playing a central role. Specifically reactive T cells are found in the spleens of regressors (animals that will regress). To determine whether progressors (animals that will not regress) can be made to regress by the transfer of specifically reactive cultured T cells, FV leukemic mice (day 14 post virus) were given two injections a week apart of $5X10^6$ T cells. The T lymphocytes were obtained from T cell cultures that were derived from mice immunized with multiple injections of $5X10^6$ UV-irradiated FV-infected cell lines. The T cell cultures were carried in 50% interleukin-2 containing medium with weekly antigenic stimulation by mitomycin C treated virus-infected cells (same as immunizing source). The cultured T cells demonstrated high levels of specific in vitro cytotoxicity directed against viral antigens. The specifically reactive T cells caused apparently permanent regression of leukemia when inoculated into progressor animals. Regression was obtained when the T cells were inoculated into fully leukemic animals; no other treatment was required. These results suggest that progressively leukemic mice can be made to regress by treatment with cultured T cells that are specifically reactive against viral antigens. (Supported in part by NIH Grant CA-33939 and grants to the AMC Cancer Research Center from Robert A. Silverberg and Richard L. Robinson.)

0394 ANTI-TAC RICIN A-CHAIN CONJUGATES SELECTIVELY INHIBIT PROTEIN SYNTHESIS IN HUMAN T CELL LEUKEMIA/LYMPHOMA VIRUS (HTLV) INFECTED LEUKEMIC T CELLS, M. Kronke, J.M. Depper, W.J. Leonard, E.S. Vitetta+, T.A. Waldmann, and W.C. Greene, NCI, NIH, Bethesda, MD 20205, and Department of Microbiology+, University of Texas, Dallas, TX 75235

Adult T cell leukemia (ATL) is comprised of malignant HTLV infected mature T cells, which express large numbers of receptors for T cell growth factor (TCGF). In an attempt to exploit the rather selective expression of this T cell activation antigen, we conjugated monoclonal anti-human TCGF receptor antibody, anti-Tac, to purified A-chain of the ricin toxin, to obtain a reagent potentially cytotoxic for ATL cells. Anti-Tac ricin A-chain conjugates (anti-Tac-A) were cytotoxic producing half maximal inhibition of protein synthesis (ID_{50}) in primary HTLV positive tumor cell lines, HTLV transformed cord blood lymphocytes, and in fresh leukemic lymphocytes from an ATL patient at concentrations ranging from 2×10^{-10} to 7.5×10^{-10}M. In contrast, monoclonal control UPC-10 ricin A-chain conjugates exhibited a 100 to 200 fold higher ID_{50}. The inhibitory effects of anti-Tac-A were completely reversed by either unconjugated anti-Tac or purified TCGF, but not by monoclonal OKT3 or OKT4, indicating that inhibition was mediated by conjugated ricin A-chain internalized via the TCGF receptor. Further, protein synthesis in HTLV negative leukemic T cell lines and EBV transformed B cell lines lacking TCGF receptors was not inhibited by concentrations of anti-Tac-A producing >95% inhibition in ATL cells. The data demonstrate that anti-Tac-A is an effective immunotoxin capable of producing selective in vitro cytotoxic effects on HTLV infected ATL cells.

0395 STRATEGIES FOR PRODUCTION OF MONOCLONAL ANTI-IDIOTYPE ANTIBODIES AGAINST HUMAN B CELL LYMPHOMAS. Ronald Levy, Kristiaan Thielemans, David G. Maloney, Timothy C. Meeker, Junichiro Fujimoto, Roger A. Warnke, Jane Bindl, Carol Doss and Richard A. Miller, Stanford University, Stanford, CA, 04305

Murine monoclonal antibodies (MAB) against the idiotype (Id) of B lymphocyte malignancies are powerful reagents for study of these diseases and potentially useful for treatment. Streamlined procedures for the production of these anti-Id MAB have been derived. Initially, the Id immunoglobulin (Ig) from non-secreting B cell tumors was "rescued" by human x mouse or human x human hybridization. These somatic cell hybridizations resulted in secretion of human Ig in 10% and 100% of the fusions, respectively. In a second step, anti- Id MAB were produced using the "rescued" Id Ig as immunogen. Our current strategy is based on a one-step procedure where the tumor cell suspension is used as immunogen. With this method of immunization and a sensitive screening assay, we are able to detect anti-Id MAB with a frequency of 1% of total hybrids. Using a pool of ten different monoclonal anti-idiotype antibodies, each reactive with the tumor of one patient, we have searched for idiotype relatedness among a subsequent panel of 50 additional tumors. No cross-reactions were found, indicating that our current strategy results in the identification of unqiue idiotypic determinants among human B cell tumors. Before a therapeutic trial, an in vitro assay is performed to determine the level of circulating antigen (Id) in the serum. Such free antigen can interfere with the binding of MAB to target cells, thus limiting therapeutic effectiveness. We have attempted to lower serum Id protein by plasmapheresis. This maneuver has had different degrees of effectiveness depending on the tumor distribution (intravascular versus extravascular).

0396 T CELLS SELECTED WITH A TUMOR-RELATED, MONOCLONAL, AUTO-ANTI-IDIOTYPIC ANTIBODY MEDIATE TUMOR-SPECIFIC DELAYED-TYPE HYPERSENSITIVITY AND LIMIT TUMOR GROWTH, Karen A. Nelson and Evan George, Fred Hutchinson Cancer Research Center, Seattle, Wa 98104 USA.

Monoclonal antibody 4.72 behaves as if anti-idiotypic to the response of BALB/c mice to a syngeneic, MCA-induced sarcoma, MCA-1490. T cells from BALB/c mice immunized with antibody 4.72 can transfer delayed-type hypersensitivity (DTH) to MCA-1490 when injected into naive mice (Nature 303,627,1983). We thus asked if T cells with the idiotype defined by antibody 4.72 were detectable in mice with MCA-1490 tumor and if lines of such T cells could be established and used in immunotherapy of this tumor. Lymphoid cells were obtained from lymph nodes adjacent to MCA-1490 tumors in BALB/c mice. These cells were incubated with antibody 4.72 coupled to biotin and with avidin-FITC. Brightly fluorescing cells were selected using a FACS-IV; brightly stained cells were not seen after treatment with a biotin-coupled control antibody and avidin-FITC. The sorted cells were cultured with Il-2 without further stimulation with antigen and have been mantained in culture for over twelve weeks. Cells from one line designated 4.72-90-3, retained the idiotype defined by antibody 4.72 and were positive for Lyt-1 and negative for Lyt-2 . When mixed with irradiated tumor cells and transferred to the footpads of naive mice, 4.72-90-3 cells mediated DTH to MCA-1490 but not to two other MCA-induced sarcomas. Likewise, 4.72-90-3 cells limited tumor growth when mixed with viable MCA-1490 cells and injected subcutaneously into naive BALB/c mice. The ability of 4.72-90-3 cells to limit the growth of established tumors will be assessed.

0397 DEVELOPMENT OF HUMAN MONOCLONAL ANTIBODIES AGAINST SYNGENEIC BREAST TUMOR-ASSOCIATED DUCTAL AND LOBULAR CELLS, Patricia A Nelson, Raymond Ranken and Emanuel Calenoff, Carcinex, Burlingame, CA .94010.

Human monoclonal antibodies reactive against syngeneic breast tumor-associated epithelial cells have been developed utilizing a two stage immortalization protocol. Patient B lymphocytes presumably exposed to tumor antigens were harvested from a tumor-involved lymph node and transformed in vitro with Epstein Barr Virus. Approximately 2% of the cultures of transformed B cells were found to be making antibodies that bound to sections of breast tumor tissue. The antibody-producing B lymphoblastoid lines not reactive against normal epithelial cells were then fused with a human myeloma cell line to form hybridomas. Of six stable hybridomas chosen for anti-breast tumor reactivity, all produce IgM and one produces both IgM and IgG. Further characterization of these human monoclonal antibodies is in progress.

0398 SEVERE DEFICIENCY OF MATURE B CELLS IN MULTIPLE MYELOMA PATIENTS: OBSERVATIONS AND HYPOTHESIS: Linda M. Pilarski[1], Michael Mant[2], Bernard A. Ruether[3], Andrew Belch[2], Department of Immunology[1] and Department of Medicine[2], University of Alberta; Department of Medicine, University of Calgary[3].

Mature B cells can be defined as sIg^+ lymphocytes. Normal individuals have 3 - 10% B cells. In contrast, in patients with multiple myeloma from 0.01 - 1% of peripheral blood lymphocytes (average = 0.3%) are sIg^+ B cells. A decrease in mature B cells could result from a) deficiencies in the entire B cell maturation pathway or b) a block in B cell maturation. Our evidence suggests that B cell maturation is blocked. Analysis of the expression of Ba-1, a B cell differentiation antigen, indicates that a large proportion of myeloma patients have normal or supra normal numbers of $Ba\text{-}1^+$ cells in their PBL. The patients $Ba\text{-}1^+$ cells are sIg^-, suggesting that B cell differentiation is blocked just prior to sIg expression. We hypothesize that this late stage block in B cell development is due to the existence of an immunoregulatory network specific for antigenic determinants expressed on sIg molecules. Support for this hypothesis comes from our observation that after EBV transformation a very large proportion (68%) of the few remaining B cells in patients secrete antibody specific for semi-private or public determinants on $F(ab)_2$ fragments. Anti-$F(ab)_2$ reactivity is virtually absent in normal B cells (0.03%). In addition, memory B cells specific for vaccination antigens appear to escape the blockade. We predict that immunoregulatory T cells, particularly T suppressor cells are responsible for the block in B cell maturation. Funded by National Cancer Institute of Canada.

0399 A NOVEL APPROACH TO PRODUCTION OF ANTI-TUMOR MONOCLONAL ANTIBODIES: ANTIBODY TO A CELL SURFACE GLYCOPROTEIN ASSOCIATED WITH TRANSFORMATION BY A HUMAN ONCOGENE,
Jack A. Roth, Philip Scuderi, Eric Westin, Robert Gallo, National Cancer Institute, Bethesda, MD 20205

Transfection is a technique for inducing transformation of normal fibroblasts (NIH 3T3) with DNA from human tumors. Our goal is to determine if these transformed cells express new surface antigens associated with malignancy. NIH 3T3 cells were transfected with DNA from a human acute lymphocytic leukemia (ALL) and transformed foci were selected for growth in soft agar. Transfected cells containing human DNA sequences were used to immunize BALB/C mice. Monoclonal antibodies were produced and screened for binding to the parental ALL, transfectant, and 3T3 cells in an enzyme-linked assay. The monoclonal antibodies designated 17-9H3 bound to ALL and transfectant but not NIH 3T3 plasma membranes. Immunoperoxidase staining confirmed this binding pattern and demonstrated the antigen was expressed on the cell surface. The antigen was strongly expressed by 5/6 cultured T cell leukemia lines, 4/8 null leukemias but not by acute mylogenous leukemias (0/8). Strong antigen expression was also noted on 15/29 solid tumors and 2/23 normal tissues, suggesting the antigen may be related to both a normal cellular oncogene homologue and transforming oncogene product. Expression of the antigen by transfectants correlated with the presence of a single 6.1 kilobase human DNA sequence. The antibody binding site of the antigen was inactivated by trypsin and glucosidase indicating the antigen was a glycoprotein. Monoclonal antibodies to oncogene related antigens may be potentially useful for cancer diagnosis and therapy.

0400 DETECTION OF VARIABLE REGIONHETEROGENIETY IN HUMAN AND MOUSE MONOCLONAL ANTIBODIES
Drew Weissman*, T. Rothstein+, and A. Marshak-Rothstein@, Departments of Microbiology and Hematology, Boston University School of Medicine, Boston, Ma., 02118

The first step in the production of anti-idiotypic antibodies against human B-cell malignancies is to isolate tumor specific immunoglobin (Ig). One approach is to fuse malignant cells and mouse myeloma cells in order to induce Ig secretion. However, tumor derived B-cell hybrids must be distinguished from contaminant normal B-cell hybrids. We have developed a rapid method of comparing monoclonal antibodies by limited proteolysis and electrophoresis. This technique has been used to demonstrate primary structure homology between different hybrids in human-mouse and mouse-mouse fusions. The method takes two days to complete, uses readily available equipment, and is only slightly more time comsuming than a radioimmunoassay. Immunoglobin is digested with chymotrypsin in SDS which results in highly reproducable banding patterns on electrophoresis. The sensitivity of this method has been judged by comparing the banding patterns of monoclonal antibodies from A/J mice directed against azophenylarsonate. Antibodies that belong to the major cross reactive idiotype family (CRI^+) as determined by amino acid sequence analysis resulted in similar but not identical patterns, whereas those that were CRI^- yielded markedly dissimilar patterns. In an example of the usefulness of this technique, multiple colonies from human B-cell leukemia fusions were compared to demonstrate that the resultant hybrids secreted Ig with the same banding patterns and came from the clonal malignancy.
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Functional Domains of MHC Molecules (Type I/Type II)

0401 CONSTRUCTION AND EXPRESSION OF HYBRID $H2K^b$ AND $H\text{-}2D^b$ GENES, H.J. Allen, Mellor, A.L., Weiss, E. and Flavell, R.A., Biogen Research Corp., Cambridge, MA 02142

We have previously reported the molecular cloning of the genes encoding $H\text{-}2K^b$ and $H\text{-}2D^b$ class I antigens in the B-10 mouse. These genes were expressed in mouse L cells and recognized by both allogeneic and virus specific cytotoxic T cells (CTL).

To identify the sites on $H\text{-}2K^b$ and $H\text{-}2D^b$ antigens recognized by CTL, we have now constructed hybrid $H\text{-}2K^b/D^b$ genes by exchanging exons between these genes. The hybrid $H\text{-}2K^b/D^b$ genes are (1) K^b exon 2 in D^b, (2) D^b exon 2 in K^b, (3) K^b exons 2 & 3 in D^b and (4) D^b exons 2 & 3 in K^b. These four hybrid genes are expressed in mouse L cells. Recognition of the hybrid K^b/D^b antigens by cloned CTL lines specific for influenza virus and restricted by either K^b or D^b is being investigated. The locations of alloantigenic determinants on the hybrid K^b/D^b antigens are also being mapped with monoclonal antibodies specific for determinants on the K^b and D^b antigens.

0402 "Functional Epitopes of Ia Molecules: Use of Ia Mutants to Predict Effects of Anti-Ia Antibodies" Hugh Auchincloss, Jr., Laurie H. Glimcher

There has been conflicting evidence on whether T cell recognition sites on the Ia molecule (histotopes) coincide with serologically defined Class II determinants.

A Class II mutant antigen presenting cell line, A19, was used in concert with a panel of I-A^k-restricted T cell hybridomas and a panel of monoclonal anti-Ia antibodies to re-examine this issue. Two anti-Ia mAb which define epitopes absent on the surface of the A19 cell line were able to block the activation by the wild-type cell of the group of T cell hybridomas for which the A19 mutant cell failed to present antigen but were unable to block the activation of the group of T cells for which the A19 served as a potent antigen presenting cell. These two antibodies, therefore, appear to define both a serologic and a functional epitope and to behave in a histotope-specific manner. These attributes, however, cannot necessarily be extended to other anti-Ia antibodies.

0403 FUNCTIONAL AND BIOCHEMICAL ANALYSIS OF MUTANT I-A^K CELL LINES, Barbara N. Beck, George G. Schlauder, Laurie H. Glimcher, Michel Pierres and David J. McKean, Mayo Clinic, Rochester, MN 55905

Several different I-A^k mutant cell lines have been isolated from the I-A^k expressing TA3 cell line, a hybrid line derived from the fusion of (BALB/c x A/J)F_1 spleen cells with the Ia^+ B lymphoma M12.4.1 of BALB/c origin. The mutants were obtained from EMS-mutagenized cells which were subsequently subjected to negative immunoselection with anti-I-A^k monoclonal antibody and complement and then to positive selection by electronic cell-sorting after staining for fluorescence with another anti-I-A^k monoclonal antibody. The selected cells were then cloned and the clones tested with a panel of I-A^k antibodies for serological phenotype. The mutant cell lines are also tested for phenotype in functional assays which include the ability to stimulate or present antigen to long-term allo- and antigen-reactive T cell lines and hybridomas and the ability to stimulate T cells in a primary mixed lymphocyte reaction. Biochemical analysis of the mutant I-A molecules expressed by these cells was done by two-dimensional gel electrophoresis and peptide mapping. To date we have identified a number of different cell lines that express mutant A^k_β polypeptides and at least one cell line that expresses a mutant A^k_α polypeptide. These studies will enable us to define the functional sites of recognition on I-A^k molecules by alloantibodies and allo- and antigen-reactive T cells.

0404 FUNCTIONAL EXPRESSION OF CLONED CLASS II MHC GENES ON B LYMPHOMA CELLS AND MACROPHAGES, Avraham Ben-Nun, Laurie Glimcher, Edmund Choi, and J. G. Seidman. Dept. of Genetics, Harvard Medical School, Boston, MA 02115

The murine I-region genes encode the two cell surface glycoproteins, the I-A and I-E, that play critical roles in determining immune responsiveness of a mouse. The genes encoding the I-A antigen (α and β genes) have been cloned and sequenced. As an approach to understanding the mechanism by which I-A antigen operates in regulating the immune response and as restricting elements in the associative recognition of antigen by T lymphocytes, the genes encoding the I-A^b antigen have been introduced into I-A^d bearing B lymphoma cells or macrophages. Recipient cells that were transfected with both α^b and β^b genes expressed levels of I-A^b antigen that were comparable to that of the endogenous I-A^d antigen and were efficient in stimulating a panel of allo-specific as well as I-A^b restricted antigen specific T cells. Cells transfected with A_α or with A_β genes only, generally could not stimulate I-A^b allospecific T cells nor I-A^b restricted antigen-specific T cells.

0405 ANALYSIS OF MHC CLASS I DOMAINS USING CTL CLONES, Jeffrey A. Bluestone*, Cynthia Palmer*, Stanley G. Nathenson†, and Steven Geier†, *National Cancer Inst, Bethesda, MD 20205 and †Albert Einstein Col. Med., Bronx, NY 10461.

Class I molecules express foreign determinants which are recognized by both alloreactive cytotoxic T lymphocytes (CTL) and alloantibodies. Serological analyses have suggested that these determinants are located in 2 or 3 epitope clusters. To examine further the relationship among determinants recognized by CTL and alloantibodies, in vitro-derived H-$2K^b$ mutant tumor cells were studied using CTL clones generated from different alloreactive combinations. Anti-H-$2K^b$ monoclonal antibodies (mAbs) representing different epitope clusters were used to immunoselect H-$2K^b$ expressing tumor cells. Mutagenized cells were treated repeatedly with a mAb plus complement. The minority of residual cells which expressed normal amounts of cell surface MHC were positively selected by Fluorescence Activated Cell Sorting using a pool of anti-H-$2K^b$ mAbs and subcloned using soft agar. Clones with reduced reactivity with the selecting antibody but normal reactivity with other anti-H-$2K^b$ mAbs were examined using a panel of CTL clones derived from in vivo derived H-$2K^b$ mutant mice (e.g. BM1, BM3, BM6, BM10 and BM11). In many of these mutant cell lines which had been selected on the basis of serological alterations, the determinant(s) recognized by CTL clones of different specificities was altered suggesting a relationship between the structures recognized by the CTL and alloantibodies. However, no obvious correlation has been detected between the serological profile of a mutant and the pattern of CTL clone reactivity. These results suggest that alloreactive T cells may recognize MHC epitope clusters spacially related to those recognized by alloantibodies but either the repertoires or the precise determinants recognized by CTL and alloantibodies different.

0406 MULTIPLE FUNCTIONAL SITES ON A SINGLE Ia MOLECULE DEFINED USING T CELL CLONES AND ANTIBODIES WITH CHAIN DETERMINED SPECIFICITY. John G. Frelinger, Minoru Shigeta, Anthony J. Infante, Patricia A. Nelson, Michel Pierres, and C.G. Fathman, Stanford University, Stanford, CA 94305.

Monoclonal antibodies were used to inhibit the proliferation of antigen reactive (C57BL6/J x A/J)F_1 restricted T cell clones. We have been able to subdivide these F_1 restricted T cell clones into two groups: one of which recognizes the $A_\alpha^k A_\beta^b$ molecule and the other group which recognizes the $A_\alpha^b A_\beta^k$ molecule. Using clones with defined reactivities, we could assign the reactivities of monoclonals to the A_α or A_β chains. By immunoprecipitation and two dimensional analysis of Ia molecules from F1 spleen cells, we could independently map the reactivities of the monoclonal antibodies to the A_α or A_β chain. To date, these two methods of chain localization of the antibody reactivity have agreed. Futher, the differential blocking of the $A_\alpha^k A_\beta^b$ restricted T cell clones suggests that there exists more than one restriction site per Ia molecule. Increasing the number of possible functional Ia restriction sites, either through combinatorial association of α and β chains or by using more than one site per molecule, increases the number of ways Ia molecules can function in antigen presentation.

0407 ANALYSIS OF H-2 DOMAINS REQUIRED FOR ALLORECOGNITION BY CTL CLONES, Lee A. Henderson, Iwona Stroynowski[+], Lee Hood[+] and James Forman, Univ. TX. Hlth. Sci. Ctr. Dallas, TX. 75235 and California Institute of Technology[+], Pasadena, CA 91109

Histocompatibility antigen (H-2 in the mouse) recognition by T lymphocytes is a required event in the regulation of cellular immunity. A precise determination of the H-2 domains essential in T cell recognition requires analysis by cloned T cells. CTL clones were generated from in vitro stimulation of dm2 (an L^d deletion mutant) with BALB/C spleen cells. For analysis of H-2 domain requirements we have used in these studies L cell transfectants which express various domains of L^d, D^d, and 27.1 (an H-2 pseudogene). Cloned cytolytic T lymphocytes specific for L^d were assayed in a standard chromium release assay with ^{51}Cr-labeled 1 cell transfectants. The results of these experiments will define the roles of the extracellular H-2 domains in allorecognition.

L.A.H. is a Fellow of The Cancer Research Institute, N.Y.

0408 ANALYSIS OF ANTIGENIC SITES ON HLA-B7, Andrew Herman, Mark Holterman, Peter Parham, Sherman Weissman and Victor H. Engelhard. Univ. of Virginia, Charlottesville, VA 22908 Stanford Univ., Stanford, CA 94305 and Yale Univ., New Haven, CT 06510.

Murine L cells expressing HLA-A2 or -B7 were isolated after DNA-mediated gene transfer (DNA-MGT) with genomic clones encoding -A2 or -B7 genes. Monoclonal antibody (moAb) binding analyses showed that the cells stably expressed high levels of -A2 or -B7 and retained all of the moAb-defined, heavy chain associated antigenic determinants, but lacked those determinants associated with human β_2-microglobulin (β_2m). These transformants could act as targets for cytotoxic T lymphocyte (CTL) clones specific for -B7 or -A2 antigens expressed on human cells. However, the CTL clones exhibited clonal variation in their ability to lyse the transformants. Since the antigens are, by serological criteria, structurally intact, these results suggest that the association of HLA heavy chains with murine, rather than human, β_2m may alter antigenic determinants that are important for CTL recognition. Further, the ability of -A2 specific CTL clones to lyse the human-mouse somatic cell hybrid CTP41 paralleled their killing of -A2 positive L cells. CTP41 expresses HLA-A2, but lacks the chromosome encoding human β_2m. DNA-MGT is being used to introduce -A2 and -B7 genes into cells expressing human β_2m. Site-directed mutagenesis is now being applied to the localization of moAb- and CTL-defined antigenic determinants on the -B7 molecule. Synthetic oligonucleotide linkers have been inserted at scattered sites in the α1 and α2 domains of -B7. These altered -B7 genes will be introduced, by DNA-MGT, into appropriate -B7 negative hosts and their biological activity assessed by use of HLA-specific moAb and CTL clones.

0409 ANALYSIS OF ANTIGEN PRESENTATION TO AND GENETIC CONTROL OF INSULIN SPECIFIC AND INSULIN ACTIVATED AUTOREACTIVE T CELLS IN H-2^b AND H-2^{bml2} MICE, Brigitte T. Huber and Paula S. Hochman, Tufts University School of Medicine, Boston, Massachusetts 02111.

Insulin specific T cell proliferation and antibody production by responder H-2^b mice is restricted to the A chain loop determinant(s) of beef insulin presented by the specific Ir gene product Ia.W39. The mutant B6.C-H-2^{bml2}(bml2) mice are unresponsive to insulin and lack Ia.W39, although they express the general Ir phenotype of wildtype H-2^b mice and differ in their I-A_β gene product by only 4 amino acids. To assess the mechanism of Ir gene control of insulin responses, insulin specific and insulin activated autoreactive T cell hybridomas (T_{HY}) were prepared by fusing T cells of beef insulin primed B6 or bml2 mice to BW5147. The cloned insulin specific H-2^b T_{HY} vary in their fine specificity, recognizing epitopes mapping to the A or B chain of insulin, and in their ability to induce polyclonal B cell proliferation and differentiation. The former T_{HY} stimulate B cells in the presence of native beef insulin, while the latter can only serve as helpers in the presence of isolated B chain, although they all produce IL-2 upon stimulation with the intact antigen. Insulin specific H-2^b or H-2^{bml2} T_{HY} see foreign antigen only in the context of B6 or bml2 APC, respectively. The majority of autoreactive T_{HY} show the same restriction to syngeneic Ia, while some recognize Ia determinants shared by the mutant and wildtype mice. These data suggest: 1) that the fine specificity of insulin activated T cells is less restricted than reflected in proliferation and PFC assays, 2) that the insulin molecule is presented by I-A^b in a very specific configuration that allows for differentiatial recognition, and 3) that the apparent retention of the H-2^b Ir phenotype by the mutant masks the generation of an altered self-I-A restricted T cell repertoire.

0410 BIOCHEMISTRY OF HLA STRUCTURAL VARIANTS WITH ALTERED SEROLOGICAL AND CELLULAR RECOGNITION DETERMINANTS, Michael S. Krangel, Shigeru Taketani, Donald Pious, William Biddison, Hergen Spits, Jan J. van der Poel and Jack L. Strominger, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724 and Harvard University, Cambridge, Ma 02138.

Peptide mapping and sequence analysis were used to investigate the structures of natural and mutagen induced variants of HLA-A2 and HLA-B7. HLA-A2 antigens have been identified which are serologically indistinguishable (or nearly so) from the predominant HLA-A2 antigen, yet which are not recognized by alloreactive or self-restricted CTLs. Comparative analysis of HLA-A2 antigens from donors M7, DR1, DB1, ANI, DK1 and KFU define two discrete subtypes; since all carry substitutions in the peptide spanning residues 147-157 of the heavy chain, this region appears critical in forming most, if not all, HLA-A2 specific CTL determinants. One HLA-B7 variant (donor CF) which fails to be recognized by an HLA-B7 specific CTL clone carries a substitution(s) in the peptide spanning residues 112-121. Immunoselection with the HLA-A2 specific monoclonal antibody BB7.2 plus complement was used to generate HLA-A2 negative mutants from the lymphoblastoid cell line T5-1. Three mutants synthesize HLA-A2 antigens which display reduced reactivity with the selecting antibody but normal reactivity with other antibodies. Two have distinct alterations in the peptide spanning residues 98-108, and the third carries a substitution at residue 161. Thus, virtually all HLA-A2 specific CTL determinants, at least one HLA-B7 specific CTL determinant and at least one HLA-A2 specific serological determinant reside in the second domain of the heavy chain. An immunoselected mutant has also been identified which secretes HLA-A2. Details will be presented.

0411 T-CELLS THAT RECOGNIZE Ia ANTIGENIC DETERMINANTS: CLONOTYPES IDENTIFIED AND ISOLATED FROM PARENT ANTI-F_1 MIXED LYMPHOCYTE CULTURES, Christopher J. Krco, Department of Immunology, Mayo Clinic, Rochester, Minnesota 55905

Previous investigations by others have resulted in the identification of combinatorial Ia antigenic determinants generated by the specific associations of A_β and E_α gene products. These combinatorial Ia determinants have been identified serologically and functionally. In an attempt to further investigate the antigenic complexity and to identify heretofore unidentified combinatorial Ia determinants mixed lymphocyte cultures have been established using parent anti-F_1 mixed lymphocyte cultures. T-cells from A/Sn mice are being used as the responder cells and F_1 spleen cells obtained from A/Sn x independent haplotype matings serve as the stimulator cells. Long-term cultured, allospecific T-cells are being isolated, cloned and characterized with respect to combinatorial Ia allospecificity. Data from T-cells isolated from one such mixed lymphocyte culture [A/Sn anti-(A/Sn x B10.D2)F_1] is summarized. The T-cells will respond when challenged with B10.D2 cells (Δ cpm 60,000) and B10.GD cells (Δ cpm 45,000). These T-cells show weak cross-reactivity against B10, B10.WB, B10.P, B10.S and B10.PL haplotype cells (Δ cpm's 10-12,000). These T-cells are inhibited in their recognition of B10.D2 cells by monoclonal anti-Ia.23 antibody 34-1-4 (49% inhibition). There is also inhibition by anti-I-A^d monoclonal antibody MK-D6 (53% inhibition) but not by 28-16-8 (anti-Ia.8; 0% inhibition) or 25-9-17 (anti-I-A^d; 13% inhibition). These cells are currently being cloned.

0412 ANALYSIS OF THE MOLECULAR HETEROGENEITY OF I-A^d MOLECULES, William P. Lafuse, Nancy E. Levy and Chella S. David, Department of Immunology, Mayo Clinic, Rochester, MN 55905

Recent DNA studies have suggested that the number of Ia genes in the mouse is limited. However, studies from this laboratory using monoclonal antibodies have indicated that considerable molecular heterogeneity exists in Ia molecules. In studies of I-A^d molecules, we found that two I-A monoclonal antibodies 17-227 and 25-9-17 immunoprecipitate additional polypeptides of 34K, 29K and 25K from B10.D2 LPS spleen extract that are not immunoprecipitated by MKD6. 17-227 and 25-9-17 monoclonal antibodies were also able to immunoprecipitate I-A^d molecules from B10.D2 extract depleted of MKD6 reactive molecules by repeated passage of extract through an MKD6 monoclonal antibody column. These studies suggest that 17-227 and 25-9-17 detect a subset of I-A^d molecules that is not immunoprecipitated by MKD6. Preliminary immunoprecipitation studies of I-A^d molecules from the Balb/c B cell lymphoma line, A20/2J suggest that the additional polypeptides immunoprecipitated by 17-227 from B10.D2 extracts are not immunoprecipitated from A20/2J. This suggests the possibility that the 17-227+, 25-9-17+, MKD6- subset is not expressed by A20/2J. Additional biochemical studies are underway to confirm the heterogeneity of I-A^d molecules. We are also examining the expression of I-A^d molecules in recombinant mice which have crossovers within the I region. B10.GD, which is a recombinant between d and b haplotypes with the crossover in the I-E beta gene, should express I-A^d molecules identical to B10.D2. However, we have found that 17-227 and 25-9-17 monoclonal antibodies precipitate 2-5 fold fewer Ia molecules from B10.GD extract than from an equivalent amount of B10.D2 extract. Additional studies are underway to determine the molecular basis for this difference in I-A^d molecules from B10.GD and B10.D2.

0413 MURINE LEUKEMIA VIRUS SEQUENCES ARE ENCODED WITHIN THE MAJOR MURINE HISTOCOMPATIBILITY COMPLEX, H-2, Daniel Meruelo, Ruth Kornreich, Anthony Rossomando, Christine Pampeno, Andrew L. Mellor, Elizabeth H. Weiss, Richard Flavell and Angel Pellicer, New York University Medical Center, New York, NY10016

The studies reported here localize, for the first time, murine leukemia viral sequences to the TL region of the major histocompatibility complex (MHC), H-2. We examined a battery of 38 cosmids isolated from two large genomic libraries constructed from C57BL/10 spleen DNA, which define 25 different Class I gene sequences. The viral probes used hybridized with only four cosmids, containing overlapping mouse sequences, which define four Class I gene related sequences in a region of 90 kilobases of DNA. The data show that two distinct viral envelope sequences are contained within the cluster. One of these sequences is situated with its 3' end next to the 3' end of a Class I sequence. The other sequence which does not contain the entire viral envelope is also proximal to the 3' end of a different Class I sequence. Hybridization of the viral probes with the H-2 cosmid clones does not appear to be due to homology between viral and H-2 sequences. Rather, the viral sequences detected appear to be linked to or inserted amid Class I genes. These findings may be significant in understanding molecular mechanisms involved in the generation of H-2 Class I gene diversity.

0414 THE IDENTIFICATION OF FUNCTIONAL T CELL RECOGNITION SITES ON I-E MOLECULES. Barbara W. Needleman[+], Michel Pierres[$], Christian Devaux[+], Patrick N. Dwyer[+], David H. Sachs[+] and Richard J. Hodes[+], [+]NIH, Bethesda, MD 20205 and [$]INSERM-CNRS, Marseille, France.

The recognition of I-E molecules by antigen-specific T cells was studied to determine if one or multiple topographical sites on the I-E molecule function as restricting elements for T cells. A panel of 14 monoclonal anti-I-E^k antibodies was used to inhibit I-E restricted antigen-specific T cell proliferation. These antibodies gave patterns of inhibition which were reproducible for each of several long term antigen-specific T cell lines and clones. The observed inhibition by the antibodies appeared to be caused by specific steric or allosteric interference with T cell recognition of antigen and Ia, and was not due to nonspecific differences in immunoglobulin subclass, affinity, or recognition of determinants on distinct subsets of I-E molecules, nor was it due entirely to interference with antigen uptake and processing by the antigen presenting cells. A single dominant pattern of inhibition was observed for each of several heterogeneous T cell lines. In contrast, however, several distinct patterns of inhibition were observed when a series of T cell clones was studied. It was therefore concluded that T cell clones can differ from each other in terms of which topographic area of the I-E molecule they recognize in association with the same nominal antigen. Based on the differences in patterns of inhibition, it was possible to identify at least three spatially definable sites on the I-E molecule which are functionally involved in the proliferative response of antigen-specific T cells.

0415 THE FUNCTIONAL ROLE OF GENETICALLY DEFINED HUMAN MHC CLASS DETERMINANTS, Pamela J. Reitnauer, Paul M. Sondel, Jaquelyn A. Hank, Cecile C. Chang, and Robert Demars, University of Wisconsin, Madison, Wisconsin 53706

The human MHC region consists of multiple class II loci with structurally similar gene products. Among the loci that have been characterized are DC/MT, DR, and SB. We are investigating the role of the class II determinants in the immune response to Epstein-Barr virus (EBV) specific antigens and to alloantigens using a series of variants generated from an EBV transformed lymphoblastoid cell line designated 721 (EBV-LCL). Three types of variants are being used in proliferative assays, all missing the paternal haplotype and one or more of the defined class II region genes of the maternal haplotype as follows: DC/MT$^+$, DR null, SB$^+$; DC/MT null, DR null, SB$^+$; DC/MT null, DR null, SB null.

Evidence from autologous primary and secondary responses to 721 and variants of 721 indicate that MHC genes in the DC/MT, DR and SB regions are each important in the response to EBV-LCL. Furthermore, there are greatly diminished but significant responses to variants that are null for all 3 of these class II loci, indicating that determinants mapping outside the defined class II region may play a role in the immune response to EBV-LCL as well. Strong allogeneic responses have been generated against mutants that lack DC/MT and DR. Moreover, marked primary and secondary responses to variants that lack all three of these defined class II products indicate that structures other than DC/MT, DR or SB may be important in the allogeneic response.

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0416 FUNCTIONAL ANALYSIS OF THE IA_β FRAME; William Roeder, Dale Wegman*, Jacques Chiller* and Richard Maki, La Jolla Cancer Research Foundation and Research Institute of Scripps Clinic*.

To study the functional expression of IA genes and to determine the nature of those sites recognized by I-helper cells, we have developed a transformation system that allows introduction of in vitro modified genes into the antigen-presenting B-lymphoma line, L10A2J.Aza^R. The IA_β genes from the k, b and d haplotypes have been cloned and an IA_β expression vector has been constructed in the plasmid pSV2-gpt. This allows the selection in HAT media of stable transformants following protoplast fusion. We have constructed recombinants in which first domain sequences are replaced by those of the other haplotypes and have introduced these chimeric genes into L10A2J to analyze the recognition requirements of a battery of IA^K specific T-helper hybridomas. We are, in addition, generating intra-first domain recombinants between the haplotypes to further define the amino acids recognized by specific T-helper cells. These studies, in concert with specific in vitro mutagenesis studies will, we hope, define those portions of the IA_β molecule recognized by T-helper cells.

0417 IDENTIFICATION OF AN ALLOANTIGENIC DETERMINANT ON THE HEAVY CHAIN OF H-2K^b CLASS I MHC ANTIGEN, Bhagirath Singh, Ester Fraga and Jana Widtman. Department of Immunology and MRC Group on Immunoregulation. University of Alberta, Edmonton, Alberta Canada.

The determinants involved in allogeneic immune responses are known to be in the heavy chains of Class I major histocompatibility antigens. The nature and relationship of these epitopes and functional properties of Class I MHC antigens is unknown. Heavy chains of some of these antigens from human and mouse have recently been sequenced and are found to contain the most polymorphism in the 61-85 region. These regions may contribute to the allo-specificity of these molecules. We have chosen the H-2K^b molecule for determining the role of this region in alloimmune responses and have chemically synthesized the 61-69 peptide fragment. This peptide when coupled to a carrier induces antibodies which are specific for H-2K^b antigen on cell surfaces. These antibodies can only be induced in mice of H-2^d and H-2^k strains but not in H-2^b (syngenic) strains. Further, this peptide induces secondary *in vitro* T cell proliferation in the lymph node T cells of H-2^d and H-2^k strain mice, which were previously primed with H-2^b cells. At present we are cloning peptide specific T cells from H-2^b and peptide primed animals for further analysis. The sequence of H-2K^b in 61-69 region is identical to H-2D^b but has about 50% residue difference with H-2K^d and H-2K^k molecules. Therefore, the 61-69 region of H-2K^b represent the first defined peptide region on Class I MHC molecules which is involved in the induction of alloimmune responses.

0418 PRODUCTION OF ANTIBODIES AGAINST SELECTED REGIONS OF MOUSE CLASS II MOLECULES BY IMMUNIZATION WITH SYNTHETIC PEPTIDES, Stanley M. Wilbur and Minnie McMillan, USC School of Medicine, Los Angeles, CA 90033

The amino acid sequences of several murine class II molecules are known. We have synthesized peptides of 8-14 amine acids which correspond to selected regions of the A and E molecules. The sites on the class II molecules were chosen using a number of criteria, including hydrophilicity, so that antibodies against the peptides might also react with denatured as well as native protein. The peptides were synthesized using solid phase techniques and purified by reverse-phase high-pressure liquid chromatography. Optimal production of antibody in mice depended markedly upon the choice of murine strain, carrier and adjuvant. Analysis using radioimmune assays demonstrated that the antisera against synthetic peptide also recognizes native Ia molecules. Monoclonal antibodies are presently being developed. The use of these antibodies as site specific probes to investigate the structure-function relationship of the class II molecules will be discussed.

Creation of the Expressed T Cell Repertoire and Self/Non-Self Discrimination

0419 Frequency of Cytotoxic T Lymphocyte Precursor Cells Specific for TNP-Modified Allogeneic Cells, Shiro Aizawa and Richard G. Miller, Ontario Cancer Institute, Toronto, Ontario, Canada M4X 1K9

The frequencies of cytotoxic T lymphocyte (CTL) precursor cells in normal mice specific for TNP-modified allogeneic and TNP-modified syngeneic cells have been compared by limiting dilution analysis. B6($H\text{-}2^b$) spleen cells were cultured at limiting dilution (125 - 2,000 cells/well) with TNP-modified syngeneic ($H\text{-}2^b$) or allogeneic ($H\text{-}2^k$) stimulator cells. Cultures were split into four aliquots and assayed against TNP-modified or unmodified syngeneic or allogeneic Con A blast targets and classified for cytotoxic activity specific for TNP-self, TNP-allo, or allo H-2 alone. The frequency of CTL precursors specific for TNP-allogeneic ($H\text{-}2^k$) cells was comparable to that of CTL precursors specific for TNP-syngeneic ($H\text{-}2^b$) cells. Similar results were obtained in the combination of $H\text{-}2^d$ responder and $H\text{-}2^k$ allogeneic stimulator. In neither case did we see a clear preference for TNP-modified self responses. Possible reasons why these results are in disagreement with those of previous workers will be discussed.

0420 Mutation-like events in the generation of T cell receptor diversity. Andrei A. Augustin, Ian A. MacNeil, Gek Kee Sim, National Jewish Hospital, Denver, CO 80206.

For obvious theoretical reasons, it is important to determine experimentally whether genes encoding for the receptor of alloreactive T cells mutate frequently and whether such mutations can contribute to the generation of T cell diversity. This study will be dedicated to the antigen specificity and function of such mutants.

We were able to detect mutation-like events experimentally both in alloreactive T cells propagated and cloned in vitro and in alloreactive (I region specific) T cell hybrids. Our basic observations are:

1. Loss of the initial known, alloreactivity in T cell clones and the appearance of a new specificity for a different alloantigen can happen simultaneously.
2. In clones of T cells exhibiting a specific pattern of crossreactivity, the appearance of variant T cell subclones which exhibit a different pattern has been noted.
3. The loss of alloreactivity can coincide with the gain of an MHC restricted specificity for soluble, conventional antigens. In this study such antigens were synthetic polypeptides for which a "gain" of reactivity could be predicted as a higher probability event on the basis of some oddities accuring in the "Ir gene controlled" responsiveness to these polypeptides.

All newly aquired antigen specificities are stable in time.

0421 A FACTOR FROM NEONATAL SPLEEN-MYELOMA FUSIONS SUPPRESS LYMPHOCYTE PROLIFERATION, Beverly E. Barton and C. Garrison Fathman, Stanford University, Stanford, CA 94305

Spleens from 1 day old DBA/2J mice were fused to the nonsecreting myeloma FO. Selected cloned cells from these fusions had an average of 64 chromosomes, morphologically resembled fibroblasts, were found to be anchorage-dependent, and secreted or shed a suppressor factor, the NBxFO factor. Dialyzed supernates of these cells were found to suppress the antigen-specfic proliferative response of cloned helper T cells at a final concentration of 0.5% or less. These supernates also inhibited the proliferative response of murine spleen cells to certain mitogens. However, the same supernates did not suppress the response of an IL-2 addicted T cell line, HT-2, to IL-2, nor did they suppress T cell help in secondary antibody formation (in an in vitro assay), although they did suppress the antigen-specific proliferation of the same helper T cells. The factor was not found to suppress the production of either IL-2 or of antibody (following stimulation of spleen cells with LPS).

Absorption analyses revealed that the target of the NBxFO factor was the accessory cell population. Further experiments indicated that the NBxFO factor blocked the proliferation of cells due to IL-1. Biochemical analysis revealed the NBxFO factor to be a protein with a molecular weight of approximately 90 Kd and a pI of approximately 4.5. The factor was found to suppress proliferative responses in 2 other species, rat and human, indicating possible therapeutic usefulness for this factor or analogues. In vivo experiments with the factor (given as ascites) showed reductions in primary MLR and in Con A mitogenic responses.

0422 THYMIC CYTOTOXIC T CELLS ARE PRIMED IN VIVO TO MINOR HISTOCOMPATIBILITY ANTIGENS, Pamela J. Fink, Michael J. Bevan and Irving L. Weissman, Stanford University Medical Center, Stanford, CA 94305

Vigorous cytotoxic T lymphocyte (CTL) activity can be generated from murine thymocyte responders cultured with spleen cell stimulators expressing foreign minor histocompatibility (minor H) antigens. This cytotoxic activity requires in vivo priming and the presence in vitro of supernatant from Con A activated spleen cells. Experiments were performed to determine whether these minor H-specific effector cells are thymocytes which have been primed in situ, contaminating cells from neighboring lymph nodes, or mature peripheral T cells which have returned to the thymus after meeting antigen in the periphery. Depletion of peripheral cells with anti-lymphocyte serum and part body irradiation demonstrates that recent thymic immigrants derived from the bone marrow contribute to the primed thymic response. In addition, thymic CTL were primed in mice in which peripheral T cell responses were completely eliminated during the in vivo priming period by repeated injections of monoclonal anti-Thy-1 antibodies. In a third set of experiments, primed antigen-activated lymph node cells were also found to contribute to the thymus-derived CTL response. Thus, the minor H-specific thymic CTL response is due both to "in situ" priming and to entry into the thymus of activated peripheral T cells, leading to speculation on the possible influence on T cell differentiation of the presence within the thymus of antigen and antigen-reactive mature T cells.

0423 ALLOREACTIVITY IS THE DUAL RECOGNITION OF CLASS II AND NON-MHC CODED PRODUCTS, Suzan Friedman, Deborah A. Sillcocks, and Harvey Cantor, Dana-Farber Cancer Institute, Boston, MA. 02115

An H-2^d derived Lyl T cell clone, selected to respond to ovalbumin and self-class II coded product, recognizes determinants on allo-class II antigens of H-2 haplotypes; b, k, and j. The genetic analysis of activation of the T cell clone revealed that allo-Class II antigen alone does not stimulate the clone. In the response to each haplotype, a non-MHC coded antigen is necessary to be presented by the spleen cells. Furthermore, the T cell clone is not tolerant to self-Class II products. By varying the background genes, the T cell clone can be induced to respond to self-Class II and non-MHC coded antigen.

The genetics of inheritance of the non-MHC coded antigen is similar to the pattern of inheritance of the A, B, O blood sugar substances: While the parental Class II antigens are co-dominantly expressed in all the F_1 strains tested, the expression of the non-MHC coded antigen is detected in a minority of the F_1 strains. Thus, the differential glycosylation of allo Class II antigens may modulate alloreactivity.

0424 ROLE OF LYMPHO-STROMAL RECOGNITION IN MURINE THYMIC LYMPHOPOIESIS, Bruno A. Kyewski and Henry S. Kaplan, Stanford Univ. School of Medicine, Stanford, Ca 94305.

Direct cell to cell interactions between thymocytes and (1) I-A/E neg. cortical macrophages, (2) I-A/E pos. medullary dendritic cells, and (3) I-A/E pos. cortical epithelial cells have been characterized by isolating and purifying multicellular lymphostromal cell complexes. These multicellular complexes represent the in vitro correlate of the interactions in vivo. The frequency of all three types of interactions strictly correlates with the ontogeny of thymic T cell maturation and is unaffected by immunization. Stromal cell-associated T cells constitute~ 3% of all thymocytes, display a surface antigen phenotype typical of immature thymocytes and are specifically enriched in dividing cells. Thymocyte-stromal cell interactions are non-random and independent of the genotype of the partner cells. During post-irradiation recovery T cells associate with stromal cells in a defined sequence indicating a temporal hierachy in intrathymic lymphostromal recognition. Only medullary dendritic cells are accessible to and capable of presenting circulating non-MHC antigens in vivo. In contrast cortical macrophages and epithelial cells do not present antigen in vivo or in vitro. Purified stromal-cell associated thymocytes, despite their immature phenotype, contain functional host-type MHC-restricted CTL precursors. These results are compatible with the proposition that all three types of interactions are sites of intrathymic lymphopoiesis ("positive selection"). The interactions differ with respect to their intrathymic location, the time course of T cell interactions, the accessory function of the stromal cells and presumably the T cell recognition structures, which are responsible for stromal-cell binding.

0425 SELF IA TRIGGERED IL-2 PRODUCTION BY NEWBORN THYMOCYTES, Edmund C. Lattime and Osias Stutman, Memorial Sloan-Kettering Cancer Center, New York, New York 10021

Interleukin-2 (IL-2) is produced by a subpopulation of thymocytes triggered by self IA/E antigens expressed on syngeneic dendritic cells. The Lyt $1^+, 2^-$ self recognizing thymocyte population is present early in ontogeny (measurable from birth until 2-3 weeks of age). IL-2 is also produced following allogeneic stimulation, however, significantly more IL-2 is produced in the self response. Following the loss of the self recognizing thymus population at three weeks, the allo-responsive cell remains and increased levels of IL-2 are induced by allo stimulation. There is no evidence of active suppression of the self IL-2 production in the adult thymus, but rather the IL-2 producing cell appears absent. At 2-3 weeks of age, a similar self IA/E triggered IL-2 producing cell (Lyt 1^+, 2^-, Ia^-, Qa 1^-, Qa 2^+, Qa 5^-) can be shown in the spleen. Studies with cloned long term T cell lines derived from Lyt 1 enriched self recognizing T cells in the spleen which grow without the need of exogenous IL-2 and require only IA^+ accessory cells show that IL-2 production is triggered by self, but not allogeneic, IA/E antigens on the accessory cell, and is fetal calf serum independent.

The presence of this self Ia recognizing IL-2 producing cell in the thymus and spleen, taken with the presence of discreet "compartments" of stromal IA^+ cells in these organs, would suggest that this type of lymphokine producing local cell-cell interaction may be involved in the differentiation of T cells in certain microenvironments, perhaps as a source of IL-2 to drive a round of proliferation as a means of "fixing" developmental changes.

0426 MIGRATION OF Ia+ CELLS INTO CULTURED FETAL THYMUS. David D. Lo, Jonathan Sprent, University of Pennsylvania, Philadelphia, PA 19104

Using isolated fetal mouse thymus, we have been studying the migration of Ia+ cells from various sources into the thymus cultured in vitro. By employing Ia-different donor-host combinations, we can use strain-specific anti-Ia antibodies to establish the origin of the Ia+ cells in the thymus. When fetal thymus is cultured for 1 week, Ia+ cells are conspicuous in both medulla and the cortex. When the thymus is cultured in the presence of Ia-compatible or Ia-incompatible marrow or fetal liver, donor derived Ia+ cells migrate into the thymus. Significantly, this is associated with a marked increase in the expression of host-type (thymic origin) Ia determinants. In the cortex, the distribution of Ia determinants is confluent and presumably reflects increased Ia expression by the epithelial cell component. Interestingly, Rat Con A Sn can substitute for bone marrow cells in causing the increased expression of host-type intrathymic Ia determinants. If cultures of Ia-compatible (Ia^b) bone marrow plus thymus (Ia^b) are cultured in the presence of anti-Ia^b antibodies (without C) the expression of Ia^b in the thymus is reduced in both the cortex and medulla. No such reduction in Ia expression occurs if Ia^k rather than Ia^b marrow is used. Here, culturing the Ia^b thymus with Ia^k marrow leads to intense expression of intrathymic Ia^b, even in the presence of anti-Ia^b antibody. Our working hypothesis is that Ia+ cells migrating into the thymus interact with cortical thymocytes as part of the T cell differentiation process. The response to "self" Ia results in the release of factors (γ-IF?) that increase Ia expression by thymus stromal (epithelial) cells.

0427 MHC RESTRICTION OF T CELL FUNCTION IN NUDE MICE RECONSTITUTED WITH NEONATAL AND ADULT THYMUSES. Dan L. Longo, Mary L. Davis, Medicine Branch, National Cancer Institute

The thymus exerts a potent influence on the recognition of antigen and self MHC by T lymphocytes. Thus, (B10.D2xB10)F_1 nude mice reconstituted with neonatal thymuses from B10.D2 mice develop T cells capable of recognizing soluble antigens only in association with I-region gene products of the d haplotype. There is no recognition of antigen in association with I-A^b gene products. The transplanted thymus has restricted the repertoire of the (B10.D2xB10)F_1 nude T cell precursors to antigen recognition in the context of the thymic MHC genotype. For unknown reasons, it has not been possible to do similar experiments using thymuses from adult mice to reconstitute nude T cell function. This could be due to the failure of the adult thymus to engraft because of its size or to the fact that the normal adult thymus is relatively dormant. Yet it appears that the adult thymus can be activated to generate more T cells when an adult suddenly loses significant amounts of its T cell mass. We reasoned that we might be able to successfully transplant adult thymuses that had been reduced in size and aroused from dormancy by treatment with antithymocyte globulin (ATS) and cortisone. Thus, thymuses from ATS and cortisone treated adult B10.D2 mice were transplanted subcutaneously into (B10.D2xB10)F_1 nude mice. Two months later their T cells responded to antigens in the context of I-A^d but not I-A^b gene products, similarly to recipients of neonatal thymus grafts. This will enable us to use thymuses from radiation-induced bone marrow chimeras to reconstitute nude mice and settle definitively the question of whether the thymic epithelium or antigen-presenting cell is the effector of MHC-restriction in the thymus.

0428 FUNCTION OF CLASS I H-2 MOLECULES EXPLORED WITH H-2 MUTANTS, C.J.M. Melief, L.P. de Waal, W.M. Kast, M.J. Stukart, Central Lab Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands.

Recent results include the following. 1) Allospecific CTL recognise conformational determinants The evidence is that the 146-162 animo acid (AA) segment in the H-2K^{bm1} molecule creates allodeterminants grossly different from those created by the identical stretch of AA in the H-2 L^d molecule. 2) H-2 K^b mutations affect the repertoire of H-2 D^b restricted Moloney virus-specific CTL. Self H-2 tolerance requirements affect the repertoire of foreign antigen-specific CTL, even if the tolerizing self H-2 molecule is not the restricting element. 3) Distinct H-2 K^b CTL restriction sites are involved in different antigenic systems, because of the divergent behaviour of K^b mutants in CTL responses to Sendai and Moloney viruses. 4) Previous studies have shown that each of nine H-2 K^b mutants shows a marked decrease in the anti-Sendai bulk CTL response when compared with the high responder strain of origin, C57B16 (B6). The bm5, bm6, bm7, bm16 and bm17 mutants were intermediate responders, bm3 and bm8 were low responders, bm11 was a very low responder and bm1 a nonresponder. Limiting dilution analysis of Sendai-specific CTL precursors from each of these strains has now shown that the frequency of Sendai-specific CTL precursors accurably reflects the capacity to respond in bulk culture. Thus, the precursor frequency was approximately 1:2,000 in B6, 1:10,000 in bm16, 1:80,000 in bm3 and bm8 and > 80,000 in bm11 and bm1. All virus-specific K^b mutant CTL clones tested also lysed B6 virus-infected target cells. These data indicate that the H-2 K^b mutants have lost Sendai CTL restriction specificities without apparent gain of new specificities, explaining their decreased or non-responsiveness and low precursor frequencies.

0429 SELF TOLERANCE APPEARS TO BE MHC-RESTRICTED, Hans-Georg Rammensee and Michael J. Bevan, Dept. Immunology, Scripps Clinic and Research Foundation, La Jolla, CA 92037

BALB/c (H-2^d) spleen cells were acutely depleted of alloreactive cytotoxic T cells (CTL) specific for B10.BR (H-2^k) cells with the BudR + light method. The surviving cells were primed in vitro with BALB.K (H-2^k) cells for 7 days, and restimulated for another 4 days, in the presence of IL-2 containing rat Con A supernatant. The resulting CTL lysed BALB.K, and to a lesser extent also B10.BR Con A blasts; this remaining reactivity on B10.BR targets, however, disappeared upon further restimulations with BALB.K. The resulting CTL line, therefore, lysed only BALB.K, and not B10.BR nor BALB/c, but did lyse (B10.BR x BALB/c)F_1 targets, indicating the recognition of a BALB minor histocompatibility (H) antigen in context of H-2^k product(s). Further testing of this CTL line revealed the following reactivity pattern: C3H was killed, while B10.A, A/J, (BALB/c x A/J)F_1, BALB.B and B10 were not killed, indicating the specificity of the CTL line as self (BALB) minor H antigen plus D^k encoded molecules. An identical reactivity pattern was observed in another CTL line derived independently with the same strain combination. Another CTL line derived from C57BL/6 (H-2^b) cells depleted of reactivity against BALB/c (H-2^d) lysed B10.D2 (H-2^d). The existence in mice of CTL capable of recognizing self minor H antigens in the context of allogeneic Class I antigens indicates that the mechanism(s) responsible for self tolerance do not involve recognition by CTL or their precursors of self antigens in the absence of a restricting Class I element. (H.-G. R. is recipient of Grant No. Ra 369/1-1 of the DFG.)

0430 The Selective Activation of Self-MHC-Specific Developing T Cells In Vitro.
Kenneth L. Rock, Edward Yeh and Baruj Benacerraf
Harvard Medical School, Boston, MA 02115

The activation requirements of developing T lymphocytes have been investigated. Thymocytes proliferate in response to exogenously added IL-1 in culture. This proliferation reflects the activation of T cells with specificity for self-Ia molecules on syngeneic accessory cells in the absence of foreign antigen. IL-1 is not directly mitogenic but rather appears to be required as a second signal for these MHC-specific thymocytes. The nature of this response has been confirmed by the analysis of thymocyte T cell hybridomas. Since Ia molecules on accessory cells may be linked to the induction of IL-1, this response could form the basis for the selected amplification of T cells with self-MHC specificity. To examine this possibility we have measured the capacity of thymocytes to induce IL-1 in culture. Thymocytes, upon interaction with syngeneic accessory cells, induce IL-1. The generation of IL-1 is dependent on Ia molecules on accessory cells. Subsequent to the induction of IL-1, these self-specific thymocytes are driven to proliferate. The features of these responses are unique to the thymus and appear to be present at all levels of thymocyte maturity. It therefore appears that this in vitro response could select and expand T cells of a restricted specificity, which may be of importance in the T cell commitment to MHC molecules that is known to occur in the thymus.

0431 ACQUISITION OF MHC DETERMINANTS BY THYMOCYTE PRECURSORS, Susan O. Sharrow, Philip J. Morrissey, Bonnie J. Mathieson and Alfred Singer. NIH, Bethesda, MD. 20205.

It has previously been shown that the Ia (Class II) determinants which murine thymocytes bear on their surface are predominantly, if not entirely, those which they have acquired from the environment in which they differentiate. In the present study, the differentiation compartment in which Ia determinants are acquired was studied by analysis of thymocytes from thymus-engrafted radiation bone marrow chimeras and thymus-engrafted nude mice using immunofluorescence and flow microfluorometry. In experimental animals in which the MHC genotype of the thymus was distinct from that of the extra-thymic host, it was observed that thymocytes acquired both syngeneic (to bone marrow genotype) and allogeneic Ia determinants from the intra-thymic environment. To examine the possibility that thymocyte precursors might also acquire host Ia determinants in the pre-thymic differentiation compartment, a variety of experimental animals were constructed in which the MHC genotype of the bone marrow stem cells, the extra-thymic host and the thymus were independently varied. It was observed that thymocyte precursors could acquire host-derived Ia determinants in the pre-thymic differentiation compartment and that the pre-thymic acquisition was specific. Namely, thymocyte precursors only acquired syngeneic but not allogeneic (to bone marrow genotype) host-derived Ia determinants in the pre-thymic compartment. These data demonstrate that the pre-thymic acquisition of host Ia determinants is specific in that thymocyte precursors appear to distinguish between syngeneic and allogeneic host Ia determinants.

0432 Internal images of I region determinants expressed by T cell receptors. Gek Kee Sim and Andrei A. Augustin, National Jewish Hospital, Denver, CO 80206.

The display of I region-like internal images by the receptor of some T helper cells would be beneficial for the generation and maintenance of a self-restricted T cell repertoire. We will report experiments which strongly suggest the existence of such epitopes.

1. When an alloreactive T cell hybridoma was used as an immunogen, it ellicited a strong T cell proliferative response in vivo and in vitro. Proliferating T cells were further hybridized. In this way we obtained two IL-2 secreting T cell hybridomas which interact specifically. None of these hybrids expresses conventional I-region molecules and one of them is alloreactive (anti-I-A).
2. When a monoclonal anti-I-A antibody is used as an immunogen one can select from the responder, proliferating T cells those which react to the antibody in the absence of any antigen presenting cells. Such T cells when hybridized gave rise to IL-2 secreting hybrids, which can be induced specifically with the anti-I-A monoclonal antibody, originally used as immunogen. The successful induction is strictly dose dependent.
3. The appropriate anti-I-A antibodies can precipitate from our internal image-bearing T cell candidates dimers of approximately 40 kd polypeptides which resemble what the consensus of the moment describes as T cell receptors.

0433 SUPPRESSION OF ANTI-SELF CTL RESPONSES BY FETAL THYMUS CELLS, Hung-Sia Teh and Margaret Ho, Department of Microbiology, University of British Columbia, Vancouver, Canada V6T 1W5.

Fetal thymus cells taken from C57BL/6X C57BL/6 female mice at 14 to 19 days of gestation were able to specifically suppress the CTL responses of non H-2^b mice to H-2^b allo-antigens. Suppression of the anti-H-2^b response was virtually complete by fetal thymus cells taken at 14 to 16 days of gestation and started to wane by day 17. Adult thymocytes were completely nonsuppressive. When fetal thymus cells were taken from C57BL/6 mice at 14 days of gestation and cultured in an organ culture system, the cultured cells were able to completely suppress an anti-H-2^b CTL response even after 19 days in culture. The suppressor cells are radiation-resistant and appear to reside within the epithelium of the fetal thymus since fetal thymus cells which were obtained by trypsinization of the whole thymus were much more suppressive than those obtained by mechanical disruption. Part of the suppression of the CTL response is due to competition by fetal thymus cells and CTL for interleukin 2. However, the non-specific suppression of the CTL response by fetal thymus cells can be completely reversed by adding interleukin 2-containing supernatants whereas the specific suppression is still observed after the addition of exogenous interleukin 2. Such a suppressor cell may be intimately involved in the induction of tolerance to self MHC antigens (Supported by MRC of Canada).

0434 Analysis of neonatally induced tolerance to MHC antigens at the clonal level. Hermann Wagner and Klaus Heeg, Dep.of Immunology, University of Ulm, 7900 Ulm, W-Germany

The mechanism of neonatally induced tolerance to MHC-antigens is controversial. In the system used here tolerance to D^d antigens is defined by 4 week acceptance of A-skin graft on CBA mice neonatally injected with 10^7 A-type spleen cells. Such mice exhibit phenotypically a 10-20 fold reduction of anti D^d cytotoxic T lymphocyte precursors (CTL-p) as assayed by limiting dilution. In agreement with the work of Nossal et al. one might conclude clonal deletion as basis of neonatally induced tolerance. However after absorption of the very same responder cells on CBA anti-A MLC cells, high frequencies of anti D^d reactive CTL-p can be demonstrated in the apparently tolerant cells, results similar to those of Stockinger. Furthermore, upon 24 h polyclonal stimulation with ConA and subsequent platine of the cells in limit dilution, high frequencies (1/200) of anti D^d reactive CTL can be demonstrated by the split culture approach. Finally, under limit dilution T cells of tolerant mice suppress antigen specificially anti D^d reactive CTL-p of normal mice. The results rule out clonal deletion as explanation of neonatally induced tolerance and favour active suppression.

0435 T CELL HYBRIDOMAS WITH DUAL SPECIFICITY FOR MLS AND H-2 DETERMINANTS: SELECTIVE LOSS OF ONE SPECIFICITY, Susan Webb, Jane Hu, Ian MacNeil, Jonathan Sprent and Darcy Wilson, University of Pennsylvania and Wistar Institute, Philadelphia, PA 19104; National Jewish Hospital and Research Center, Denver, CO 80206

Like H-2 determinants, Mls gene products stimulate strong primary mixed lymphocyte responses. In several respects, responses to Mls determinants are quite different from responses to either H-2 alloantigens or self + X antigens: Mls determinants cannot be detected serologically, do not generate cytotoxic T cell responses, do not appear to act as transplantation antigens and do not induce lethal graft-versus-host reactions. Previously, we have shown that a portion of T cell clones reactive to Mls determinants can also proliferate in response to H-2 determinants. Significantly, the pattern of anti-MHC reactivity seen among this panel of Mls reactive clones appeared to be random. These findings, coupled with the unusual nature of T cell responses to Mls determinants, suggested to us that T cell recognition of Mls may be fundamentally different from T cell recognition of either H-2 alloantigens or self + X antigens, in which case, Mls recognition might occur via a totally independent set of receptors. We have tested this question using karyotypically unstable T cell hybridomas. Our data show that subclones of dual reactive hybridomas having specificity for both Mls and H-2 can lose the ability to react to either H-2 determinants or Mls determinants while maintaining reactivity for the other ligand. This ability to lose H-2 reactivity independently of Mls reactivity supports the notion that the recognition units are encoded on different chromosomes.

0436 ANALYSIS OF HELPER AND EFFECTOR POPULATIONS IN LYMPH NODES OF NEONATALLY TOLERANT MICE, Peter J.Wood, Phoebe Strome, and J.W.Streilein, University of Texas Health Science Center, Dallas, TX 75235

Lymph node (LN) cells, from mice made tolerant of H-2 antigens as neonates by the injection of semiallogeneic spleen and bone marrow cells, fail to proliferate (mixed lymphoycte response, MLR) or to generate cytotoxic T lymphocytes (T_cs) in response to the tolerogen. Since previous results had suggested that this failure to respond was not due to suppression, we investigated whether the non-reactivity was due to a lack of antigen reactive cells. Limiting dilution assays were performed to measure the frequency(i) of cells capable of proliferating in response to tolerogen, (ii) IL-2 producers, and (iii) of T_c precursors (pT_c), in the LNs of tolerant mice. Using the combinations of B10.A tol B10 (whole H-2) and AQR tol 6R (class II), it was found that the frequency of MLR cells and IL-2 producers toward the tolerogen was reduced to that of the frequency against self. When T_c were generated in the presence of rat spleen Con-A culture supernatant as a source of help, significant though reduced cytotoxicity could be generated against the tolerogen with LN cells of B10.A mice tolerant of B10. Limiting dilution studies confirmed that tolerant LN cells contained 5-10% of the numbers of pT_cs to the tolerogen compared to normal B10.A mice. Thus the lack of cytotoxicity responses in tolerant mice is not necessarily due to a lack of effector cell precursors (pT_c) but can be explained by clonal deletion of helper cells. The possibility that inappropriate activation of pT_c in vivo could result in anti-tolerogen directed responses may have significance to autoimmune pathogenesis mechanisms.

0437 **MECHANISMS OF T CELL REPERTOIRE SELECTION - CHARACTERIZATION OF AUTOREACTIVE, Ia-SPECIFIC, IL-2 PRODUCING THYMOCYTE HYBRIDOMAS FROM THYMOCYTE CULTURES,** **Edward Yeh, Baruj Benacerraf and Kenneth L. Rock, Harvard Medical School, Department of Pathology, Boston, MA 02115.**

The H-2 specificity of the thymus plays a pivotal role on the acquisition of the T cell reprertoire. Generation and selection of T cell specificities may involve both thymic and extra-thymic processes. Our laboratory has demonstrated that thymocyte proliferation to IL-1 requires Ia recognition on an adherent, radioresistant, Ia-bearing cell. Furthermore, purified thymic T cells can be driven to expand in the presence of only Ia-bearing cells. To extend these observations, we have used somatic cell hybridization to generate thymocyte hybrid clones. Hybridomas from T cell blasts derived from thymocyte cultures with or without exogenous IL-1 were obtained. A high frequency of self-specific hybridomas have been identified. The specificity of these clones has been mapped to both the I-A and I-E subregion. These hybrids are Lyt 1^+ and have their activities blocked by specific monoclonal antibodies against either I-A or I-E. We will discuss the implication of our findings and present other relevant data.

0438 INDUCTION OF SELF-TOLERANCE IS H-2 RESTRICTED, R. Zamoyska, P. Matziner & H. Waldmann, Department of Pathology, University of Cambridge, Cambridge, UK.

In the past it has been shown that the induction of self-tolerance in T cells does not appear to be restricted by the major histocompatibility complex (MHC). However, this apparent unrestriction could have resulted from host processing of the antigen. We present data which show that in experiments where the potential for antigen processing is minimised the induction of T cell tolerance is absolutely restricted by the MHC.

Thymectomised (B10 x B10D2)F_1 mice (AxB) were irradiated, reconstituted with syngeneic bone marrow and grafted with combinations of thymuses of parental MHC types, either syngeneic to the recipient (A+B) or bearing multiple minor histocompatibility antigen differences (A'+B'). These animals were primed and tested for the ability to generate cytotoxic T cells to minor HA bearing targets of the thymus type (A'&B') or to control unrelated minor incompatible cells (A"&B"). The results are presented in the table below. Tolerance induction was shown to be MHC restricted in animals which received one parental thymus, eg, A, + one minor incompatible thymus of the other parental MHC type, eg, B'. These animals were only tolerant of the minor antigens in combination with the parental MHC type with which they appeared in the thymus (B') but could respond to the same minor antigens in combination with the other parental MHC type (A').

Thymuses grafted	Response to:	A(B10)	A'(BALB.B)	A"(C3H.SW)	B(B10D2)	B'(BALB/c)	B"(DBA/2)
B10 + B10D2 A+B		-	+	+	-	+	+
BALB.B + BALB/c A'+B'		-	-	+	-	-	+
B10 + BALB/c A+B'		-	+	+	-	-	+

0439 TOLERANCE TO SELF M LOCUS MEDIATED BY T SUPPRESSORS CELLS. Marc Zeicher, Université libre de Bruxelles, 1640 Rhode-St-Genèse, Belgium.

We describe in naive Mls a mice a subset of T cells suppressing the proliferative response of Mls b strain T cells to Mls a lymphocytes in a primary in vitro mixed lymphocyte reaction. The phenotype of that subset is characterized and the role of I-A, I-E, I-J, IgH allotype loci in the interaction between suppressor and proliferating cells is specified. These suppressors cells could be an example of mechanism of unresponsiveness to self antigens actively maintained in adult mice. It is possible that, in addition to a central process of clonal deletion/inactivation induced by self antigens during early ontogeny,, there is a safety mechanism that operates to prevent autoimmunity when cells with autoimmune potential escape the central mechanism or mutate at a later state of maturation.

The Isotype Switch: Genetic, Physiological and Cellular Mechanisms

0440 INDUCTION OF ISOTYPE-SPECIFIC IMMUNOGLOBULIN mRNAs BY MITOGENS AND T CELL-DERIVED LYMPHOKINES, Sharon L. Jones, Judy Layton, Peter Krammer, Ellen S. Vitetta and Philip W. Tucker, University of Texas Health Science Center at Dallas, Dallas, Texas 75235

In B lymphocytes, the switch in isotype synthesis depends on the rearrangement of immunoglobulin (Ig) genes. The regulation of expression of these genes is influenced by lymphokines derived from T cells. These lymphokines may act by inducing DNA rearrangements or changes in the levels of isotype specific RNA (i.g. transcription, processing, half-life, etc.). We have cultured murine B cells with lipopolysaccharide (LPS) and T cell-derived lymphokines containing the lymphokine B cell differentiation factor for IgG_1 (BCDFγ). B cells isolated from normal BALB/c splenocytes, which express predominantly $sIgM^+$ and sIgD (less than 5% $sIgG^+$) were cultured for 2 to 6 days with LPS and BCDFγ-containing supernatants. The cytoplasmic RNA was isolated and subjected to Northern or dot blot analysis using the appropriate cloned probes to identify γ1 and γ3 mRNA. LPS alone induced an increase in the levels of the secreted form of the γ3 heavy chain mRNA (1.8 kb) by day 3 with no significant induction of γ1 mRNA until day 5. LPS + BCDFγ induced a marked increase in the expression of γ1 mRNA by day 2, and this γ1 mRNA continued to increase until day 6 at which point the experiments were terminated. Concomitant with the increases in γ1 mRNA, there was a decrease in the expression of γ3 mRNA. These data suggest that lymphokines secreted by T cell lines can influence the levels of isotype-specific mRNA in activated B cells.

0441 LYMPHOKINE-MEDIATED ACTIVATION OF T CELL DEPENDENT IgA ANTI-POLYSACCHARIDE RESPONSES, Martin F. Kagnoff, Susan L. Swain, and Peter D. Murray, University of California, San Diego, CA 92093

IgA responses are important to host immunity at mucosal surfaces. We previously showed that mice produce substantial T cell dependent IgA responses to α(1,3) glucan determinants on the bacterial capsular polysaccharide dextran B1355 in vivo and in culture. The present studies demonstrate that the requirement for T cells for the activation of the IgA anti-α (1,3) dextran B1355 response can be replaced by T cell derived non-antigen specific helper factors.

Supernatants from the activated T cell lines cr-15 and (DL)C.C3.11.75 containing interferon and T cell replacing factors supported differentiation of dextran-stimulated B cells to IgA anti-α (1,3) glucan antibody forming cells. Supernatants with interleukin 2 activity did not support optimal antigen-driven plaque forming cell responses, but synergized with supernatants having interferon and T cell replacing factor activity in the production of IgA anti-α (1,3) glucan responses. Supernatants from the T cell lines B6.11 and (DL)A.4 containing B cell growth factor activity did not support activation of IgA anti-α (1,3) glucan PFC. Studies with highly purified T cell derived lymphokines clearly demonstrate that interferon-γ plays a key role in the antigen-driven differentiation of murine B cells to IgA antibody-forming cells.

0442 THE IgH CHAIN SWITCH IN PROLIFERATING B CELL POPULATIONS, Amy L. Kenter and T.H. Rabbitts, MRC Laboratory of Molecular Biology, Cambridge, England

LPS has been shown to act as a B cell mitrogen causing the proliferation and differentiation of murine splenic B cells to Ig secreting plasma cells. It was previously established by others that the Cμ gene is lost in LPS treated spleen cells. We have studied the kinetics of Cμ gene rearrangement, in response to LPS, by Southern and Northern filter hybridization analysis. These studies suggest that the Cμ gene is being deleted by a complex mechanism, which may include sister chromated exchange.

0443 ALLOTYPE SWITCH VARIANTS IN CULTURED MONOCLONAL PRODUCING HYBRIDOMAS, Thomas J. Kipps and Leonard A. Herzenberg, Stanford University, Stanford, CA 94305.

We fused the non-immunoglobulin secreting BABL/c myeloma line NS-1 with spleen cells from BAB/25 X AKR/J mice that had been immunized with dansyl-keyhole limpet hemocyanin. From this fusion we cloned hybridomas producing monoclonal anti-dansyl antibodies of either the "b" or "d" allotype of the BAB/25 or AKR/J parent, respectively. These allotypes can be distinguished using a solid phase radioimmunoassay employing monoclonal anti-allotypic antibodies. Cultures of cloned IgG1-producing hybridomas were stained with fluorescene-conjugated goat antibodies specific for the mouse IgG2 isotype and subjected to "switch variant" selection using the fluorescence activated cell sorter. After repeated rounds of sorting, we were successful in generating several different switch variant "families", with both IgG2a and IgG2b producing switch variants being isolated from IgG1 producing parents. Although in most cases the allotypes of the switch variant families were conserved, in one family we noted a switch in allotype along with the switch in isotype. The fine specificities of the anti-dansyl antibodies produced by each member of this later switch variant family were compared by analysing the emission fluorescence of the purified antibodies binding free dansyl-lysine with excitation at 330nm. The spectra of the antibodies from individual members of this family were identical to one another but distinct from the spectra obtained from anti-dansyl antibodies obtained from members of other switch variant families. This suggests that the same variable region gene is expressed by all members of this switch variant family, and that interchromasomal recombination may account for this allotype switch.

0444 PEYER'S PATCH T CELL CLONES COLLABORATE WITH COMMITTED IGA B CELLS VIA Fcα R, Hiroshi Kiyono, Max D. Cooper, John F. Kearney, Suzanne M. Michalek, William J. Koopman and Jerry R. McGhee, University of Alabama in Birmingham, Birmingham, AL 35294.

We have isolated and characterized several T cell clones from murine Peyer's patches (PP) which exhibit $_{+}$antigen$_{-}$specific helper activity mainly for IgA responses (PP Th A clones). These Thy 1.2^{+}, Lyt-1^{+}, Lyt-2^{-} cells bear Fc receptors for IgA (Fcα R) which are important in isotype derived help, since monoclonal IgA (but not IgM or IgG) blocks the IgA response. Mutant forms of IgA which lack amino acid segments in the C_H3 domain, do not affect PP Th A cell function for IgA responses. The nature of B cells required for IgA responses was addressed by either removal of, or enrichment for, surface IgA (sIgA^{+})$_{+}$B cells from PP or depletion of sIgA^{+} B cells from splenic B cell cultures. Removal of sIgA^{+} B cells from either splenic or PP B cell cultures by FACS or by panning significantly reduced the B cell population which productively collaborated with cloned PP Th A cells. Enrichment of sIgA^{+} B cells from PP, upon culture with PP Th A cells and antigen, resulted in higher IgA responses than occurred in total PP B cell cultures. PP Th A cells did not support IgA responses in cultures of splenic B cells from mice 1 to 14 days of age, but supported full responses in young adult (5-7 weeks) B cell cultures. These results suggest that the Fcα R on PP Th A cells is important in IgA isotype help, and the C_H3 domain of α chain is crucial for the recognition of the precommitted B cell.

0445 DIFFERENCES IN THE SYNTHESIS OF IMMUNOGLOBULIN ISOTYPES DURING A MALARIA INFECTION, Jean Langhorne and Richard Asofsky, Laboratory of Microbial Immunity, National Institutes of Health, Bethesda MD 20205.

Infection of mice with Plasmodia gives rise to marked alterations in immunoregulation, resulting in polyclonal activation and apparent immunodepression. Our studies indicate also that the specific antibody response to P. chabaudi adami is restricted to certain isotypes during a primary infection; namely a predominant and persistent IgM and lower but detectable IgG_{2a}, IgG_{2b} and IgG_3 responses. Specific IgG_1 antibodies are not detectable. This suggests that either P. chabaudi antigens do not stimulate an IgG_1 response, or the IgG_1 response is suppressed. The polyclonal response in similarly restricted, in that, throughout an infection with P. chabaudi IgM-secreting cells are the majority (50%) of immunoglobulin-secreting cells in the spleen. Although there is an increase in the number of IgG_1 plaque-forming cells (PFC), they contributed to less than 5% of the total PFC response. Studies of the specific PFC response to sheep erythrocytes and to the terpolymer poly(Glu^{60} Ala^{30} Tyr^{10}) (GAT) during infection indicated that, although the total PFC response was relatively unaffected by the infection, the IgG_1 response to these antigens was preferentially suppressed. The IgM, IgG_{2a}, IgG_{2b} and IgG_3 responses were unaffected or enhanced compared with those of immunised, uninfected mice. The data suggest that there is selective suppression of IgG_1 responses (but not those of other isotypes) to several antigens during infection with P. chabaudi.

0446 A PROKARYOTIC DELETION SYSTEM WHICH USES A DNA SEQUENCE (DELTA) INVOLVED IN IMMUNE SWITCHING, Karl G. Lark, Paul Keim, Andrew Tliveris and Edward Meenan, University of Utah, Salt Lake City, UT 84112

The Red recombination system of phage lambda causes specific deletions within a highly repeated, interspersed sequence which occurs in the Kangaroo Rat D. ordii. These deletions are not mediated by the RecA system of E. coli, but do occur when Red function is replaced by the Red analogue, Lambda reverse or RecE. (MGG (1982) 188:27-36). The end points of the deletions suggested that they might be determined by nucleotide sequence. DNA was cloned into a Red-, lambda vector, from which it was subcloned into a pUC9 plasmid. The plasmid was grown in a RecE+ host and this plasmid DNA used to transform a RecE- host. We have determined the DNA sequence of deletions isolated in this manner, as well as the DNA from the boundary regions within the parent plasmid DNA. The deletion boundaries in the parent plasmid contain a DNA sequence, which also occurs at the point at which the DNA switches between the two flanking regions. This sequence occurs in vertebrates at switch points in the immunoglobulin and histocompatibility systems. We shall discuss the use of a prokaryotic, permissive-nonpermissive, deletion system as a prototype for studying the switching of genes in the immune system.

0447 STUDIES ON THE MECHANISM OF ACTION OF T CELL-DERIVED B CELL DIFFERENTIATION FACTOR(S) FOR IgG (BCDFγ), Judith E. Layton, Peter H. Krammer, Jonathan W. Uhr and Ellen S. Vitetta, University of Texas Health Science Center, Dallas, Texas 75235

We have previously described a T cell-derived lymphokine which induces increased levels of IgG_1 secretion in LPS-stimulated B cells and has been termed BCDFγ. BCDFγ (or other factors produced by the same T cell line) also causes suppression of IgG_2 and IgG_3 secretion. The purpose of this study was to determine whether BCDFγ might be inducing isotype switching or whether it is an amplification factor acting on cells that have already switched to IgG_1 production. A limiting dilution culture system was used to show that a T cell supernatant containing BCDFγ caused a 16-fold increase in the precursor frequency of IgG_1-secreting clones and a 6-fold decrease in the frequency of IgG_3-secreting clones. To determine whether BCDFγ acted on cells already expressing surface IgG (sIgG), splenic B cells were cultured with LPS for 2 or 3 days, stained with anti-γ, and the $sIgG^-$ blasts were sorted directly into microtiter trays by a fluorescence-activated cell sorter. Few $sIgG^+$ cells were detected but removal of the brightest 10% of cells had no effect on the frequency of clones secreting IgG_1 in response to BCDFγ. Secretion of IgG_1 and IgG_3 showed a low degree of correlation: a substantial proportion of IgG_1-secreting clones did not secrete IgG_3. Thus, the switch to IgG_1 in some clones may be independent of prior IgG_3 secretion.

0448 ISOTYPE-SPECIFIC HELPER T CELL REGULATION: A REQUIREMENT FOR CLASS COMMITTED B CELLS, Jerry R. McGhee, Hiroshi Kiyono, John F. Kearney, Suzanne M. Michalek, William J. Koopman, and Max D. Cooper, Univ. Alabama in Birmingham, Birmingham, AL 35294.

The precise role of T cells in B cell isotype commitment and differentiation to plasma cells synthesizing a particular isotype is currently under intensive investigation. Two broad alternatives have been proposed. One suggestion is that T cells or soluble factors can direct B cell isotype switching; the alternative hypothesis is that T helper (Th) cells can preferentially select isotype-committed B cells and influence their growth and differentiation into antibody producing cells. We have examined this issue in studies using cloned Th cells from murine Peyer's patches (PP), a central IgA inductive site, and B cells from both PP and spleen. PP T cells were derived from mice orally primed with sheep erythrocytes (SRBC) and have been cloned and maintained in continuous culture for 2 yr. These Thy 1.2^+, $Lyt\text{-}1^+$ cells bear Fc receptors for IgA (FcαR), but not FcμR or FcγR, and preferentially support IgA anti-SRBC responses in splenic or PP B cell cultures. In addition, some of these clones support low IgM responses, and others low but significant IgG subclass responses as well. Removal of surface IgA ($sIgA^+$) B cells from splenic or PP B cell cultures selectively reduced IgA responses, while enrichment of $sIgA^+$ B cells from PP by FACS or panning, upon culture with cloned Th cells gave enhanced IgA responses. Removal of $sIgA^+$ B cells did not affect IgM responses, and $sIgA^+$ enriched B cell cultures showed negligible IgM responses. We conclude that isotype-specific Th cells can preferentially collaborate with IgA committed B cells.

0449 PREFERENTIAL LOCALIZATION OF T CELL HELP (T_H) FOR THE IGA RESPONSE IN THE GUT-ASSOCIATED LYMPHOID TISSUE (GALT) OF CAF_1, MICE. Julia M. Phillips-Quagliata, Margaret Arny, Patricia Kelly-Hatfield and Michael E. Lamm. New York University Medical Center, New York, N.Y. 10012.

Differential distribution of IgA-specific T_H in favor of GALT has been proposed to account for the high proportion of IgA-producing plasma cells at mucosal sites. When, however, T_H are titrated into cultures of hapten-primed or unprimed splenic B cells in the presence of appropriate hapten-carrier conjugates, GALT T_H primed *in vivo* with enterically administered sheep erythrocytes (SRBC) (T_H-SRBC) exhibit no more help for IgA responses than subcutaneously primed peripheral node (PN) T_H-SRBC. Moreover, GALT T_H primed with Keyhole limpet hemocyanin (T_H-KLH) exhibit much less help for all isotypes than PN T_H-KLH, apparently because T cell tolerance of soluble antigen is rapidly induced via the enteric route. On the other hand, when T cell-replacing factors contained in the supernatants of different Concanavalin A-stimulated T cell populations are compared, it is evident that factors capable of helping IgA responses titrate independently of other growth and differentiation factors present in the supernatants and that GALT produces relatively higher amounts of IgA helper factor than PN or particularly than spleen. When help provided to the same pool of primed splenic B cells by PN T_H-KLH and PN T_H-SRBC is compared, the former support much lower IgA responses relative to other isotypes than the latter. It appears that subsets of primed B cells capable of responding to non-cognate interaction with T_H have greater potential to give rise to IgA PFC than those requiring cognate T_H. The generation of high IgA responses at mucosal sites may depend partly on the presence in GALT of high proportions of T cells capable of making IgA helper factors in response to signals from antigen non-specific stimuli like Con A. It may also reflect a preponderance of B cells capable of making IgA responses to particulate antigen in the presence of non-cognate T cell help, the only kind of help to escape tolerance induction on enteric priming. Supported by NIH Grants CA 20045, AI 15071.

0450 INDEPENDENT SWITCHING AND EXPRESSION OF IgG1, IgG2 AND IgG3 ISOTYPES, Yvonne J. Rosenberg, NIH, Bethesda, MD 20205

To examine the mechanisms leading to Ig isotype-restricted secretion by B cells and the role of T cells in the process, *in vivo* and *in vitro* experiments were done using immunologically abnormal mice e.g. either autoimmune strains (MRL-*lpr/lpr*, BXSB, NZB) which spontaneouslyproduce large numbers of cells secreting Ig of several isotypes or *xid*- bearing mouse strains (CBA/N, MRL.*xid*) which exhibit a selective low-responsiveness in the IgG1 class following immunization with certain T cell-dependent antigens. Using a reverse PFC assay as a measure of Ig production, *in vivo* studies with autoimmune strains suggest that (i) direct switching from S_μ to any constant region gene is possible (ii) almost all combinations of Ig isotypes can be observed and (iii) in most cases switching from $S_\mu \rightarrow S_\gamma$ or S_α but not both occurs. That the expression of each IgG class may be independently controlled by different T cells is indicated by two series of experiments in which (i) several long-term T cell lines derived from MRL-*lpr/lpr* have been shown to provide IgG2 or IgG3 restricted help when cultured with normal control B cell and (ii) CBA/N mice, low responders for IgG1 are selectively deficient in helper cells capable of inducing IgG1 but not IgG2 secreting cells. Studies with BXSB,$(NZB \times BXSB)F_1$ or congenic NZB.BXSB also show that accelerated increases in IgG1 and IgG2 levels appear to be controlled by different genes than IgG3 in that while the former require both a BXSB Y chromosome factor and (an) autosomal gene(s), elevated IgG3 secreting cell numbers can occur in the presence of the BXSB Y chromosome only. These results suggest that as with IgA and IgE, each IgG class is independently regulated and does not require switching through the IgG3 gene.

0451 OCCURRENCE OF Bγ MEMORY CELLS IN THE COURSE OF IMMUNIZATION WITH A TI-2 ANTIGEN IS T-CELL DEPENDENT, Walter Schuler, A. Schuler & E. Kölsch, University of Münster, Department of Immunology, Domagkstr. 10, D-4400 Münster, FRG.

The TI-2 antigen dextran B1355S (Dex) elicits in BALB/c mice a primary immune response largely restricted to IgM. It fails to give raise to anamnestic responses in situ. Yet, in spleens of Dex-primed euthymic BALB/c mice specific Bγ memory cells can be demonstrated upon adoptive transfer of spleen cells into irradiated Ig-allotype congeneic BALB.Igh^b mice which are non-responders to Dex. Activation of Dex-specific Bγ memory cells is T-cell independent as shown by T-cell depletion prior to cell transfer. On the contrary, occurrence of Dex specific memory cells is dependent on the presence of functional T-cells: memory cells are not demonstrable in spleens of Dex-primed athymic BALB/c nu/nu mice, which show a pronounced primary IgG response to Dex, but only in primed euthymic BALB/c or T-cell reconstituted BALB/c nu/nu mice. Syngeneic recipients, though irradiated, are non-permissive towards transferred Dex-specific Bγ memory cells. This also applies to immune-defective male (CBA/NxBALB/c)F1 hybrids which do not mount humoral responses to TI-2 antigens. The latter finding makes it unlikely that non-permissiveness is caused by humoral factors but suggests a cellular suppressor mechanism acting upon Dex-specific memory cells. From our results we conclude that the ultimate differentiation of Dex-specific B-cells into IgG secreting plasma cells is blocked in euthymic mice by a pre-established specific T-cell mediated suppressor mechanism which prevents clonal exhaustion and allow accumulation of Bγ memory cells. (Supported by DFG, SFB 104)

0452 SURFACE IMMUNOGLOBULIN G IN HUMAN BCELL LYMPHOMAS AS REVEALED BY MONOCLONAL ANTIBODIES, Erlend B. Smeland,[1] Roy Jefferis[2] and Tore Godal[1], The Norwegian Radium Hospital[1], Montebello, OSLO 3, Norway, University of Birmingham[2], Birmingham, U.K.

IgG subclass expression on cells from lymph node biopsies of seventeen sIgG positive human B cell lymphomas has been studied. Subclass specific monoclonal antibodies were used in an immunoradiometric assay. Ten were also tested by immunofluorescence using a biotin-avidin system. Thirteen of the lymphomas expressed IgG only, while four co-expressed other Ig-isotypes as well. Morphological investigations revealed that fourteen lymphomas originated from germinal centre cells. IgG1 was found to be the dominant subclass in nine lymphomas (53%), IgG3 in seven (41%), IgG2 in one (6%), while none expressed IgG4 (<6%). Multiple IgG subclasses could not be detected in any of the lymphomas tested.

This distribution of sIgG subclasses accords with the distribution observed in normal tonsillar lymphocytes in man. This indicates that the distribution reported here reflects the distribution in normal peripheral lymphoid tissues. Since the switching to sIgG in peripheral lymphoid tissues appear to take place in germinal centres, and the great majority of our lymphomas were of germinal centre cell origin, the observed distribution most likely reflects differentiational events related to switching in germinal centres of lymph nodes and tonsils.

0453 IgA SWITCH T CELLS IN PEYER'S PATCHES, Warren Strober, Hidenori Kawanishi, National Institutes of Health, Bethesda, MD 20205

To explore mechanisms of IgA regulation, we have established Con A-induced cloned T cell lines originating from PP and spleen. Cloned T cells derived from PP were found to suppress LPS-induced IgM and IgG synthesis and secretion by co-cultured PP B cells; in addition, whereas the PP cloned T cells did not bring about IgA production, they did cause the appearance of large number of cells expressing IgA. On the other hand, cloned T cells derived from spleen had little or no effect on LPS-induced IgM synthesis and secretion by PP B cells; in addition, whereas they did suppress IgG production, they neither brought about IgA production nor the appearance of sIgA-expressing cells. These studies provide evidence for the existence of a new type of T cell in PP, a switch T cell, which is able to induce B cells to undergo class-specific switches from IgM to IgA. These switch T cells are probably responsible for the fact that PP are a major source of mucosal IgA B cells.

In additional studies, we have shown that post-switch IgA-bearing B cells, i.e., cells pre-cultured with PP cloned T cells, have the capacity to undergo terminal differentiation into IgA producing plasma cells, provided they are exposed to helper T cells (uncloned) and an appropriate mitogenic stimulus. We conclude, therefore, that the development of PP B cells into IgA-producing plasma cells requires two steps: one which involves heavy chain switching to IgA and which is governed by IgA class-specific switch T cells, and the other which involves terminal differentiation of post-switch B cells and which is governed by helper T cells.

0454 PRESENCE OF SINGLE TRANSCRIPTS CONTAINING C_μ, $C_{\gamma 2b}$ AND C_α SEQUENCES, Gayle Woloschak and Charles Liarakos, Dept. Cell Biology, Mayo Clinic/Foundation, Rochester, MN 55905 and Dept. Biochem., Univ. Arkansas, Little Rock, AR 72205

Total cellular poly A+ RNA was isolated from TEPC15 myeloma cells (α,k) and characterized by Northern blot analysis. Successive hybridization to the C_α-specific probe pl07αR5 revealed the presence of four RNA species having lengths of approximately 11.5, 4.1, 3.0 and 2.3 kb. Hybridization of the blot to C_μ- and $C_{\gamma 2b}$-specific probes demonstrated the presence of both μ- and γ_{2b}-hybridizing bands as well. These molecular species were found to be similar in electrophoretic mobility to the 11.5 and 2.3 kb RNA species identified by C_α-specific hybridization. These data establish the presence of Poly A+ RNA specific for C_α, C_μ, and $C_{\gamma 2b}$ sequences in TEPC15 cells. In addition, because only one chromosome in the TEPC15 cell line contains C_μ and $C_{\gamma 2b}$ genes (the other chromosome has deleted all C_H genes except C_α), this work provides evidence for the production of a long primary transcript (perhaps the 11.5 kb RNA species) containing least C_μ, $C_{\gamma 2b}$ and possibly C_α sequences. The data also suggest the possible transcription of RNA from each of the two rearranged C_H-containing chromosomes. Further experiments identified similar high molecular weight RNA species in mouse spleen and Peyer's patch tissues having lengths of up to 13.5 kb. Primer extension experiments using Peyer's patch total cellular Poly A+ RNA demonstrated that a C_α-probe was able to prime for the synthesis of $C_{\gamma 2b}$-specific RNA sequences while various negative controls (such as C_μ, C_k) could not. These data provide evidence for the existence of single RNA transcripts containing C_μ, $C_{\gamma 2b}$, and C_α sequences. (Supported in part by ACS No. IM-348).

I-J: Genetics, Biochemistry and Function

0455 CHARACTERIZATION OF AN I-J RESTICTED SUPPRESSOR FACTOR FROM A T CELL LEUKEMIA, Michael J. Daley[1], Masataka Nakamura[1], Paola Ricciardi[2], Luciano Adorini[2] and Malcolm Gefter[1]. Department of Biology, M.I.T.[1], Cambridge, MA 02139 and Laboratory of Radiopathology, Centro Studi Nucleari Casaccia[2], Rome, ITALY.

The immune system is a complex network of cell lineages and subsetswhich interact when the host is challenged with foreign antigen. Various approaches to simplify the complexity of this system have utilized isolated clones of T and B cells. However, many of these lines, especially of the T cell lineage, suffer from the instability of the expressed biological activity. Our approach has been to isolate normal functional T cells and immortalize them by the infection with radiation leukemia virus (RadLV) of the mouse. Such a functional clone , LH-8, was established from C57BL/6 spleenic T cells. Previous studies had shown that the B6 animal is genetically a non-responder to HEL because of the activated suppressor cells. LH-8 secretes a highly active suppressor factor(s) which is I-J restrictedand can inhibit the in vivo secretory cell response to a variety (HEL, TEL, TNP-BSA and ARS-KLH) of antigens, but not to others (REL, TNP-KLH and SRBC). The factor is secreted into the culture media and can be partially purified based upon a variety of properties. The factor can be salted out of solution by 90% NH_4SO_4(SAS) but not 45% SAS; the factor can be absorbed and eluted from a sepharose column coupled with an anti-$I\text{-}J^b$ purified monoclonal antibody but not when coupled with an anti-$I\text{-}J^d$ monoclonal; the factor has an affinity for the Fc portion of some purified monoclonal antibodies which is unrelated to their isotype. The cell surface phenotype of the LH-8 line is: mIg(-), Thy-1(++++), MØ-FcR (-), MAC-1(-), Lyt-1(-), Lyt(++), $I\text{-}J^b$(5-30%,+) and $H\text{-}2^b$(++). We are currently further characterizing the biological, biochemical and molecular aspects of this factor.

0456 TWO COMPLEMENTING GENES CONTROL CELL SURFACE I-J EXPRESSION, Karen K. Klyczek, Harvey Cantor, and Colleen E. Hayes, University of Wisconsin, Madison, WI 53705, and Harvard Medical School, Boston, MA 02115

A troublesome paradox concerning the murine I-J subregion has confounded immunologists; I-J DNA sequences were not found in the central I-subregion position described by immunogeneticists. We investigated $I\text{-}J^k$ determinant expression on peripheral T lymphocytes using a B10.A(3R) anti-B10.A(5R) serum, Waltenbaugh $I\text{-}J^k$-specific monoclonal antibodies, and our $I\text{-}J^k$ specific monoclonal antibodies. Our experiments show that the $I\text{-}J^k$ molecule results from the action of at least two complementing genes; one is in H-2, the other is not. Some $H\text{-}2^k$ strains do not express $I\text{-}J^k$ molecules. When these are crossed with C57BL/10, the F_1 hybrid T cells display $I\text{-}J^k$ specificities. Complementation tests suggest that B10.A(3R) and B10.A(5R) differ outside rather than inside H-2; (3R X B10)F_1 hybrid T cells express $I\text{-}J^k$ determinants. Using Taylor recombinant inbred strain sets and congenic mouse strains, we mapped both the H-2 and the non-H-2 I-J-controlling loci. We named the non-H-2 I-J controlling locus Jet for its control of $I\text{-}J^k$ expression by T cells. Cellular $I\text{-}J^k$ molecules appear to be glycoproteins. They are removed by extended trypsinization; inhibiting either protein synthesis or glycosylation prevents T cell $I\text{-}J^k$ reexpression.

0457 MOLECULAR ANALYSIS OF I REGION RECOMBINANTS, Joan A. Kobori, Astar Winoto, Lloyd Smith, Karyl Minard, Erich Strauss, and Leroy Hood, California Institute of Technology, Pasadena, CA 91125

Restriction enzyme site polymorphisms have been used to correlate the molecular maps of six murine major histocompatibility complex I region recombinants with the genetic maps derived from recombinational analyses of serological markers. The DNA that maps between the I-A and I-E subregions is approximately 1.5 kilobases (kb) in length and is contained within the E_β gene. These data were obtained by restriction mapping of isolated lambda phage clones and plasmid subclones of four parental I region haplotypes (B10.D2, B10.A, C57BL/10, ASW) and six I region recombinants (B10.GD, B10.A(4R), B10.A(5R), B10.A(3R), B10.HTT, B10.S(9R)). This 1.5 kb region should encode the I-B and I-J subregions. At the molecular level, the two recombinants classically used to define the I-J subregion B10.A(5R), which is $I\text{-}J^k$, and B10.A(3R), which is $I\text{-}J^b$, are identical. DNA sequence analysis of the region determined to contain the I-J subregion is in progress. These data may enable us to identify an open reading frame for a putative partial or complete coding sequence for an I-J polypeptide or may enable us to state unequivocally that no additional non-E_β coding sequences exist in the genetically mapped I-J region. Kronenberg et al. (PNAS 80:5704, 1983) were unable to detect the expression of mRNA homologous to E_β in suppressor T cell lines serotyped as $I\text{-}J^+$. Together these data suggest that the gene for the I-J polypeptide may not be encoded between the I-A and I-E subregions.

0458 A MOLECULAR APPROACH TO THE GENES ENCODING THE I-J, Tsu, AND IDIOTYPIC SPECIFICITIES ON SUPPRESSOR T CELLS, Ellen Kraig and Mitchell Kronenberg, University of Texas Health Science Center at San Antonio and the California Institute of Technology

Murine T-cell hybridomas often secrete functional, antigen-binding factors whose structures have been characterized using specific serologic reagents. For example, suppressor T cells (T_S) express Tsu, a T-cell alloantigen that appears to be associated with antigen-binding polypeptides. Tsu is encoded by genes on chromosome 12, linked to the immunoglobulin C_H locus (Owen et al., J. Exp. Med. 153:801, 1981). Furthermore, T cells often express idiotypic determinants characteristic of the predominant immunoglobulin synthesized by B cells in response to the same antigen. Nonetheless, we have shown that T cells do not transcribe gene segments from the B cell V_H gene repertoire (J. Exp. Med. 158:192 & 210, 1983). Lastly, T_S cells and their secreted factors express I-J serological determinants thought to be encoded by genes in the I region of the MHC. However, we were unable to detect an RNA transcript deriving from this region in $I\text{-}J^+$ T_S hybridomas (PNAS 80:5704, 1983).

Currently we are pursuing a molecular approach to the I-J and idiotypic specificities, making no a priori assumptions about the locations of the genes that encode these determinants. We are constructing T-cell cDNA libraries in a bacterial expression vector; they will be screened directly with serological reagents specific for I-J, idiotype, and Tsu. Progress on the identification of appropriate cDNA clones will be reported.

This work is being done in collaboration with Drs. Judy Kapp, Carl Pierce, Craig Sorensen, and JoAnn Trial (Washington Univ. Med. School), Tomio Tada (Tokyo Univ.), and Leroy Hood (Caltech).

0459 ALLO-IJ EXPRESSION IS REQUIRED FOR MAINTENANCE OF CLASS II TOLERANCE, Kendall M. Mohler, Phoebe G. Strome, and J.Wayne Streilein, UTHSCD, Dallas, TX. 75235

Clonal deletion and active suppression have been advanced as mechanisms responsible for neonatal transplantation tolerance. Recent studies in our lab have demonstrated an active suppressive mechanism in mice tolerant alone of Class II antigens. Since many suppressor cell systems contain IJ^+ elements, we have examined the requirement for expression of host or chimeric (allo) IJ determinants in maintaining the tolerant state.

Adult tolerant mice were injected with anti-IJ monoclonal antibodies (Mab) specific for host IJ, allo IJ or irrelevant IJ. Mab directed at host IJ (or an irrelevant IJ) antigen did not abolish tolerance - these mice retained their tolerated skin grafts. However, anti-IJ Mab directed at allo IJ determinants of chimeric cell origin abolished tolerance in mice tolerant of whole H-2 or Class II, alone, disparities. Surprisingly, this treatment did not abolish tolerance in mice across an IJ thru D, disparity. Thus, the allo anti-IJ treatment was only effective when the tolerance envolved an I-A disparity. These results fail to indicate that tolerance to Class I antigens is maintained by an allo IJ-dependent mechanism. However, tolerance to Class II antigens is maintained by an active mechanism that is dependent upon chimeric allo-IJ determinants.

0460 Suppressor-like T Cell Clones which Recognize Class II $E^k_\beta E^/_\alpha$ Antigens

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We have obtained a cell line (SAC-9) and several clones from an allogeneic bone marrow chimera (SJL→AKR) using limiting dilution techniques. These cells were donor in origin (i.e. $H\text{-}2^s$ and Thy-1.2^+) and have been maintained in IL-2 conditioned medium without stimulator or feeder cells for over a year. SAC-9 clones are Lyt-1^+2^- and proliferate in response to stimulator cells expressing the k haplotype of H-2, but not to b, d, p, r, f, q, or s; weak stimulation by j was found. Using cells from recombinant congenic strains, we found that although the E_α locus controlled expression of the stimulating antigen, the antigenic determinant was expressed on E_β or the combinatory structure of $E_\alpha E_\beta$. Surprisingly, SAC-9 clones responded to stimulation by B10.A(3R), B10.A(5R), B10.S(9R) and B10.HTT cells in which E_β gene has been assigned to the b or s haplotypes. Based on recent molecular genetic findings that recombination may have occurred within E_β gene sequences of these strains, we hypothesize that their E_β gene may encode an epitope from k which stimulates SAC-9 clones. That is, the E_β gene product may be a "chimeric" of b/k or s/k in these strains. Such a hypothesis has implications for the specificity of antibodies used to define I-J and raises the question of whether E_β and I-J are the same. Various monoclonal and alloantisera specific for E_α, E_β, or $I\text{-}J^k$ can block stimulation of SAC-9 clones in MLC. Preliminary studies show that SAC-9 clones suppress alloreactivity in vivo and in vitro. This could be interpreted to indicate that E_β can act as an immune suppression gene.

0461 CHARACTERIZATION OF MONOCLONAL ANTIBODIES GENERATED AGAINST ONE AND TWO CHAIN L-GLUTAMIC ACID^{60}-L-ALANINE^{30}-L-TYROSINE^{10}(GAT)-SPECIFIC SUPPRESSOR FACTORS.

Craig M. Sorensen and Carl W. Pierce. Jewish Hospital of St. Louis, St. Louis, MO 63110.

A panel of monoclonal antibodies has been generated by fusing spleen cells from rats hyper-immunized with HPLC-purified, $H2^b$-derived single chain(372b3.5) or two chain(372d6.5) suppressor factors (TsF) specific for the antigen GAT with the non-secretor B cell myeloma SP2/0-Ag14. Monoclonal antibodies derived from immunization with the single chain TsF fall into two functional categories: those that reverse suppression by the TsF; and those that induce antigen-specific suppression. All antibodies absorb only the immunizing TsF with three exceptions: C2.11 and C4.5 also bind single chain TsF of other haplotypes. A4.8 binds two chain TsF of the same haplotype; and, A4.10 binds other single chain TsF from mice of the B10 background. Antibodies derived from immunization with the two chain factor are divided into three sets: 1) those that recognize only the antigen-binding chain (ABC+); 2) those that recognize only the I-J determinant-bearing chain, 3) those that recognize only the intact molecule, presumably specific for a combinatorial determinant. With the exception of D2.2, an ABC^+ specific reagent, these antibodies fail to react with other two chain or single chain TsF from either H2 matched or mismatched mice. Comparison of conventional monoclonals raised against I-J determinants with the monoclonals directed against TsF I-J determinants should help in defining products of this region of the H2. Finally, a set of monoclonal BALB/c anti-C.B20 reagents define $IgH\text{-}1^b$ derived single chain TsF, the ABC^+ from $Igh\text{-}1^b$ derived two chain TsF, or both single and two-chain $Igh\text{-}1^b$ derived TsF. Further screening with these reagents may also define other T cell alloantigens similar to those previously reported.

0462 CHARACTERIZATION OF POLY($GLU^{50}TYR^{50}$) SUPPRESSOR T CELL DEFECTS IN 'NONSUPPRESSOR' B6 AND A/J MICE.

Carl Waltenbaugh and Huan-Yao Lei. Northwestern University Medical School, Chicago, IL 60611

Poly($Glu^{50}Tyr^{50}$) (GT) injection of $H\text{-}2^{d\ or\ k}$ mice induces specific suppressor T (Ts) cells and $I\text{-}J^k$-bearing factors (TsF) which suppress plaque forming cell (PFC) responses to GT as GT-MBSA (GT-methylated bovine serum albumin). A/J ($H\text{-}2^a$) and B6 ($H\text{-}2^b$) mice are not suppressed by GT injection. A/J mice lack $Ts1$ cells, cannot produce $GT\text{-}TsF_1$; however, they can be suppressed by $GT\text{-}TsF_1$. We have produced $GT\text{-}TsF_1$-secreting T hybrids from $GT\text{-}TsF_1$-injected A/J mice. TheseA/J-derived, $I\text{-}J^k$-bearing factors fall into two categories: an early acting, anti-idiotypic $GT\text{-}TsF_2$ and a late-acting $GT\text{-}TsF_3$. The target cell for both $GT\text{-}TsF_2$ and $GT\text{-}TsF_3$ is a T cell. B6 mice produce, but are not suppressed by, $GT\text{-}TsF_1$; however B6 mice are readily suppressed by $GT\text{-}TsF_2$ and $GT\text{-}TsF_3$, indicating that B6 mice are deficient only in Ts_2 cells. Our data show that both strains have Ts defects early in the suppressor pathways (Ts1 for A/J and Ts2 for b6), while both A/J and B6 mice show normal $Ts3$ and TsF_3 activity. $GT\text{-}TsF_3$ acts upon a cyclophosphamide-sensitive, Thy-1^+, Lyt-$1^+,2^-$(Ts4) cell which releases a non-specific suppressive signal to the plaque forming cell.

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Strategies for Controlling Transplant Rejection and GVH

0463 STUDIES ON A MURINE MODEL OF SPONTANEOUS RECURRENT ABORTIONS AND OF THEIR IMMUNOLOGICAL TREATMENT BY PREIMMUNISATION OF MOTHERS WITH ALLOGENEIC SPLEEN CELLS,MINOR LOCI DISPARATE. Gérard CHAOUAT and Jean-Pierre KOLB-U 255 Inserm-Institut Curie,Paris-France.

We have observed that CBA/J females mated with DBA/2 males,but not these mated with Balb/c males,display a high rate of resorbtions (Day 12 of pregnancy).This prompted us to analyse the effects of preimmunisation of CBA/J females with Balb/c splenocytes prior to mating.This manoeuvre significantly reduces the number of resorbtions,bringing it back to the one observed in the CBAxCBA combination(slightly higher than C_3HxC_3H). This effect correlates with :

a) suppressor cell induction at systemic level,assayed in a CBA/J vs DBA/2 CML.

b) high levels of seric IgG,and especially IgG_1,in"vaccinated"animals (in cooperation with T.G. WEGMANN,by a solid phase radio-immunoassay.

c) greater ability of the trophoblastic cells from CBAxDBA/2 placentae to suppress an NK assay if coming from vaccinated mice.

The anti abortion effect is cell and serum transferable.It can be obtained whether mice are immunised i.p. or locally.Its mechanisms will be discussed .

0464 NATURAL SUPPRESSOR (NS) CELLS FROM ADULT MICE. IN VITRO CULTURE AND CLONING. Birgit Hertel-Wulff and Samuel Strober, Stanford University, CA 94305

We hereby report the establishment and cloning of long-term in vitro lines of murine, splenic natural suppressor cells. They were obtained more than 20 months ago by culturing spleen cells from mice given total lymphoid irradiation. The cloned natural suppressor cells were co-cultured with responder and stimulator cells from a variety of strains and showed non-specific inhibition of (^{3}H)-thymidine incorporation in the primary MLR. 1 x 10^5 co-cultured cells per well suppressed the MLR by a mean of 93% as compared to cultures without co-cultured cells. The natural suppressor cells did not change the kinetics of the proliferation in the MLR and lacked general cytotoxic nature as well as natural killer activity. Suppression occurred for immune recognitive proliferations only. By analysis on a FACS III more than 90% of the cloned splenic natural suppressor cells stained with anti-Thy-1.2 antibodies after two months of culture. More than 95% of the cells carried H-$2K^d$, D^d as well as asialo-GM1. Less than 5% stained with anti-Lyt 1.2 or -Lyt 2.2 antibodies, and less than 2% of the cells carried immunoglobulin, Mac-1, F4/80, 2C2, H-2I-A^d or H-2I-E^d. Because of the property of suppression of immune recognition processes the isolated cells may represent a population involved in development of immunoregulation.

0465 MECHANISMS OF SPECIFIC ACCEPTANCE OF SKIN GRAFTS IN MIXED ALLOGENEIC AND XENOGENEIC CHIMERAS, Suzanne T. Ildstad, Sherry Wren, David H. Sachs, NIH, Bethesda, MD 20205

Transplantation between genetically disparate individuals continues to require non-specific immunosuppressants to control the rejection reaction. Models utilizing bone marrow transplantation in an attempt to induce specific tolerance in adult animals have been limited by graft-versus-host reactions and a level of generalized immunoincompetence resulting from a failure of appropriate immune cell interactions in the reconstituted host. We have recently shown that reconstitution of lethally irradiated B10 mice with T cell depleted syngeneic (B10) plus allogeneic (B10D2) or xenogeneic (F344 rat) bone marrow results in long term survival of animals and specific prolongation of donor-type full thickness tail skin grafts. These animals promptly reject third party grafts in a time course identical to unirradiated controls (MST = 8 to 14 days). These animals have now been examined for relative immunocompetence, patterns of reconstitution, and in vitro reactivity. In preliminary experiments these mixed reconstituted animals appear to be immunocompetent in both B cell and helper T cell function as assessed by PFC responses to SRBC immunization in vivo. In contrast, fully allogeneic chimeras were incompetent in the same assay. By CML and MLR assays these animals are specifically tolerant to donor type cells and normally reactive to mouse and rat third party cells. These results suggest a model for the induction of specific transplantation tolerance without loss of immunocompetence and with potential application to solid organ transplantation.

0466 THE EFFECTIVENESS OF RICIN A CHAIN CONJUGATED ANTI-Ia ANTIBODIES IN SUPPRESSION OF MLR. Donald Kaplan and Michael Bjorn, Cetus Corporation, Emeryville, CA 94608; Kazuhiko Nakahara and G. Garrison Fathman, Stanford Medical School, Stanford, CA 94305.

The potential use of a mouse anti-Ia monoclonal antibody conjugated with a plant toxin (ricin A-chain RTA) as an immunosuppressive agent for organ transplantation was examined by studying the mixed lymphocyte reaction (MLR). In initial observations in a murine system utilizing strains A/J and C57B1/6 (B6), it was shown that an RTA conjugated anti-I-E^K monoclonal antibody (13.4-RTA) would kill Ia bearing cells of strain A/J (I-E^K) but not of strain B6 whose cells lack I-E determinants. Studies reported elsewhere demonstrated that 1 μg of the 13.4-RTA/10^6 spleen cells would effectively remove stimulatory cells from the spleen but had no effect on responder lymph node cells similarly treated (i.e., 1 μg/10^6 cells). Taking advantage of the finding that such anti-I-E antibodies react with rat spleen cells bearing class II MHC molecules, we utilized these toxin conjugated antibodies to inhibit MLR in rats. The 13.4-RTA conjugates were more effective in removing stimulatory activity from cells in MLR than either antibody plus complement treatment or continuous blocking by antibody. Assuming that the MLR is an in vitro representative of graft rejection, we suggest such RTA conjugated antibodies might be useful for pretreatment of organ allografts for transplantation across major histocompatibility barriers.

0467 HOST-SPECIFIC NONREACTIVITY IN ANTIBODY-FACILITATED BONE MARROW CHIMERAS, Susan A. McCarthy, Phillip Gambel, Erwin Diener and Thomas G. Wegmann, Univ. of Alberta, Edmonton, Alberta, Canada, T6G 2H7

We have generated murine antibody-facilitated bone marrow chimeras in the genetic combination $P_1 \rightarrow (P_1 \times P_2)F_1$ by the simultaneous injection of anti-P_2 monoclonal antibody and P_1 bone marrow cells. These (unirradiated) animals display no overt signs of graft-vs-host disease in vivo. Our preliminary results from bulk culture experiments indicated that the genotypically P_1 (parent) lymphoid cells present in the spleens of these F_1 host animals do not respond in vitro, in assays of proliferation and cytotoxic cell generation, to host alloantigens presented by P_2 or $(P_1 \times P_2)F_1$ stimulator cells; these same spleen cell populations do respond to unrelated third party alloantigens. We are now investigating the basis of this host-specific nonreactivity in detail. In modified bulk culture in vitro assays, we are separately analyzing the anti-host reactivities of the cytotoxic T lymphocyte (CTL) compartment and the helper T lymphocyte (HTL; Interleukin-2 producer) compartment, since specific nonreactivity in either or both of these responder cell subsets could be responsible for the observed failure to generate an anti-host cytotoxic response. By using limiting dilution analysis (LDA) protocols, we shall then examine whether host-specific nonreactivity in the CTL and/or HTL populations (identified in our modified bulk culture assays) is a consequence of reduced frequencies of host-specific precursor cells or a consequence of active suppression of host-specific responder cells. Knowledge of the mechanism(s) by which this "acquired" nonreactivity to (genotypically) non-self determinants is achieved may help us to identify the mechanism(s) responsible for nonreactivity to "true" (genotypically) self determinants. Supported by AHFMR and MRC

0468 CYCLOSPORINE INDUCES SPECIFIC UNRESPONSIVENESS TO ALLOGRAFTS IN THE MONGREL DOG: GRAFT SURVIVAL AND CYTOLYTIC T CELL ACTIVITY, A. Norin, E. Emeson, S. Kamholz, K. Pinsker and F. Veith, Montefiore Med. Ctr. - Albert Einstein Col. of Med. New York 10467

The possible development of cyclosporine (Cys) induced unresponsiveness was investigated in a single lung transplantation model in the mongrel dog. Persisting in vivo unresponsiveness was observed in 6 recipients by open lung biopsies, plain chest roentgenograms, radionuclide blood perfusion scans of the lungs and by lung survival of >649, >575, >201, 95, >30, and >26 days after Cys therapy was terminated. Specific tolerance to donor alloantigens was monitored in vitro in ^{51}Cr release assays of direct cytolytic T cell (CTC) activity of lung and blood lymphocytes and in assays of the generation of CTC activity in mixed lymphocyte cultures (MLC). Direct cytotoxicity of the recipients' T cells was usually detected in the lung but not in the blood >30 days after transplantation. One way MLC of recipient (R) blood cells and donor lymphocytes were deficient in the ability to generate cytotoxicity to donor target cells, even though a proliferative response was observed. The lack of responsiveness was not due to inadvertent antigenic matches since the same MLC combinations were able to generate CTC activity before transplantation. Specificity of unresponsiveness was shown in that MLC of R x 3rd party lymphocytes were fully capable of generating high levels of cytotoxicity and the donor lymphocytes were able to generate CTC in MLC with 3rd party T cells. These results indicate that the inability of canine recipients to reject their lung allografts after appropriate Cys therapy, is associated with a systemic inability to generate CTC on exposure to specific alloantigens of the donor. Supported by NIH grant HL 17417 and the Manning Foundation.

0469 SUPPRESSION OF T CELL IMMUNITY TO GRAFT ALLOANTIGENS BY MONOCLONAL ANTIBODY IMMUNOTHERAPY, Linda L. Perry and Ifor R. Williams, Emory University, Atlanta, GA 30322

Antibodies specific for cell surface glyoproteins encoded by the I-A or I-E subregions of the H-2 gene complex block the activation of I-A- or I-E- restricted Lyt-1+ T cells, respectively in vivo or in vitro. In addition to interfering with T cell-antigen presenting cell interactions, anti-I-A antibody treatment in vivo results in the activation of Lyt-2+ suppressor T cells (T_S) specific for the immunizing antigen. T_S function is required for the complete inhibition of Lyt-1+ T cell activity, as shown by the absence of antibody effects in cyclophosphamide-pretreated animals. In the present studies, monoclonal antibodies specific for host I-A or I-E gene products were used to inhibit T cell-mediated rejection of multiple minor alloantigen-disparate skin grafts. Inhibition is dose dependent and results in a 2-3 fold prolongation of graft survival (mean survival time of 11 days in controls versus 21 to 28 days in treated groups). Treatment is associated with a significant decrease in host DTH and cytotoxic T cell responses to graft alloantigens and the appearance of T_S capable of adoptively inhibiting alloantigen-specific DTH reactivity. Preliminary results suggest that similar treatments may enhance survival of class I alloantigen-disparate grafts, but not of grafts across combined class I plus class II histocompatibility barriers. These distinctions may be related to the differential mechanisms of T cell recognition of minor, class I, and class II alloantigens, as will be discussed. These experiments provide evidence for the potential benefits of monoclonal antibody immunotherapy in controlling clinical transplant rejection due to minor and/or isolated class I HLA incompatibilities.

0470 Class II MHC Antigen Matching as a Means to Achieve Long Term Renal Allograft Survival in Miniature Swine, Mark D. Pescovitz and David H. Sachs, Immunology Branch, NCI NIH, Bethesda, Md. 20205

The benefit of class II MHC antigen matching to renal allograft survival, in the absence of immunosuppression, has been studied in partially inbred, MHC-recombinant, miniature swine. Permanent renal allograft acceptance was found in 30% of recipients of class-II-only matched grafts at 6 months, which was equal that of complete-MHC matched grafts. Class-I-only matched grafts, although prolonged over complete-MHC mismatched grafts, were all rejected by 30 days. Analysis of recipient donor combinations suggested that several genetic factors, including gene dose and a possible Ir-gene, regulated the prolonged survival of class II matched grafts. The mechanism of the prolonged survival has been examined and has been shown to be associated with:(1) the specific prolongation of subsequent skin grafts from the renal donor but not from third party donors; (2) the presence of suppressor cells in peripheral blood which specifically inhibit an MLR between renal donor and recipient haplotype cells. In addition, an anti-donor-MHC antibody response was found to coincide with a self-limited rejection episode which peaked at 2 weeks. This antibody response has been shown to be predominantly IgM by 2-mercaptoethanol sensitivity. By contrast, animals that reject similarly matched skin or kidney grafts mounted a predominantly IgG response. Thus the long term survival appeared to associated with the absence of an IgM to IgG class switch in the anti-donor-MHC antibody response. These MHC recombinant swine therefore provide a model for mechanistic investigations of the role of selected MHC differences in renal allograft survival.

0471 STABILIZATION OF ISLET ALLOGRAFTS BY TREATMENT OF RECIPIENTS WITH ULTRAVIOLET IRRADIATED DONOR SPLEEN CELLS, Stephen J. Prowse, Michael Agostino, and Kevin J. Lafferty, Barbara Davis Center for Childhood Diabetes, Denver, CO. 80262, and Transplantation Biology Unit, J.C.S.M.R., Canberra, Australia.

Culture of mouse pancreatic islets in an oxygen-rich atmosphere before transplantation facilitates long-term allograft survival without the use of immunosuppression. A comparison of the capacity of ultraviolet (UV) irradiated and live spleen cells of donor origin to induce allograft rejection showed that UV-irradiated spleen cells were not immunogenic; live spleen cells were immunogenic and their injection triggered allograft rejection. Following treatment with irradiated spleen cells from about day 30 post-transplantation, recipient animals were able to withstand subsequent challenges with 10^6 and 10^7 viable donor spleen cells. Untreated animals rejected their graft when challenged with 10^6 donor spleen cells. That is, treatment with UV-irradiated cells stabilized the islet allograft by inducing a state of tolerance. Subsequent transplantation of stabilized animals with uncultured thyroids of both donor origin and from a third party strain, demonstrated that the tolerance was specific. In vitro test of immune reactivity showed this tolerance was not due to the deletion of antigen reactive cells.

0472 ELIMINATION OF NATURAL KILLER (NK) ACTIVITY WITH AN IMMUNOTOXIN TA-1-RICIN. R.R. Quinones,S.M.Azemove,R.J.Youle,and D.A.Vallera,University of Minnesota,Minneapolis,MN 55455 and NIMH, Bethesda MD 20205. We report,for the first time,an immunotoxin (IT) effective in eliminating NK function. The immunotoxin was synthesized by covalently conjugating TA-1, a monoclonal antibody , reactive with large granular lymphocytes, T cells, and monocytes, to intact ricin. Cells were pretreated in the presence of lactose blockade to render the IT antigen specific. TA-1-ricin effectively eliminated NK activity against K562 and Molt-4 tumor cell lines at a dose of 500 ng/ml, even when pretreatment was followed by stimulation with inteferon. Control anti-human-T-cell-ITs,not reactive with large granular lymphocytes, were not inhibitory to NK activity even at doses of 2000 ng/ml. Our data demonstrates that IT can eliminate mature preprogrammed cytolytic effector cells. The NK purgative effects of TA-1-ricin, in combination with TA-1-ricin's T-cell eliminating and hematopoietic stem cell sparing properties, make it a promising reagent for the in vitro purging of immunocompetent cells from allogeneic bone marrow inoculums to decrease the risk of graft-versus-host-disease with transplantation across MHC barriers.

0473 DISCORDANCE BETWEEN IN VITRO AND IN VIVO ALLOREACTIVITY PHENOTYPES OF H-2 TOLERANT MICE. J.W.Streilein,P.Strome,P.Wood,K.Mohler,R.Gruchalla,R.Berkompas,UTHSCD,Dallas,TX. Lymphoid cells from H-2 congenic B10 mice bearing healthy skin allografts following neonatal inoculation of semiallogeneic hematopoietic cells are uniformly and specifically non-reactive in vitro (MLR,CML). As we have perturbed experimentally this tolerant state, we have become aware that the in vivo and in vitro phenotypes of alloreactivity are not always concordant. When immunocompetent cells are placed in vivo in an environment in which allo-Ia tolerance exists or is imposed (i.e. adoptive transfer of allo-Ia tolerant lymphoid cells, and infusion of normal, syngeneic lymphoid cells into Ia tolerant mice), the cells retain a modest capacity to respond to appropriate Ia alloantigens in MLR, but are unable to effect rejection of Ia-disparate skin grafts. When allo-Ia and H-2 tolerance is broken by intraperitoneal administration of monoclonal anti allo-I-J antibodies, skin graft rejection occurs, but is not accompanied by acquisition of in vitro alloreactivity. Moreover, lymphoid cells from mice that fail to become tolerant following neonatal inoculation of H-2 semiallogeneic cells respond only feebly in MLR and fail completely to respond in CML to the relevant H-2 alloantigens. It is our feeling that where skin allograft survival occurs in the face of in vitro alloreactivity, an active suppression mechanism operates in vivo to protect incompatible skin grafts from endogenous alloreactive cells. When graft rejection is not attended by the appearance of in vitro alloreactivity, we conclude that the predominant effector mechanism responsible for graft rejection has not been measured by the in vitro assay, and we presume that the relevant effector of graft rejection is the T_{DTH} lymphocyte.

0474 BONE MARROW TRANSPLANTATION AS A PRECONDITIONING MOIETY FOR ORGAN TRANSPLANTATION, Daniel A. Vallera, John M. Donhowe and Michael W. Steffes. Dept. of Therapeutic Radiology and Laboratory Medicine/Pathology. University of Minnesota, 55455

Bone marrow transplantation was used to condition lethally irradiated mice for the acceptance of unrelated organ grafts. Donor-recipient combinations were histoincompatable and developed lethal GVHD with a 100% incidence unless protected by donor T cell depletion prior to transplantation. Depletion was accomplished using monoclonal antibody plus complement or immunotoxin. Typing peripheral blood mononuclear cells 30-60 days post-transplant showed that these animals were chimeric (90% donor haplotype).

Chimeras were rendered diabetic using streptozotocin. The diabetic state was reversed following intrasplenic infusion of isolated adult islets from either donor or recipient strain combinations. Diabetic chimerias rejected third party islets demonstrating that engraftment was not attributed to nonspecific immunosuppression of the transplanted mice. Removal of the spleen 2-3 mo post-islet transplant resulted in precipitous increases in blood glucose levels combined with diminishing body weights signaling a return of the diabetic state. Tissue samples from chimeras showed that intact islets were present in the spleen.

Chimeric splenocytes from islet-infused mice were tested in PHA and MLR assays. Cells were responsive to mitogens, but PHA responses were reduced as compared to normal controls while LPS stimulation resulted in hyper-responsiveness. Stimulation of chimeric cells in MLR assays with donor or recipient alloantigen resulted in no response in several animals while cells were responsive to third party alloantigens.

Thus bone marrow transplantation can be used as preparative moiety for the induction of specific unresponsiveness followed by mismatched organ transplantation. Supported by NIH Grant CA-31618 and 36725.

0475 ELIMINATION OF IMMUNOCOMPETENT LYMPHOCYTES FROM DONOR BONE MARROW BY IN VITRO TREATMENT WITH METHYLPREDNISOLONE. Axel Zander, Herbert Chow, Jonathan Yau, Sulabha Kulkarni, Lap Huynh, Karel Dicke. The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, 6723 Bertner Avenue, Houston, Texas 77030. Purging of allogeneic bone marrow in vitro with MP has been used for the elimination of immunocompetent cells for the prevention of GVHD. Normal human BM-mononuclear cells (MNC) were tested for E-rosette (E+), mitogen (PHA) stimulation, and CFU-GM before and after incubation with 0, 5, 10, 15 mg/ml of MP at 37°C for 1 hr. While a loss of about 50% of total CFU-GM was noted after such treatment, E+ cells were reduced by 95% and PHA response of BM-MNCs was reduced by 100% as compared to the baseline thymidine uptake of BM-MNC. Peripheral blood (PB) MNCs were treated similarly with MP and monitored for the change in the T4+, T8+, E+ cell populations and the response to PHA stimulation. Complete abrogation of PHA response was achieved at MP dose of 15 mg/ml. T4+ and 8+ cells were reduced by 85% and 60%, respectively. High dose in vitro MP treatment leads to an inverted helper/suppressor cell ratio. Six patients with acute leukemia received marrow purged by methylprednisolone. Mononuclear cell reduction was achieved by 40% (21%-69%). E-rosettes were reduced to .4% (0%-1.5%) CFU-GM from 0%-154/10 plated cells. All patients had engraftment by blood counts and bone marrow morphology. Acute GVHD grades III to IV occurred in two patients. In conclusion, chemoseparation with methylprednisolone is feasible and engraftment can be obtained. The follow-up is too short to draw definite conclusions about the effectiveness of MP treatment as prevention of GVHD.

Phylogenetic Origins and Diversification of the MHC

0476 EVOLUTION OF HISTOCOMPATIBILITY GENES: MOLECULAR CLONING AND ANALYSIS OF MHC-RELATED GENES IN THE PROTOCHORDATE BOTRYLLUS sp. MONTEREY. Jayne s. Danska, Howard K. Gershenfeld, Virginia L. Scofield, Irving L. Weissman and Hugh O. McDevitt. Stanford Medical School, Stanford C.A. 94305. Although the aquisition of allografts in mammals only results from experimental or clinical efforts (except pregnancy), colonial Tunicates undergo transplantation in nature. The protochordate Botryllus possesses a polymorphic histocompatibility locus that determines the acceptance or rejection of new individuals into a parabiotic colony. On the hypothesis that control of these functions is exerted by genes ancestral to the major histocompatibility complex (MHC) of mammals, we have sought DNA sequences in Botryllus homologous to MHC class I and class II genes. To this end genomic libraries have been constructed and probed with cDNA clones for mammalian MHC genes. We have identified clones which anneal at moderate to high stringency to HLA-B7 and I-E ; efforts to isolate HLA-DR and beta-2 microglobulin related sequences are in progress. We have studied the number and pattern of Botryllus genomic restriction fragments homologous to the MHC-related (MHC-r) clones. DNA prepared from individuals colonies which have been typed at the fusion locus has been subjected to Southern blotting analysis to determine whether MHC-r polymorphic resrtiction fragments are correlated with self/not-self recognition. Nucleotide sequencing of MHC-r genomic and/or cDNA clones will permit comparison of tunicate and mammalian sequences providing a novel phylogenetic perspective on amino acid conservation, codon usage, domain structure and inter and intra-molecular associations among proteins central to immunological recognition.

0477 **Xenogeneic CTL recognize alloantigenic determinants on the HLA-A2 molecule-implication for a model of T cell recognition.** **Victor H. Engelhard and Christopher Benjamin,** Dept. of Microbiology, University of Virginia Medical School, Charlottesville 22908
Clones of murine CTL were federated by immunization with the human lymphoblastoid cell line, JY (HLA-A2,2; -B7,7,-DR4,6). Assignment of clonal specificity was made with target cells sharing only one of these antigens with JY. Assignments were confirmed by the ability of the clones to be blocked by alloantigen specific monoclonal antibodies and by the ability to recognize gene products expressed after transfection of murine L cells. All clones assigned as reactive with the class I antigens A2 or B7 were unreactive with 8-10 other HLA-A antigens and 10-12 other HLA-B antigens. Most HLA-A2 specific clones could discriminate between the prototype HLA-A2 antigen against which they were raised, and HLA-A2 variants expressed on the cell lines DR1, M7, and DK1. The patterns of recognition exhibited by individual clones were consistent with known biochemical differences in these molecules. This suggests that all of these clones are recognizing determinants which are the same or closely linked to alloantigenic determinants recognized by human CTL. In contrast, murine monoclonal antibodies against HLA antigens are directed primarily against species-specific, monomorphic determinants. Based on this result, together with the observation that this xenogeneic CTL response is entirely directed at MHC products, a model is proposed in which two sites on the HLA molecule, one highly conserved and one polymorphic, are recognized by two corresponding linked sites on the T cell receptor. As a consequence, initial recognition of HLA antigens via a class I-specific receptor site would limit the location of polymorphic structures which could be recognized by a second receptor site. Other supporting evidence for this model will be presented.

0478 BIOCHEMICAL IDENTIFICATION OF MHC ENCODED MOLECULES IN XENOPUS, Martin F. Flajnik, James F. Kaufman, Patricia Riegert, and Louis Du Pasquier, Basel Institute for Immunology, Basel, Switzerland

By immunization with blood cells after skin grafting, we have produced Xenopus alloantisera specific for eight different MHC haplotypes that had previously been defined functionally by MLR, CML, skin grafting, and T-B collaboration assays. A 42-44 kilodalton (kd) molecule and a 12 kd molecule are co-precipitated from radioactively-labeled lymphocytes or erythrocytes. These molecules are likely homologous to class I molecules (heavy chain and $\beta 2$ microglobulin) described for higher species. Some alloantisera also precipitate a band at 32-34 kd which is also recognized by an anti-DR light chain xenoantiserum. This band resolves into two bands after treatment with tunicamycin or endoglycosidases. These two bands are likely homologous to the α and β chains of previously described class II molecules. Studies of these molecules throughout ontogeny are in progress.

0479 CHARACTERIZATION OF AN EXPRESSED CLASS I GENE FROM THE RABBIT. Patrice Marche, Mark Tykocinski, Thomas J. Kindt and Edward E. Max, LIG, NIAID, National Institutes of Health, Bethesda, Maryland 20205

A cDNA library was constructed from mRNA of the rabbit cell line RL5; a clone (pR9) was shown to correspond to the previously reported amino terminal sequence of the single class I RLA molecule detected in the RL5 cells. We constructed a genomic library from RL5 DNA using the vector λJ1. Thirty clones hybridizing with the pR9 cDNA probe were isolated. The gene corresponding to the cDNA pR9 was detected by analysis of exon III restriction fragments using a probe specific for this region. Two clones showing the same fragmentation pattern as pR9 - were further characterized. One clone, 19-1, was fully sequenced and showed 100% homology with pR9 cDNA sequence. Furthermore, the transcriptional regulation sequences such as TATA box, and the splicing signal were found. These data suggest that the 19-1 clone corresponds to the gene encoding the RLA molecule expressed by RL5 cells. The genetic organization and the nucleotide sequence of the 19-1 gene display a striking homology with human class I genes. Analysis of a second clone, 5.2, has revealed 97% homology in exon III to 19-1. As the exon III encodes the C1 domain which is highly polymorphic in all other comparisons, this unexpected localized conservation of sequence suggests that the two genes result from a recent duplications. Alternatively an active mechanism of gene conversion may operate on the rabbit class I genes.

0480 GENETIC DIVERSITY OF CLASS II HISTOCOMPATIBILITY GENES IN WILD MICE, Edward K. Wakeland, Thomas J. McConnell, Beree Darby and John E. Coligan, University of Florida, Gainesville, FL 32610 and NIAID, Bethesda, MD 20205

We are studying the class II genes and gene products of H-2 haplotypes extracted from wild mouse populations of Mus m. domesticus, Mus m. musculus and Mus castaneus. The class II gene alleles from these wild mice can be grouped into several closely-related "families" on the basis of similarities in their A molecules. Class II gene alleles within the same family encode antigenically similar A molecules which are identical in more than 90% of their tryptic peptides, while A molecules encoded by alleles in separate families are identical in less than 70% of their tryptic peptides. These findings suggest that the evolution of A_α and A_β in Mus has not resulted in the production of a continuous spectrum of genetic variance. Instead, a limited number of allelic families predominate in the wild mouse populations we have studied. Tryptic peptide fingerprinting and protein sequencing studies of the A_α and A_β subunits encoded by 2 alleles within the A^k family have identified regions within these polypeptides which are distinguishable by allogeneic structural variations. The A_α subunits of W12A and B10.STC90 differ from A^k_α in 2 adjacent tryptic peptides which span positions 44 to 71 from the amino terminus of A^k_α. The A_β subunit of W12A differs from A^k_β by allogeneic structural variations effecting amino acid positions 28 (I to X) and 95 (L to X). No other structural variations were detected by tryptic peptide fingerprinting and protein sequencing comparisons of these A^k-related molecules. These results indicate that class II gene alleles grouped in the same allelic "family" may differ by simple amino acid interchanges within the first amino terminal domains of their subunits.

0481 STUDIES ON MHC RESTRICTED KILLING OF VIRUS-INFECTED XENOPUS CELLS. D. Watkins and N. Cohen, University of Rochester, School of Medicine, Rochester, New York 14642

A unique model to study the ontogeny of thymic education has recently been developed in the frog, Xenopus. At 24 hours postfertilization, chimeras are created so that the anlagen of the thymus are derived from a frog of one MHC haplotype and the hemopoietic stem cells are derived from a second frog of another MHC haplotype. These chimeras have already been used to study restriction of antibody responses and graft rejection reactions in vivo. We are now beginning to explore thymic education of MHC restriction of virus-specific cytotoxic T cells in vitro. To this end, fibroblast cell strains have been derived from cardiac tissues of Xenopus laevis-gilli (LG15) cloned frogs of the XLA haplotype a/c, and from MHC homozygous (haplotype r) albino X. laevis laevis. Cell strains have also been derived from the lung and the kidney of the partially inbred MHC homozygous (haplotype j) J strain of X. laevis laevis. We have demonstrated that J strain cells are susceptible to infection with frog virus 3 (FV3), a pox-like herpes-like virus, that inserts virus-specific proteins into the host membrane. Spleen cells of J strain frogs immunized with virus in vivo and restimulated in vitro with virus-infected J strain cells can lyse MHC identical targets, providing that the targets are virus-infected. Whether this killing is MHC restricted and virus-specific is currently being determined.

T Cell Membrane Antigens: Biochemistry, Molecular Biology, Cognitive and Other Functional Aspects

0482 ANTIGEN-SPECIFIC INDUCTION OF INTERLEUKIN-2 RECEPTORS ON MURINE CTL CLONES. M.E. Andrew*, V.L. Braciale and T.J. Braciale. Washington University, St. Louis, MO 63110 Interleukin-2 is produced by a subset of T lymphocytes and has been demonstrated to be necessary for the proliferation of cytotoxic T lymphocytes (CTL) which bear receptors for this lymphokine. In unprimed animals, antigen has been shown to induce expression of the IL-2 receptor (IL-2R) by CTL precursors, but the subsequent fate of the receptors is unknown. We have cloned lines of IL-2-dependent CTL which also require periodic antigen stimulation to maintain proliferation. We can demonstrate that antigen induces IL-2R expression, which, over a number of cell divisions, decays until the CTL are refractory to the mitogenic stimulus of IL-2. Concommitant with the changes in IL-2R expression, the CTL display changes in morphology and in expression of other cell surface markers. In contrast, the antigen-specific lytic potential of these CTL remains constant as the cells cycle from a quiescent to active state, demonstrating that not all functions of the cells are down-regulated. These cloned lines provide an ideal system in which to study the mechanism whereby antigen induces expression of the IL-2R.

0483 ANTIBODIES TO THE T4(Leu 3) MOLECULE BLOCK CTL-TARGET CELL BINDING. William E. Biddison and Stephen Shaw, NIH, Bethesda, MD, 20205

The present study examines the potential role of the T4 molecule in functional cell-cell interactions between target cells and human CTL clones that are specific for HLA class II alloantigens encoded by the SB locus. There were marked differences (greater than 30 fold) between the 7 SB-specific clones studied with respect to their susceptibiltiy to inhibition by anti-T4 antibodies. We wished to test the hypothesis that such variation among the clones would be due to differences in clonal "affinity" for antigen. To quantitate differences among the CTL clones in the tightness with which they bind target cells, the clones were analyzed using a previously published assay of susceptibiltiy of CTL-target cell conjugates to dissociation in the presence of unlabelled targets. (Balk and Mescher, J. Immunol. 127:51, 1981). The results revealed that the clones that were most susceptible to inhibition by anti-T4 were the weakest target cell binders, and vice versa. Anti-T4 antibody could partially induce dissociation of functional CTL-target cell conjugates in the absence of any added cold targets. For the "highest affinity" clone, such anti-T4 antibody-induced dissociation could be observed at 4^{o} but not 23^{o}. These results indicate that the T4 molecule is functionally involved in target cell binding by CTL, and raise the possibility that although it is easiest to demonstrate the function of the T4 molecule in "low affinity" clones, that function may also be operative in the "high affinity" clones.

0484 THE ANALYSIS OF THY-1 GENE. Mei-hui Teng, Ross Basch, New York University,New York,NY 10016; Jack Silver, Michigan State University, East Lansing, MI 48824, Joel Buxbaum New York University & New York VA Medical Center, New York, New York 10016.

Thy-1 is found on murine neural, dermal and lymphoid tissues. Thymic T-lymphocytes fully express the antigen on their surfaces. In vitro, prothymocytes can be induced to express the antigen by several thymic peptides. The amino acid sequences of solubilized Thy-1 molecules from both rat and mouse (Thy-1.1, Thy-1.2) have been reported.

The mouse Thy-1 gene was studied by Southern blotting with a DNA probe isolated from a rat cDNA clone. Preliminary observations indicate that there is one Thy-1 gene. No restriction digestion differences were observed among brain, thymus and liver. The gene was also present in identical forms in two prototype mouse tumor cell lines which were Thy-1 (+) and Thy-1 (-). Several genomic clones containing the Thy-1 gene have been isolated from a Balb/c library and a tentative restriction map has been constructed.

The expression of Thy-1 mRNA in various cell types was studied by Northern blotting. There is a single 2kb poly A containing mRNA found in normal rat and mouse tissues and mouse tumor cell lines.

These results suggest that the Thy-1 gene is present as a single copy in DNA from all tissues studied. Gene rearrangement is not required for its expression in differentiated T-cells. The expression of the antigen in both lymphoid and non-lymphoid tissues is associated with identical single RNA transcripts which are larger than the size expected to code for a mature polypeptide of 14,000 daltons. This observation suggests a possible role for post-transcriptional regulation in the expression of Thy-1 antigen.

0485 BIOCHEMICAL AND FUNCTIONAL CHARACTERIZATION OF THE MAMMALIAN REOVIRUS RECEPTOR ON MURINE T CELLS USING ANTI-IDIOTYPIC ANTIBODY, Glen N. Gaulton, Man Sung Co, John H. Noseworthy and Mark I. Greene, Harvard Medical School, Boston, MA 02115

Studies have been conducted to elucidate the biochemical structure, cellular distribution, and modulation of the mammalian reovirus receptor. A syngeneic monoclonal anti-idiotypic antibody (87.92.6) was prepared by injection of purified monoclonal id$^+$ antibody (G5), with specificity to the reovirus type 3 hemagglutinin (HA3). Anti-id has been shown to bind to reovirus receptors on a variety of cell types and can block viral binding. Previous work has demonstrated that this anti-id binds to 45% of Ly2$^+$ T cells and when added to T cells stimulated in vitro with Con A suppresses the proliferative response by 80%. Similar suppression observed upon reovirus exposure was mediated by the activation of Ts cells. It has also been shown that anti-id binds reovirus specific Tc and blocks the lytic activity of a reovirus specific Tc line to both reovirus infected P815 cells and to anti-id hybridoma (87.92.6) cells. We report here on the capping of reovirus receptors following anti-id binding and its linkage to cytoskeletal elements, on the kinetics of binding of ^{125}I-labeled anti-id to immune and somatic cells, and on the biochemical characterization of the reovirus receptor. Anti-id binding was abrogated by prior treatment of cells with either protease or tunicamycin suggesting that the receptor is a glycoprotein. Anti-id bound to a single band on western blots of purified cell membranes run on SDS-PAGE. Similar analysis of membrane proteins on IEF gels identified the charge characteristics. The relationship of the immune receptor on T cells to that on somatic cells will be discussed.

0486 THE ACCESSORY FUNCTION OF THE MURINE T CELL SURFACE MOLECULE, L3T4. Julia L. Greenstein John Kappler, Philippa Marrack and Steven J. Burakoff. Dana Farber Cancer Institute, Harvard Medical School, Boston, MA and National Jewish Hospital, Denver, CO.

The role of the L3T4 molecule in the recognition of class I antigens was investigated. 3DT52.5, a self reactive T cell hybridoma, was demonstrated to be specific for D^d by reactivity to L cells that were transfected with the D^d gene. Recognition of D^d by 3DT52.5 induced the production of IL-2 and the cytolysis of D^d bearing targets.

3DT52.5 was shown to express the L3T4 surface molecule. Previous work has shown an association of the class II MHC recognition of effector T cells with the expression of the L3T4 marker. These experiments suggest that L3T4 interacts with class II molecules in distinction to the antigen receptor which sees antigen in association with class II molecules. These two (or more) interactions lead to a cumulative avidity of interaction which triggers T cell function. The expression of L3T4 by a class I reactive T cell appears to contradict the data which implicate the L3T4 association with the recognition of class II molecules. This paradox was investigated by antibody blocking of IL-2 production and cytolysis on Ia$^+$ and Ia$^-$ targets. 3DT52.5 recognition of D^d on Ia$^+$ targets is inhibited by anti-L3T4(GK1.5) and L3T4 interacts directly with a non-polymorphic determinant of Ia. However, anti-L3T4 does not inhibit 3DT52.5 recognition of D^d, Ia$^-$ cells. These results imply that while 3DT52.5 has antigen specificity for the D^d molecule alone, recognition of Ia via the L3T4 surface molecule can influence, but is not essential for, the triggering of effector T cell function. Therefore, the L3T4 molecule serves an accessory function to the T cell antigen receptor.

0487 T CELL ACTIVATING PROPERTIES OF AN ANTI-THY-1 MONOCLONAL ANTIBODY, Kurt C. Gunter, Thomas R. Malek and Ethan M. Shevach, NIAID, NIH, Bethesda, Md 20205

Cell surface antigens important in T cell functions may be studied by producing monoclonal antibodies (Mabs) to cloned populations of T cell hybridomas and screening these antibodies for their ability to stimulate or inhibit T cell functions. Taking this approach, we have immunized rats with mouse T hybridomas and screened these antibodies for their ability to augment or inhibit interleukin 2 (IL2) secretion by the T hybridoma used for immunization when stimulated with antigen plus presenting cells. One Mab, designated G7, was detected which stimulated IL2 from every functional T hybridoma tested. This Mab was also shown to induce IL2 secretion from normal splenic T cells. When splenic T cells were treated with the G7 Mab, a proliferative response was seen that was equivalent to that mediated by mitogens such as concanavalin A. Furthermore, the G7 Mab was shown to stimulate expression of IL2 receptors by fluorescence activated cell sorting (FACS), using a Mab to the IL2 receptor. FACS analysis revealed the antigen stained by G7 to be T cell specific. Immunoprecipitation of surface iodinated thymocytes and T cell hybridomas followed by sodium dodecyl sulfate polyacrylamide gel electrophoresis showed Mab G7 to react with a major antigen of 28 kilodaltons (kd) on T hybridomas and 28-32kd on thymocytes. Sequential immunoprecipitation with an anti-Thy-1.2 Mab indicated that the antigen recognized by G7 was Thy-1. The functional properties of G7, and tissue distribution and chemistry of the antigen bear some similarity to the OKT3/Leu4/UCHT-1 Mabs which recognize the T3 determinant in the human system.

0488 DEMONSTRATION AND ISOLATION OF HUMAN T CELL IMMUNOGLOBULIN-CROSS-REACTIVE MOLECULES WITH ANTIBODIES RAISED AGAINST FVμ AND AGAINST A NEOPLASTIC T CELL LINE, D. Haegert, M. Trudel and M. Timm, Department of Pathology, Montreal General Hospital, Montreal, Quebec, H3G 1A4

Recent serological and genetic studies indicate that the mammalian T cell antigen receptor is related to serum immunoglobulin (Ig) molecules. In an attempt to study Ig-cross-reactive molecules in human T cells chicken antibodies were raised against human Fvμ fragments and against a human neoplastic T cell line (CCRF-HSB-2); the antibodies were absorbed on then acid eluted from Sepharose-Fvμ and Sepharose-F(ab')$_2$ respectively. Whereas anti-CCRF-HSB-2 antibodies bound to approximately 90% of normal human peripheral T cells, anti-Fvμ antibodies bound to about 20% of T cells. Materials cross-reactive with F(ab')$_2$ (F(ab')$_2$-CRMS) were then isolated from CCRF-CEM and CCRF-HSB-2 T cell lines. These F(ab')$_2$-CRMS were shown to express determinants similar to or identical with F(ab')$_2$-cross-reactive determinants expressed in normal T cell membranes and to correspond closely to T cell antigen binding molecules. Competitive radioimmunoassay with ^{125}I labelled CCRF-HSB-2 derived F(ab')$_2$-CRMS indicates that the determinants recognized in the F(ab')$_2$-CRMS are only weakly cross-reactive with Ig. Immunoprecipitation experiments using anti-Fvμ and anti-CCRF-HSB-2 antibodies and various F(ab')$_2$-CRMS provide evidence for human T cell receptor heterogeneity.

0489 ORGANIZATION OF LOCI ENCODING LEUKOCYTE ALLOANTIGENS IN SEVERAL DISTINCT GENE CLUSTERS, Ulrich Hammerling, Nobuhiko Tada and Shoji Kimura, Memorial Sloan-Kettering Cancer Center, New York, NY 10021.

We have surveyed over forty of the loci which control leukocyte (Ly) alloantigens in the mouse. They have been found to map to eight clusters on seven chromosomes. The prototypes are Mls (I) (roman numeral indicates chromosome number) H-3 (II); Ly 6 (II); Lyb 2 (IV) Lyt 2 (VI) Igh (XII); H-2 (XVII); Lyt 1 (XIX). Almost every family includes antigens which are expressed on stem cells or early hemopoietic progenitor cells, as well as antigen(s) expressed on developmentally intermediate lymphocytes and other characteristics of mature and terminally differentiated cells. It is hypothesized that the control of chronological expression of sets of required surface molecules is the reason why gene clusters are formed and maintain in the murine genome.

0490 LECTIN-DEPENDENT CELLULAR CYTOTOXICITY OF H-2+ AND OF H-2- TARGETS IS BLOCKED BY ANTI-LYT 2 and ANTI-LFA-1, Thomas Hünig, Institut für Virologie und Immunbiologie, Versbacher Str. 7, D-8700 Würzburg, West Germany.

The predominant expression of the Lyt 2 antigen on H-2-class I restricted T-cells and the susceptibility of cytolysis mediated by these cells to inhibition by anti-Lyt 2 antibodies has led to the hypothesis the Lyt 2 functions as an MHC class-specific receptor by interacting with monomorphic determinants on class I molecules (1). The recent observation that anti-Lyt 2 also blocks lectin-dependent cellular cytotoxicity (LDCC) (2), and the availability of a β_2 microglobulin defective (and thus $H\text{-}2^-$)thymoma (3) that is nevertheless to lysed in LDCC (4), has made it possible to test whether blocking by anti-Lyt 2 does in fact indicate an interaction of the Lyt 2 antigen with H-2 class I molecules. Both alloreactive and polyclonally induced CTL lysed Con A-pulsed $H\text{-}2^+$ and $H\text{-}2^-$ thymoma cells. More importantly, anti-Lyt 2.2 inhibited LDCC of both targets similarly. In contrast to the allele-specifictiy observed for the inhibition with anti Lyt 2.2, anti-LFA-1 inhibited LDCC of $H\text{-}2^+$ and $H\text{-}2^-$ targets irrespective of the effector cells' Lyt 2 allele. It is concluded that anti-Lyt 2 can block T-cell mediated cytolysis by a mechanism distinct from interfering with the postulated interaction of Lyt 2 with H-2 class I molecules. 1) Swain, S. L. 1983. Immunol. Rev. 74:129.2) Bonavida, B., et al. 1983. Immunol. Rev. 72:119. 3) Hyman, R., and Stallings, V. 1976. Immunogenetics 3:75.4) Bevan, M. J., and Hyman, R. 1977. Immunogenetics 4:7.

0491 TRANSFECTION OF MOUSE L CELLS FOR CELL SURFACE LYMPHOID DIFFERENTIATION ANTIGENS AND SUBSEQUENT GENE AMPLIFICATION. Paula Kavathas, Charles Chu and Leonard A. Herzenberg. Stanford University, Stanford, CA 94305

Transfection of mouse L cells for cell surface differentiation antigens is often successful. Stable transferents were obtained for 11 out of 13 antigens tried. These include Leu-1, Leu-2, Ly-1, Ly-2, ThB, FcR, T200, Thy 1, L3T4, BLA-1, and BLA-2. After cotransformation with thymidine kinase and cellular DNA, antigen transferents are found among 10^3 - 10^4 TK^+ transferents using fluorescein-conjugated monoclonal antibodies and a fluorescence-activated cell sorter. The source of DNA for transfection can be either from expressing or from nonexpressing tissue or cell lines. An unusual finding is that spontaneous gene amplification is observed in 25-50% of Leu-2 transferents whereas none of the other antigen transferents have demonstrated this phenomena. Transfection of mouse L cells for cell surface antigens will be useful for cloning genes coding for lymphoid differentiation antigens

0492 CELL SURFACE STRUCTURES INVOLVED IN THE HUMAN CYTOLYTIC T LYMPHOCYTE RESPONSE, Alan M. Krensky, Carol Clayberger, Julia Greenstein, Elizabeth Robbins, Timothy A. Springer, and Steven J. Burakoff, Harvard Medical School, Boston, MA 02115.

Human cytolytic T lymphocyte (CTL) lines specific for a variety of MHC antigens (HLA-A2, HLA-DR6, and DC1) were used to define cell surface structures involved in lymphocyte-target cell interactions. Monoclonal antibodies (mAbs) recognizing LFA-1 (180K, 95K), LFA-2 (Leu5/OKT11-49K), LFA-3 (55-65K), and Leu 4 (OKT3-19K) antigens blocked cytolysis by all three CTL lines. Monoclonal antibodies recognizing Leu2a (OKT8) inhibited cytolysis by the Leu2a+, HLA-A2-specific CTL line and mAbs recognizing Leu2a (OKT4A, B, and E) inhibited cytolysis by the Leu3a+, HLA-DR6-specific CTL line, supporting the hypothesis that Leu2a/Leu3a mAbs define T cell surface structures involved in the recognition of MHC antigens (PNAS 75: 2365, 1982). No mAb recognizing either Leu2a or Leu3a blocked cytolysis by the Leu2a+, DC1-specific CTL, suggesting that an as yet undefined cell surface structure may be involved in DC recognition.

Monoclonal antibodies which recognize LFA-1, LFA-2, Leu2a, Leu3a, and Leu4 blocked cytolysis by binding to the effector cell while anti-LFA-3 and anti-MHC (HLA-A,B, DR, and DC) mAbs inhibited by binding to target cells. Furthermore, using a fluorescein-labelled target cell binding assay, anti-LFA-1, LFA-2, LFA-3, and Leu2a/Leu3a mAbs were shown to block conjugate formation while anti-Leu4 mAb did not.

Thus, at least six cell surface molecules (Leu4, Leu2a, Leu3a, LFA-1, LFA-2, and LFA-3), in addition to the antigen receptor and specific target antigen, are involved in the CTL-target cell interaction.

0493 ANALYSIS OF T CELL ACTIVATION IN A TUMOR CELL MODEL SYSTEM. By Ulf Landegren, Jan Andersson and Hans Wigzell. Dept. of Immunology, BMC, 751 23 Uppsala, Sweden.

The T cell communicates with the external world via a number of cell surface molecules. We have previously shown that the antigens denoted T3 and T8 are both requisite for the lytic action by T cells but that only antibodies against the T8 antigen prevent the conjugation to target cells (Landegren et. al. J. Exp. Med. 155-1579, 1982).

The T cell lymphoma line Jurcat responds to mitogenic trigger by the release of T cell growth factor. The stimulation can be shown to obey rules similar to those regulating the activation of normal T cells. A requirement for specialized accessory cells can be obviated by the addition of a phorbol ester to the culture. Mitogenic monoclonal antibodies with specificity for the T3 antigen effectively induce the release of growth factor. In the case of OKT3, accessory cells greatly augment this release whereas UCHT1 shows no such dependency. Alternate means of stimulating the lymphoma include mitogenic lectins and oxidative modification of the Jurcat cells or of another added tumor cell e.g. K562. Furthermore, a weak stimulatory effect was obtained by two unmodified cell lines, Raji and U715.

The stimulation of Jurcat by Con A can be hindered by agents normally interfering with T cell function. OKT 11 monoclonal antibodies abrogate stimulation with a 50% maximal effect at 100 ng/ml. A rabbit anti HLA DR serum depresses responses as does OKT4 antibodies. The response is extremely sensitive to inhibition by the drug Cyclosporin A.

The Jurcat cell line offers a convenient model system for the assessment of the nature of molecular interactions in lymphocyte responses.

0494 MEMBRANE STRUCTURES ASSOCIATED WITH THE MURINE T CELL RECEPTOR, James P. Allison and Lewis L. Lanier, University of Texas Cancer Center, Science Park, Smithville, Texas 78957; Becton Dickinson Monoclonal Center, Inc., 2375 Garcia Ave., Mountain View, CA 94043

A murine monoclonal antibody, 124-40, generated against a C57BL/Ka x-ray induced T lymphoma reacts with a clonotype specific determinant. The 124-40 antibody immunoprecipitated a glycoprotein composed of disulfide-linked subunits of 39,000 and 41,000 mw. In an attempt to determine whether this clonotype specific glycoprotein was associated with other membrane structures, glycoproteins on the surface of 125I radiolabelled C6VL T lymphoma cells were cross-linked using a cleavable cross-linking reagent, dimethyl 3,3'-dithiobis-propionimdate. 2HCl. The cross-linked or sham treated cells were solublized in lysis buffer containing 0.5% NP-40 and immunoprecipitated with the 124-40 monoclonal antibody, or a control antibody and analyzed by SDS-PAGE gel electrophoresis under reducing and non-reducing conditions. In the cross-linked, but not the sham preparation, additional structures were observed in the 124-40 precipitates. Under reducing conditions, two additional structures were observed, which were similar in nature to the human Leu 4/T3 structure. These studies indicated that similar structures may be associated with the T cell receptor of man and mouse.

0495 MONOCLONAL ANTIBODIES IN THE CD3 GROUP REACT WITH THE P23,P25 POLYPEPTIDE. J.A. Ledbetter, P.J. Martin, M. Braun, & J.A. Hansen, Genetic Systems Corp., Seattle WA 98121

Monoclonal antibodies in the CD3 group (T3, Leu-4, etc) react with a molecular complex (Tp19-29) that shows a close physical association with the antigen receptor (clonetype) on IL-2 dependent T cell lines. The Tp19-29 complex is thought to be involved in early events in T cell responses to antigen, since all antibodies in this group directly stimulate T cell proliferation, and the Tp19-29 complex quickly dissappears from the surface of T cells after exposure to antigen. Our immunoprecipitation experiments of surface labeled antigen from peripheral blood T cells resolve four noncovalently associated bands of 19K, 23K, 25K and 29K daltons on SDS gels. These four bands consist of at least two distinct polypeptide chains; the p23 and p25 bands contain intrachain disulfide bonds and are closely related to each other since they show identical partial protease cleavage products on PAGE. The p19 and p29 bands do not contain intrachain disulfide bonds and appear to be distinct from the p23, p25 bands. The p23, p25 bands are derived from a basic cytoplasmic precursor of 27,000 daltons. In Western blots, our anti-Tp19-29 antibodies react with the p23 and p25 bands but not the p19 or p29 bands. Since all the antibodies in this CD3 group, including UCHT-1, T3 38.1, 8961, 64.1, Leu-4 and G19-4 show cross blocking, it is likely that all the antibodies react with the p23, p25 polypeptide chain and that the p19 and p29 polypeptides do not express epitopes that have yet been recognized by monoclonal antibodies.

0496 PURIFICATION OF THE HUMAN RECEPTOR FOR T-CELL GROWTH FACTOR (TCGF) AND IDENTIFICATION OF ITS PRIMARY TRANSLATION PRODUCT, W.J. Leonard, J.M. Depper, R.J. Robb*, M. Kronke, P.B. Svetlik, N.J. Peffer, T.A. Waldmann, and W.C. Greene, NCI, NIH, Bethesda, MD 20205 and *Central Research Division, E.I. duPont de Nemours and Co., Glenolden, PA 19036

We have previously identified anti-Tac as a monoclonal antibody that recognizes the human TCGF receptor. We have prepared an anti-Tac immunoaffinity column, purified the receptor to homogeneity, and used the purified receptor protein to prepare a rabbit heteroantiserum. Both the heteroantiserum and anti-Tac precipitate the identical protein from ^{35}S-methionine labeled HUT-102B2 cells. The polyadenylated fractions of total RNA from peripheral blood T cells stimulated with PHA and PMA and from HUT-102B2 cells were isolated on oligo-dT cellulose and translated in a wheat germ lysate cell free translation system. Identically sized (M_r=34,500) primary translation products corresponding to the TCGF receptor were identified following heteroantiserum immunoprecipitation of translations with both mRNAs. This confirms that the receptor on HUT-102B2 cells (M_r=50,000) and that on PHA activated T cells (M_r= 55,000) differ in post-translational processing. We have previously identified a nonglycosylated putative peptide precursor of 33,000 daltons in anti-Tac immunoprecipitations from cells studied in pulse-chase experiments in the presence of tunicamycin. The apparent difference between this peptide precursor and the primary translation product presumably reflects the cleavage of a signal peptide. That anti-Tac precipitates the 33,000 dalton band but not the primary translation product suggests that some conformational changes or signal peptide cleavage are necessary for anti-Tac recognition.

0497 MONOMERIC IGG MODULATES STIMULATION OF T CELLS BY OKT3, Richard J. Looney and George N. Abraham, University of Rochester, Rochester, N.Y. 14642

Normal human serum has been reported to inhibit the activation of PBMC's by OKT3. We identified the Fc portion of intact IgG as the major inhibitor in humanserum of this OKT3 induced stimulation. Hypogammaglobulinemic serum, IgA, IgM, and F(ab')$_2$ of IgG were not inhibitory relative to intact IgG or Fc of IgG. Inhibition by IgG subclasses correlated with their ability to bind to the monocyte Fc receptor i.e. IgG1 & IgG3 > IgG2 & IgG4. Inhibition was competitive.By extrapolating our results, we would predict that in peripheral blood, which has about 10mg/ml of IgG, 10µg/ml of OKT3 would be needed for optimal stimulation. In contrast, in fetal calf serum only 1ng/ml is required.

If monocytes are removed from PBMC's, no stimulation by OKT3 occurs. To reconstitute stimulation, ILl and paraformaldehyde fixed monocytes must both be added to the monocyte depleted PBMC's. Thus, the monocyte serves a dual role.

Further investigation will focus on why the monocyte Fc receptor potentiates OKT3 stimulation, and on the effect of T cell or monocyte activation on OKT3 stimulation.

The relevance of these lines of investigation is, in part, that regulation of the idiotype network probably involves similar interactions between the T cell antigen recognition complex and antibody.

0498 PLURIFORM EFFECT OF ANTI-LFA1 MONOCLONAL ANTIBODY ON HUMAN LYMPHOCYTE AND GRANULOCYTE FUNCTIONS. LACK OF GRANULOCYTE LFA-1 IN A PATIENT WITH SEVERE RECURRENT INFECTIONS, Frank Miedema, Pedro A.T. Tetteroo, Wim G. Hesselink, Ron S. Weening, Dirk Roos, Cornelis J.M. Melief, Central Lab. Netherlands Red Cross, Blood Transf.Center, Amsterdam.

A monoclonal antibody (mcab), CLB54 directed to the human lymphocyte function associated antigen number 1 (LFA-1) was raised to the peripheral blood lymphocytes of a Tγ lymphocytosis patient. The mcab was selected by inhibition of the Natural Killer (NK)- and Killer (K)-cell activity of the patients lymphocytes. CLB54 had a strong inhibiting effect on the K and NK function of normal PBL and also inhibited the cytotoxic activity of CTL clones specific for either Class I or Class II HLA molecules. The membrane molecule recognized by CLB54 was shown to consist of 3 polypeptides with MW of 190 (α), 155, and 95 (β) kD. The mcab reacts with T-cells, B-cells, monocytes and granulocytes. It stains normal Tγ cells and Tγ cells of Tγ lymphocytosis patients 2-3 fold stronger than normal T-cells. Modulation experiments showed that LFA-1 and Fc receptors on Tγ cells are independent membrane structures both required for K-cell activity. CLB54 inhibited the phorbol-myristate-acetate stimulated chemiluminescence of granulocytes. Moreover a patient with recurrent bacterial and fungal infections due to a dysfunction on the activation mechanism of the granulocytic oxidative metabolism was shown to lack LFA-1 on the membrane of this granulocytes.

0499 ISOLATION OF CYTOLYTIC GRANULES FROM A CLONED T-CELL LINE AND CHARACTERIZATION OF THE CYTOLYTIC T-CELL PROTEINS (PERFORINS)

E.R. Podack, Scripps Clinic and Research Foundation, 10666 North Torrey Pines Road, La Jolla, California 92037

Cytolysis of tumor cells by cloned NK and TK cells appears to involve the participation of dense granules of killer cells and the formation of ultrastructural membrane lesions on target membranes. The dense granules of a cloned allospecific T-cell line were isolated after nitrogen cavitation by Percoll density gradient centrifugation. The granules (ρ=1.10-1.12 g/ml) were free from other cell organelles as judged by electron microscopy and marker enzyme assays.

In the presence of 1-3 mM Ca the isolated granules displayed strong cytolytic activity. More than 90% lysis of S194 ensued with 5 µg granule protein in less than 30 min at 37°. YAC-1 tumor cells were resistant to lysis by isolated granules whereas sheep and rabbit erythrocytes were highly susceptible.

Incubation of sheep erythrocytes with isolated granules resulted in Ca dependent formation of ultrastructural membrane lesions formed by insertion of 160 Å wide 160 Å long tubular complexes. Extraction of these complexes and SDS polyacrylamide gel electrophoresis revealed their composition of three major proteins of molecular weight 59k, 38k and ~20k. Since similar proteins are detected in the isolated granules, it is suggested they represent the cytolytic effector proteins that lyse target cells by complex formation in the target membrane, a process that may be accompanied by formation of transmembrane channels.

0500 REGULATION OF EXPRESSION OF THE MOUSE LYMPHOCYTE T200 ANTIGEN: EXPRESSION OF T200 IN L CELLS AFTER TRANSFECTION WITH LYMPHOMA DNA, William C. Raschke, David L. DeWitt, La Jolla Cancer Research Foundation, La Jolla, CA 92037.

The mouse lymphocyte T200 antigen has a 190 kd form expressed predominantly by T cells and a crossreacting 220 kd form present on most B cells. The transfection of the lymphoma T200 gene into L cells has provided a means of evaluating the regulation of expression of the T200 glycoproteins. Although the T200 molecules expressed by the parent BW5147 line and the T200-positive L cell are similar in size, around 190 kd, differences in expression were observed. The fully glycosylated T200 is 3 kd larger in the lymphoma, whereas the nonglycosylated T200 molecules are the same size in the two cells. The level of expression of T200 at the BW5147 cell surface is 4 times greater per cell and 8 times per unit surface area than the transfected L cell. These results argue for a specific coding sequence in the T lymphoma for the smaller form of T200 and not a larger, precursor, B cell form, unless T lymphocytes and L cells have the same posttranscriptional modification systems for T200. Although the slight difference in size of the BW5147 and L cell T200 molecules can be explained by glycosylation differences, the same is not true for the difference between the 190 kd T lymphocyte form and the 220 kd B lymphocyte form. The removal of high mannose and complex carbohydrates with Endo F leaves a size difference of about 35 kd. Whether the two forms of T200 are different gene products, the result of gene rearrangement during development, or the consequence of posttranscriptional or posttranslational events is currently being investigated.

0501 HUMAN T-CELL LEUKEMIA VIRUS (HTLV) AND HLA CLASS I ANTIGEN EXPRESSION, M. Reitz, M. Clarke, L. Seigel, S. O'Brien, D. Mann, and R.C. Gallo, National Cancer Institute, Bethesda, Maryland 20205

HTLV is a retrovirus etiologically associated with certain human adult T-cell leukemias and lymphomas. All T-cells infected with and expressing HTLV express apparent extra HLA Class I allotypic determinants. These determinants can be detected with 4D12, a monoclonal antibody against a polymorphic determinant present on the HLA B5, 15 and A 11, 29, 30 cross-reactive groups. Several types of evidence suggest that these extra determinants may be virally encoded. (1) Hybridization of an HLA Class I cloned DNA probe is obtained to part of the env region of HTLV. No hybridization is obtained with HLA Class II probes, and no hybridization of the Class I probe is obtained with HTLV II, which does not induce the 4D12 antigen in infected cells; (2) In somatic cell hybrids using an HTLV infected cell line (HUT-102) and hamster fibroblasts, expression of the 4D12 antigen does not segregate with chromosome 6, and appears only in provirus-containing cells; and (3) Expression of viral RNA and the 4D12 antigen are low or absent in uncultured peripheral blood T-cells from HTLV-infected persons, and increase in short-term culture with the same kinetics. Further studies are in progress to definitively determine whether or not the 4D12 antigen is a viral protein.

0502 SYNTHESIS AND EXPRESSION OF IA ANTIGENS IN AN ALLOREACTIVE DERIVED T CELL CLONE, Sujay K. Singh, Christopher J. Krco, William P. Lafuse and Chella S. David, Mayo Clinic, Rochester, Minnesota 55905

We analyzed the endogenous synthesis of Ia antigens by an alloreactive derived T cell clone. Clone PLT-17.2.C1 was derived by limiting dilution cloning from an alloreactive cell population B10.K anti-B6 and has been in culture for over a year in the absence of feeder cells on exogenous growth factors. The expression of T cell markers and Ia antigens on their cell surface was examined by using monoclonal antibodies and cytofluorometry. The cells were positive for Thy.1, Lyt.1 and Lyt.2 and negative for cell surface immunoglobulins. Both anti-I-A^k (H40-292.6, H39-64.5 and 17-227) and I-E^k (H7-8.26, H40-39.4 and 14-4-4) detected syngeneic Ia determinants on the surface of PLT-17.2.C1 cells. The Ia antigens of stimulator B6 (H-2^b) cells could not be detected using A^b specific monoclonal antibodies, 25-9-17, 25-5-16 and 28-16-8. The Ia antigens from these cells migrate as α and β peaks of conventional Ia antigens. These evidences suggest that Ia antigens expressed by PLT-17.2.C1 are endogenously synthesized and not passively acquired during culture. These cell lines secrete multiple lymphokines into the culture medium as shown by their ability to support the growth of other lymphocyte populations. Since it has been shown that lymphocyte derived interferon can induce the synthesis of Ia antigens in non-expressor T cells, we are in the process of purifying various lymphokines from PLT-17.2.C1 conditioned medium and examining their role in the induction of Ia antigen synthesis in other T cell lines.

0503 CELL-CELL CONTACT PROTEINS ON THE SURFACE OF CLONED CYTOTOXIC T LYMPHOCYTES (CTL). Michail V. Sitkovsky, Martin S. Schwartz and Herman N. Eisen, Massachusetts Institute of Technology, Cambridge, MA 02139

A photosensitive, heterobifunctional, cleavable crosslinking reagent (XL) was used to isolate CTL surface proteins ("contact proteins") that make contact with target cells (TC) and possibly participate in delivering a "lethal hit" in transient CTL:TC conjugates. The TCs were coated diffusely on their surface by XL•Con A (i.e. Con A to which XL was bound covalently through one of its two functional groups) and then mixed with ^{125}I-labeled CTLs. The resulting conjugates were exposed to UV light to induce crosslinking and then extracted with detergents. Antibodies to Con A were added, immunoprecipitating soluble complexes that consisted of contact proteins covalently bound to XL•Con A which, in turn, was bound noncovalently by the anti-Con A antibodies. Reductive cleavage of S-S bonds in XL yielded ^{125}I-labeled CTL proteins, which were analyzed by SDS PAGE. Of 12 immunoprecipitated bands 7 seem to be "contact proteins": i.e. they were not immunoprecipitated if the CTL:TC conjugates were not UV-irradiated, or if XL was omitted. The labeled CTL contact proteins had apparent MWs (in Kd) of approximately 200,160,120,105, and 80, plus faint bands of about 38 Kd and 33 Kd. The approach described may provide a new way to isolate CTL surface proteins of functional significance for TC recognition and lysis.

0504 PRELIMINARY CHARACTERIZATION OF A SOLUBLE IMMUNOSUPPRESSIVE MOLECULE FROM DBA/2 SPLEEN CELLS, Anthea Tench Stammers, J. J. Kevin Steele, Thomas Maier and Julia G. Levy, University of British Columbia.

In a previous publication we described a monoclonal antibody (B16G) which appeared to recognize T suppressor cells and a T suppressor factor (TsF) in the spleens of DBA/2 mice. The present study involves preliminary characterization of the material with which B16G reacts. It was found that the B16G-reactive protein (putative TsF) could be absorbed and eluted specifically from a B16G immunoadsorbent column. Material eluting from the B16G column reacted with B16G in an ELISA and appeared to run as two or more bands of 40-45 Kd molecular weight in SDS-PAGE. The eluted material was biologically active (i.e. suppressive) in the standard assay (MLR of DBA/2 splenocytes with B10.BR targets), and its suppressive activity was abrogated by the addition of B16G to the MLR cultures. Sephadex G-150 chromatography of the B16G reactive material showed that it occurred naturally as a dimer of 80-90 Kd molecular weight which broke down into subunits of 40-45 Kd under reducing conditions.

0505 THE ANALYSIS OF THY-1 GENE. M. Teng, R.S. Basch, New York University, New York; J. Silver, Michigan State University, East Lansing; and J.N. Buxbaum, New York University & New York VA Medical Center, New York, 10010.

Thy-1 is found on murine neural, dermal and lymphoid tissues. Thymic T-lymphocytes fully express the antigen on their surfaces. In vitro, prothymocytes can be induced to express the antigen by several thymic peptides. The amino acid sequences of solubilized Thy-1 molecules from both rat and mouse (Thy-1.1, Thy-1.2) have been reported.

The mouse Thy-1 gene was studied by Southern blotting with a DNA probe isolated from a rat cDNA clone. Preliminary observations indicate that there is one Thy-1 gene. No restriction digestion differences were observed among brain, thymus and liver. The gene was also present in identical forms in two prototype mouse tumor cell lines which were Thy-1 (+) and Thy-1 (-). Several genomic clones containing the Thy-1 gene have been isolated from a Balb/c library and a tentative restriction map has been constructed.

The expression of Thy-1 mRNA in various cell types was studied by Northern blotting. There is a single 2kb poly A containing mRNA found in normal rat and mouse tissues and mouse tumor cell lines.

These results suggest that the Thy-1 gene is present as a single copy in DNA from all tissues studied. Gene rearrangement is not required for its expression in differentiated T-cells. The expression of the antigen in both lymphoid and non-lymphoid tissues is associated with identical single RNA transcripts which are larger than the size expected to code for a mature polypeptide of 14,000 daltons. This observation suggests a possible role for post-transcriptional regulation in the expression of Thy-1 antigen.

0506 THE EXPRESSION OF HUMAN T CELL DIFFERENTIATION ANTIGENS ON MURINE T CELLS BY DNA-MEDIATED GENE TRANSFER. Ofra Weinberger, Alan M. Krensky, Steven J. Burakoff and Jonathan Seidman. Departments of Genetics and Pediatrics, Harvard Medical School, Boston, Mass. 02115.

In order to understand the role of the differentiation antigens in immune function, it will be necessary to define the structure of the proteins and the genes which encode them. For the study of the regulation of gene expression, it would be preferable to transfer genes encoding proteins expressed during differentiation into the cell type which normally expresses these genes of interest. We have transfected genomic DNA from a human cytotoxic T cell clone into a murine cytotoxic T cell hybridoma, Cloned murine T cell populations stably expressing the human T cell antigens, LFA-2(Leu5) and OKT4, have been selected by continual FACs selection with specific monoclonal antibodies. Both LFA-2 and OKT4 are antigens associated with T cell functions, e.g. antigen specific proliferation and cytotoxicity. Functional studies on the newly expressed antigens are in progress.

0507 THE ROLE OF THE T3 ANTIGEN IN HUMAN T CELL ACTIVATION, Arthur Weiss, John Imboden, Dolores Shoback, Roberta Kamin, Robert Wiskocil and John Stobo, Howard Hughes Medical Institute, University of California, San Francisco, Ca. 94143

The human T cell leukemia, Jurkat, served as a model to assess the role of the T3 antigen complex in human T cell activation. When Jurkat cells were stimulated with anti-T3 monoclonal antibodies, a second stimulus, phorbol myristate acetate (PMA), was required for the production of interleukin-2 (IL-2) and gamma-interferon (IFN). Studies to examine the cellular and molecular events by which these stimuli exerted their effects were undertaken. A T3 negative mutant of Jurkat (S.5), was used to assess the relative requirement for the T3 antigen and the role of T3 in the process of activation. Whereas, S.5 produced no detectable IL-2 in response to anti-T3 antibodies and PMA , equivalent levels of IL-2 were obtained when S.5 and Jurkat were stimulated with a calcium ionophore and PMA. Antibodies specific for T3 increased free cytoplasmic Ca^{++} in Jurkat cells, but only the calcium ionophore increased free cytoplasmic Ca^{++} in S.5. These results suggest that the activation signal mediated by the T3 antigen complex regulates intracellular Ca^{++} concentration. Utilizing cDNA probes (obtained from Genentech, Inc.), studies were designed to examine the molecular events occurring during activation. Unstimulated Jurkat cells as well as cells stimulated with anti-T3 or PMA only contained no detectable IL-2 or IFN specific RNA. IL-2 and IFN specific RNA were detected only in cells which had received both stimuli. These results demonstrate that the two stimuli exert their effects at pre-translational levels.

0508 A FUNCTIONAL SUBSET OF HUMAN T HELPER CELLS DEFINED BY A MONOCLONAL ANTIBODY TO THE IL-2 RECEPTOR. Paul Yoshihara, David Regan, Denis Burger, VA Medical Center, Portland, OR 97201.

Human T helper cells (T_H, OKT-4+ cells) are heterogeneous with respect to biological function. We have prepared monoclonal antibodies to subsets of cells in the T_H family and attempted to relate phenotypic expression of surface markers to functional properties of the subsets. Mice were immunized with cloned, human T_H lymphoma cells and after fusion hybrids were screened for production of antibodies against the T_H subset from which the lymphoma was derived. A variety of screening patterns was observed including one defining a minor population of normal T_H cells. This hybrid culture was cloned (designated $T_H5.2$) and the resulting monoclonal antibody characterized as a cytotoxic IgM isotype recognizing 3-5% of normal blood mononuclear cells (about 20% of blood T_H cells). Addition of $T_H5.2$ to lymphocyte cultures produced significant augmentation of mitogen and antigen-induced proliferation particularly when the stimulant concentration was suboptimal. Depletion of $T_H5.2$+ cells by sorting or cytotoxicity eliminated antigen responsiveness as well as suppressing (65-80%) mitogen-induced proliferation. This monoclonal antibody also blocked the ability of IL-2 dependent cell lines (HT-2) to respond to IL-2. Taken together the data suggests that this antibody recognizes the IL-2 receptor.

Factors Involved In B Cell Stimulation: Toward a Molecular Definition

0509 A MONOCLONAL ANTIBODY (AE3.D3) WITH MITOGENIC PROPERTIES FOR MURINE B CELLS, Michèle Allouche*, Ross S. Basch and Joan W. Berman, Department of Pathology,NYU Medical Center NY, NY 10016. *Present Address : Dept. of Hematology, Hop. P.Brousse, 94800 Villejuif

A monoclonal antibody, AE3.D3, derived from the fusion of rat splenocytes, immunized against mouse brain, with the myeloma SP2, has been produced which has the property of inducing the proliferation of mouse lymphocytes. The mitogenic effect is highest in spleen and lymph node cells, where up to a 10-fold stimulation of ^{3}H-TdR incorporation is observed. B lymphocytes are the most susceptible to this proliferative stimulus, and they are induced to differentiate into plaque-forming cells. T lymphocytes and "null" cells (defined by the absence of Thy-1 or Ig on their surface) do proliferate, although to a smaller extent. The T cell subpopulation, isolated from either spleen or thymus, requires additional "helper factors" in order to proliferate. The mitogenic response is not genetically restricted, and B-cell deficient mouse strains such as C3H/HeJ and CBA/N, as well as T-cell deficient strains such as BALB/c nude mice, are capable of responding to the stimulus of AE3.D3.
Using immunofluorescence, we also examined the distribution of AE3.D3-positive cells in various lymphoid organs. The highest percentage of stained cells is found in the spleen (28 %) and lymph node (18 %), whereas only 14 % of the bone marrow and 5 % of the cells of the thymus are brightly stained with AE3.D3.

0510 B CELL GROWTH FACTOR (BCGF): A SENSITIVE BIOASSAY USING HAPTEN-BINDING MURINE PLASMACYTOMA CELLS AS INDICATORS, A. Altman, Department of Immunology, Scripps Clinic and Research Foundation, La Jolla, California 92037

Attempting to establish a sensitive, reproducible B cell growth factor (BCGF) bioassay, we ascertained the ability of an in vivo variant of the murine DNP-binding, IgA-secreting plasmacytoma, MOPC-315, to respond to lymphokine (LK) preparations. We hypothesized that the known inability of this ascitic variant to grow in vitro may reflect a B-cell-tropic growth factor(s) requirement. In the absence of LK, the proliferation of MOPC-315 cells in culture was very low. However, LK preparations of polyclonal or T cell monoclonal origin, including those lacking demonstrable T cell growth factor (IL2) activity, as well as interleukin 1 (IL1)-containing supernatants from the macrophage cell line P388D1 stimulated the proliferation of the myeloma cells up to 25 fold. On the other hand, purified IL2 or T cell mitogens had no detectable effect. Stimulation could be detected with as few as 500 myeloma cells per well, as early as 24 hrs. after stimulation. In contrast to the ascites line, an in vitro-adapted variant of the same plasmacytoma, MOPC-315/P, displayed a high level of constitutive proliferation which was not modified by LK preparation addition. However, the MOPC-315/P line, which does not secrete significant levels of DNP-binding IgA, appeared to be sensitive to putative B cell differentiation factor(s) (BCDF) inasmuch as it responded to different LK preparations with a marked increase in the number of anti-DNP plaque forming cells. These results indicate that distinct myeloma variants, producing an easily-quantifiable and well-defined idiotype, can serve as sensitive indicators in BCGF and BCDF bioassays.

0511 CELL CYCLE-RELATED EXPRESSION OF RECEPTORS FOR B CELL DIFFERENTIATION FACTORS (BCDFμ). K. Brooks, J.W. Uhr and E.S. Vitetta. Department of Microbiology, University of Texas Health Science Center at Dallas, Dallas, Texas 75235

Cloned, neoplastic murine B cells (BCL_1) were used to examine lymphokine-mediated differentiation of B cells to IgM antibody-forming cells (AFC). BCL_1 cells respond to T cell-derived B cell differentiation factor(s) (BCDFμ) in a cell-cycle-dependent manner. The maximal response to BCDFμ is seen when cells are stimulated during S and/or G2 of the cell-cycle. A 2 hr pulse with BCDFμ is sufficient to induce IgM secretion when given to cells synchronized at G2/M. This limited exposure to BCDFμ initiates a chain of events resulting in altered mRNA processing, enhanced IgM synthesis and secretion of IgM. The cell-cycle-dependent expression of the BCDFμ receptor is also indicated by the preferential capacity of cells in S and G2 to absorb BCDFμ activity from a T cell supernatant.

0512 B CELL ACTIVATION: PROVISION OF SECOND SIGNALS FOR G_0 TO G_1 TRANSITION BY ANTI-IA ANTIBODIES. John C. Cambier, Kenneth M. Coggeshall, Lesley R. Harris and Olivera J. Finn, National Jewish Hospital, Denver, CO 80206 and Duke University Durham, NC 27710.
It is well established that generation of humoral immune responses to most protein antigens requires interaction of B cells with antigen, antigen specific Ia restricted T cell help, and nonspecific macrophage and T cell derived factors. Interaction of quiescent B cells with antigen and restricted T cell help promotes entry of these cells into cell cycle, i.e. G_0 to G_1 transition. Upon subsequent interaction of G_1 cells with nonspecific T cell derived factors, cells proliferate and differentiate into antibody forming cells. Palacios et al. (PNAS 80:3456) have recently reported that certain anti-Ia antibodies promote normally thymus dependent anti-SRBC antibody responses in the absence of T cells. These findings suggest that anti-Ia antibody mimic the T cell signal in its action on B cells. Here we report further studies of the ability of anti-Ia antibodies to "replace" T cells in in vitro B cell immune responses. Results demonstrate that certain monoclonal anti-DR/DC antibodies which crossreact with mouse I-A/E determinants promote anti-SRBC responses in vitro. Optimal promotion of B cell responses by anti-Ia requires presence of antigen, as well as supernatants containing IL1, IL2, BCGF and BCDF. The anti-Ia antibodies partially inhibit anti-SRBC responses generated in the presence of SRBC primed and irradiated helper T cell populations. Finally, these antibodies synergize with receptor immunoglobulin crosslinking ligands in stimulating quiescent (G_0) B cells G_0 enter G_1. Results indicate that membrane associated Ia molecules may play an important signal transducing role in the antigen and T cell dependent promotion of G0 to G1 by B lymphocytes transition during humoral immune responses.

0513 ROLE OF PHOSPHATIDYLINOSITOL HYDROLYSIS AND PROTEIN KINASE C ACTIVATION IN EARLY B CELL ACTIVATION EVENTS, Kenneth M. Coggeshall and John C. Cambier, National Jewish Hospital, Denver, Co 80206. Murine B cells treated with specific antigen or rabbit anti-mouse immunoglobulin (RAMIG) undergo a rapid loss of membrane potential and a subsequent increase in surface I-A antigen expression. We have proposed that these events are important consequences of "first signals" in thymus-dependent B cell activation. In view of this we have begun investigations of the mechanism by which B cell surface Ig crosslinking and depolarization are coupled. We have observed that certain phorbol esters are able to induce B cell depolarization suggesting that PKC activation may be an important coupling event. Nishizuka et al. have suggested that PKC activation is linked to phosphatidylinositol (PI) hydrolysis and the generation of diacylglycerol (DG). Maino et al. have demonstrated activation of PI hydrolysis in RaMIg-treated B cells. We hypothesize that this PI hydrolysis results in sequential PKC activation and alterations in ion flux manifest by depolarization. Here we describe our efforts to test this hypothesis by examining the effects of artificially inducing or inhibiting the production of DG. We have observed that exogenously added phospholipase C or DG is able to induce depolarization in B cells in a dose-dependent manner. We have further observed that RaMIg-induced but not phorbol-induced depolarization is blocked by raising intracellular cyclic AMP, a condition known to inhibit DG production. We suggest this is because receptor-mediated activation of PK-C requires DG production and thus is susceptible to cyclic AMP inhibition while direct activation of PK-C is not. Results are consistent with the notion that DG production and PK-C activation are important biochemical events in receptor Ig mediated transmembrane signalling in B cells.

0514 REQUIREMENT OF LIPOXYGENASE METABOLISM FOR INTERLEUKIN ACTIVTY, William L. Farrar Laboratory of Molecular Immunoregulation, NCI-Frederick Cancer Research Facility, Frederick, MD. 21701

Several investigations have suggested that products of arachidonic acid metabolism have modulatory effects on the development of cellular immunity. This report documents the role of arachidonic acid metabolism on interleukin 1 (IL 1) induction of interleukin 2 (IL 2) productionand on IL 2 regulation of intracellular cyclic GMP levels, gamma interferon (IFN γ) production as well as lymphocyte proliferation. Utilizing pharmacological inhibitors of phospholipase A_2 (PLA) we have demonstrated that PLA activation is required for both IL 1 induction of IL 2 production and IL 2 regulation of proliferation and IFN γ production by mature T lymphocytes and natural killer cells. In addition, a panel of inhibitors of arachidonic acid metabolism were used to determine whether cyclooxygenase or lipoxygenase pathways were required for the respective interleukin activities. The results suggest that both IL 1 and IL 2 utilize the lipoxygenase pathway of arachidonic acid metabolism and not the cyclooxygenase pathway. Lipoxygenase activity was required for IL 1 induction of IL 2 production and IL 2 regulation of cellular proliferation, IFN γ production and modulation of intracellular cyclic GMP. The same requirements for products of lipoxygenase metabolism were seen when phorbol myristic acetate (PMA) was used a a stimulant which suggests that PMA and interleukins mode of action for stimulation-secretory events may utilize similar cellular metabolic pathways.

0515 OPPOSING REGULATORY MECHANISMS FOR B CELL ACTIVATION AS MEDIATED BY CYCLO-OXYGENASE AND LIPOXYGENASE PRODUCTS OF ARACHIDONIC ACID, Michael G. Goodman and William O. Weigle, Scripps Clinic and Research Foundation, La Jolla, CA 92037

The immunoregulatory effects of the oxidative metabolites of arachidonic acid (AA) on proliferation of B lymphocytes were assessed in a serum-free culture system. Activation of B cells by a) membrane-directed ligands, and b) intracellular activators was regulated by AA metabolites in a very distinct fashion. Thus, exogenous cyclo-oxygenase products (particularly PGE_1 and PGE_2) amplified the response to anti-immunoglobulin antibodies, whereas lipoxygenase products damped this response. B cell activation with 8MGuo (an intracellular activator), in contrast, was inhibited by cyclo-oxygenase products and remained relatively unaffected by lipoxygenase products. This pattern of results was confirmed in studies with pathway inhibitors. Moreover, when liberation of endogenous arachidonate was induced by stimulation of phospholipase A_2 with melittin, inhibition of the response to each class of activator was counteracted with the appropriate pathway inhibitor. These results suggest that the two major groups of arachidonic acid oxidation products function as a system of counterbalancing regulatory influences serving to modulate B cell activation occurring at the plasma membrane, and to down-regulate B cell activation at the intracellular level.

0516 IMMUNOLOGICALLY DISTINCT B CELL-STIMULATION FACTORS FROM A T CELL HYBRIDOMA B151. T. Hamaoka, S. Ono, Y. Hara, Y. Takahama and K. Dobashi, Osaka University Medical School, Fukushimaku, Osaka, 553, Japan.

We previously established a monoclonal T cell hybridoma B151 which spontaneously releases selected B cell-stimulation factor(s) without any other known lymphokine activities. We report here that the B cell-stimulation activities of B151 comprise two factors with distinct immunological and physicochemical properties. One of the factors, designated herein as B cell growth and differentiation factor (BGDF)-1, preferentially acts on antigen-primed B cells and their corresponding neoplastic BCL_1 cell line. By contrast, BGDF-2 acts on antigen-non-primed naive B cells as well as neonatal B cells. B cells from DBA/2Ha mice do not respond to the BGDF-1, whereas the same cells respond to BGDF-2. By contrast, CBA/N B cells become responsive to BGDF-1 after antigen-priming, but not to BGDF-2. Moreover, the addition of solubilized membrane fraction from BCL_1 cells selectively blocks activity of BGDF-1 activity but not BGDF-2. A combination of reverse phase HPLC, labelling, gel filtration analysis and ligand target binding reveals that BGDF-1 has a MW of 50 Kd whereas BGDF-2 had a MW of 30 Kd. BGDF-1 activity shows binding to lectin-columns of LBA, DBA, SJA, SBA and LPA but not to WGA, LcH and Con A. In contrast, BGDF-2 activity does not bind to any of the lectin-columns. Thus B151 T cell hybridoma releases two distinct B cell stimulation factors which may represent either a single peptide with and without carbohydrates or different peptides encoded by two distinct genes.

0517 DIFFERENTIATION OF A B CELL LYMPHOMA IS HELPED BY I-E RESTRICTED, ANTIGEN-SPECIFIC T CELLS. Geoffrey Haughton and N. J. LoCáscio, University of North Carolina, Chapel Hill, N. C. 27514.

CH12 is a monoclonal B cell lymphoma of B10.H-2^aH-4^b p/Wts (2^a4^b) mice, the cells of which bear membrane IgM (κ) of a single idiotype, reactive with sheep and chicken Rbc and with bromelain-treated autologous mouse Rbc. The cells express $K^kA^kE^k$ and D^d antigens. During the latter stages of growth in vivo or in vitro, a small proportion (<3%) of the cells differentiate to secrete hemolytic antibody as measured by the Cunningham assay for plaque forming cells (PFC). We cultured CH12 cells with antigen and spleen cells from primed animals, and assayed for PFC induction. Differentiation was induced by cells from SRbc primed 2^a4^b mice in the presence of SRbc or ChRbc but not rabbit or human erythrocytes. Activity was depleted by treatment of the spleen cells with anti-Thy-1 or anti-Lyt-1 but not anti-Lyt-2 plus complement Helper cells could also be induced by priming 2^a4^b mice with ChRbc but not rabbit or human Rbc. Use of H-2 congenic mice, showed that help was H-2 restricted and specifically, that matching for $I\text{-}E^k$ was required. Matching for $I\text{-}A^k$ alone was inadequate for transmission of help. Induced differentiation of CH12 did not occur when the cells were cultured with any of a variety of non-specific T cell helper factors, even in the presence of SRbc. However, LPS induced CH12 to differentiate whether or not SRbc were also present. These characteristics resemble those of normal resting (as opposed to activated) B cells and since CH12 cells are continuously dividing, establish that transition from the "resting" to the "activated" state is not an inevitable consequence of entry into the mitotic cycle.

0518 MODULATION OF SURFACE Ia EXPRESSION ON HUMAN B LYMPHOCYTES. John H. Kehrl Atsushi Muraguchi, and Anthony S. Fauci, LIR, NIAID, NIH, Bethesda, MD 20205.

Using flow cytometry techniques, changes in surface Ia (DR and DS) expression on human B lymphocytes were correlated with changes in the cell cycle following stimulation with anti-μ. The effect of interleukin 1 (IL 1) and interferons on Ia expression on resting B cells was also examined. A population of resting B lymphocytes was cultured in vitro with 100 μg/ml of anti-μ and immunofluorescently stained for DR and DS at various times following stimulation. Detectable increases in DR and DS expression were found within 8 hours, and the major increases (two- to four-fold) in DR and DS expression occurred over the next 48 hours. Using cell cycle inhibitors and propidium iodide staining, it was demonstrated that the enhanced DR and DS expression following anti-μ stimulation began during G_0 to G_1 transition and increased as the cells progressed through G_1 phase. During S and G_2/M phase there were minimal further increases in surface Ia. Although prolonged exposure of B cells to anti-μ was required for cellular activation, cell size enlargement and progression into S phase, a brief exposure to anti-μ, insufficient for cellular activation, markedly enhanced Ia expression. Thus, anti-μ-stimulated resting human B lymphocytes rapidly increase their surface Ia expression. This increase occurs predominantly prior to entrance into S phase and can occur in the absence of significant cellular activation. Interferons have been reported to modulate surface Ia expression on a human lymphoid B cell line and on monocytes; however, neither α or γ interferon, nor purified IL 1 modified surface DR expression on normal resting human B cells.

0519 HYBRIDOMA 2.4G2: DISSOCIATION OF THE B LYMPHOCYTE TRIGGERING ACTIVITY FROM THE MONOCLONAL ANTI-Fc IgG RECEPTOR ANTIBODY, Marinus C. Lamers, Susan E. Heckford, and Howard B. Dickler, Immunology Branch, National Cancer Institute, Bethesda, MD 20205

We have previously reported that monoclonal antibody specific for mouse Fc IgG receptors (purified by affinity chromatography using goat $F(ab')_2$ anti-rat IgG from supernatants of 2.4G2) can induce both proliferation and differentiation to antibody secretion of normal B lymphocytes. Additional analysis has now shown that a copurified substance triggers the B cells in that the activating activity can be distinguished from the 2.4G2 antibody by several criteria. 1. Different preparations of 2.4G2 antibody with identical binding capacity for Fc IgG receptors differ markedly in their ability to trigger B lymphocytes. 2. Removal of the anti-Fc IgG receptor antibody from an active preparation by absorption with insolubilized mouse anti-rat kappa antibody and the P388D1 macrophage-like cell line does not result in loss of B cell triggering activity. 3. The active principal has a molecular weight of less than 50,000 Daltons. 4. Preparations which trigger mouse B cells can also induce rat spleen cells to proliferate and human peripheral blood cells to secrete antibody, while 2.4G2 antibody does not bind to rat or human lymphocytes. We are currently characterizing the active principal.

0520 B-CELL HELPER FACTORS, H. James Leibson, N. Roehm, A. Zlotnik, P. Marrack, and J.W. Kappler, National Jewish Hospital, Denver, CO, 80206

We have identified three non-specific helper factor preparations which synergize in stimulating murine B-cell antibody responses to sheep red blood cells (SRBC). The first is the IL-1 containing supernatant (Sn) of the macrophage cell line p388D$_1$. The second is the gamma interferon (IFNγ)-containing, Con A-stimulated Sn of the T-cell hybridoma FS7-20.6.18. We have recently domonstrated that the helper activity of this Sn can be replaced by IFN produced in E. coli via a recombinant DNA clone. The third factor preparation is produced by Con A stimulation of the T-cell hybrid FS6-14.13. This preparation contains both interleukin-2 (IL-2) and B-cell growth factor (BCGF). The essential helper factor in this supernatant co-purifies with IL-2 rather than BCGF in Sephadex G-75 and C_8 reverse-phase HPLC chromatographies. We are currently testing IL-2 produced via a recombinant DNA clone to confirm this point. Our current hypothesis is that IL-1, IL-2, and IFNγ play essential roles in this B-cell response, and that the effectiveness of the SRBC antigen in activating SRBC-specific B-cells via cross-linking of surface immunoglobulin precludes the need for BCGF.

0521 TRANSLATION AND CLONING OF A T HYBRIDOMA-DERIVED B CELL REPLICATION FACTOR, Waldemar Lernhardt, Robert J. Deans, Nicolas Fasel, Fritz Melchers and Randolph Wall, Molecular Biology Institute, University of California, Los Angeles, CA 90024

We have identified a T cell hybridoma which produces B cell replication factor (BRF) upon stimulation with concanavalin A. To our knowledge, this hybridoma does not produce other lymphokines such as Interleukin I and II, T cell replacing factor and colony stimulating factor. This hybridoma, therefore, is an ideal source for the molecular characterization of BRF. We are currently purifying the BRF protein. Extracted mRNA from the induced hybridoma can be successfully translated into BRF activity by Xenopus laevis oocytes. No such activity can be obtained by translating mRNA from the uninduced hybridoma nor from BW5147, the parent lymphoma line. By cDNA cloning we have identified two clones coding for BRF. These clones are presently being characterized and will be employed to identify genomic sequences coding for BRF.

0522 PRODUCTION OF HUMAN BCGF FROM A T-CELL LINE AND ASSAY USING MURINE CELLS. Frances S. Ligler, E. I. du Pont de Nemours and Company, Glenolden, PA 19036

A proliferation assay for human B cell growth factor (BCGF) was developed that is, in our hands, more sensitive than the anti-IgM costimulator assay using isolated human B cells. The assay uses anti-Thy 1+C'-treated murine spleen cells incubated for 24 hours with LPS. Blasts are isolated using a Percoll® gradient and incubated for three days in the presence or absence of BCGF. Typical cpm of ^{3}H-thymidine incorporated in 18 hours by 2x10^4 cells is 200 background, 400 with PHA, 5000 with LPS and 9000 with BCGF.

Two types of cells are used to produce human BCGF in serum-free medium: PBL from platelet pheresis residues stimulated with RAJI and IM-9 cells and an unstimulated human T cell line. Both of these systems also produce low levels of TCGF. However, TCGF activity can be absorbed onto Sepharose-coupled rabbit anti-TCGF without reducing the BCGF activity.

0523 Characterization of the Gene for B Cell Maturation Factor, Richard Maki, Christopher Paige[1], Charles Sidman[2], Max Schrier[1]
La Jolla Cancer Research Foundation, La Jolla CA., [1] Basel Institute for Immunology, Basel, Switzerland, [2] Jackson Labs, Bar Harbor, ME.

We are attempting to identify the gene for B cell maturation factor (BMF). The cell line EL4 can be induced to secrete BMF after exposure to phorbol myristate acetate (PMA). RNA extracted from these cells can be shown to code for BMF activity when injected into frog oocytes. The assay for BMF we are currently using is the conversion of 70Z/3 cells from surface IgM negative to surface IgM positive. Normally, 70Z/3 does not express surface IgM but, after exposure to BMF, the cell line responds by beginning to synthesize light chain and becomes surface IgM positive. We have recently fractionated the mRNA from EL4 on a sucrose gradient and identified one peak of BMF activity. A cDNA library is being constructed from this fraction of RNA and will be used to screen for the BMF gene.

0524 STAGE SPECIFIC ACTION OF T CELL FACTORS IN B CELL GROWTH AND DIFFERENTIATION, Kenji Nakanishi, NIAID, NIH, Bethesda, MD 20205

B lymphocytes cultured with anti-IgM antibodies divide and differentiate into Ig synthesizing cells in the presence of cofactors. Proliferation depends upon B cell growth factor (BSF-p1) and IL-1; differentiation requires the further addition of B15-TRF (supernatant of B151K12 T hybridoma cells) and EL-TRF (pI-4.5 fraction of supernatant of PMA-induced EL-4 cells). The ordered action of these factors is indicated by experiments showing that anti-IgM stimulated B cells precultured with BSF-p1 can be stimulated to synthesize Ig by subsequent culture in B15-TRF and EL-TRF without additional BSF-p1, implying that the differentiation of such cells is independent of BSF-p1. Anti-IgM stimulated B cells precultured with BSF-p1 and B15-TRF require both B15-TRF and EL-TRF in order to differentiate into Ig synthesizing cells. Since it has previously been shown that B15-TRF must be added early in culture while the addition of EL-TRF can be delayed until 24 hours before assay, it appears that B15-TRF is required throughout the differentiation process. Studies of IgM mRNA expression in stimulated B cells indicate that culture of B cells in anti-IgM and BSF-p1 causes enhancement in mRNA hybridizing with cDNA probes for membrane μ (μ_m) and for total μ (μ_t). Treatment of such cells with B15-TRF strikingly reduces μ_m mRNA without changing μ_t suggesting that it mainly alters mRNA splicing patterns. Addition of EL-TRF to cultures containing BSF-p1 and B15-TRF causes a substantial enhancement in μ_t mRNA but μ_m remains at the very low levels found with BSF-p1 and B15-TRF. The results presented here emphasize the distinctive and ordered function of T cell factors in B cell proliferation and differentiation.

0525 HUMAN B-CELL INDUCING FACTOR (BIF) FOR IgM, IgG and IgA PRODUCTION: INDEPENDENCE FROM IL2, Peter Ralph and Karl Welte, Sloan-Kettering Institute, Rye, NY 10580

We described an assay for BIF using T-depleted B cells from blood and spleen that are mitogenically stimulated with Staphylococcus bacteria (Sac)(J. Immunol. 127:1044, 1981). BIF also stimulates secretion in IgM and IgG cell lines (Eur. J. Immunol. 13:31, 1983; Cell. Immunol. 79:36, 1983). We have purified BIF 11,000-fold from PHA/DAUDI-induced blood lymphokine using methods for IL2 purification (J. Exp. Med. 156:454, 1982). Factor(s) inducing IgM, IgG and IgA production copurify throughout the biochemical fractions. Most IgG and IgA-secreting cells come from a small fraction of B cells in blood that have already switched (Eur. J. Immunol. 12:506, 1982). However, in vitro production of IgG and IgA from spleen cells derives from $\mu^+\delta^+$ precursors (Clin. Immunol. Immunopathol. 25:114, 1982), and the activity of BIF on spleen cells suggests that this factor may play a role in switching. BIF is about 18KD and is separable from IL2 on dye affinity columns and HPLC. IL1 and IL2 up to 100 U/ml have no BIF activity, and they do not affect optimal or suboptimal concentrations of IL2-free BIF in induction of Ig secretion. (IL1 is probably saturating in the assay due to endogenous production by monocytes.) However, three sources of recombinant IL2 and a pure, conventional cell line IL2 induce Ig secretion at 10(4) U/ml or higher. This concentration may not be physiological, but it suggests that the BIF receptor has a low affinity for IL2 and that BIF and IL2 may be homologous proteins.

0526 TRIGGERING OF A UNIQUE B CELL LYMPHOMA BY RECEPTOR: LIGAND INTERACTIONS, David W. Scott and Mark Ling, Duke Medical Center, Durham, N.C. and Immunology Unit, University of Rochester Cancer Center, Rochester, N.Y. 14642

We previously have described a spontaneous B cell lymphoma, called NBL, which originated in a Swiss nude mouse. This line has been adapted to in vitro culture and repeatedly subcloned. In the presence of either monoclonal or affinity purified heterologous anti-μ chain reagents, this cell line increases its rate of incorporation of tritiated thymidine into acid precipitable DNA. This effect occurs in the absence of serum or filler cells, but requires 2-ME and does not occur with anti-H-2 or anti-β_2 microglobulin reagents. Furthermore, NBL can increase both surface and cytoplasmic μ chain (including μ_S chain) synthesis in the presence of LPS or non-specific T cell factors. Thus, NBL can respond to extrinsic signals regulating its growth and differentiation. Since NBL binds murine leukemia viruses at or near its surface IgM receptors, we have investigated the effects of this virus: receptor interaction on the growth and differentiation of this unique B lymphoma line. The role of receptor cross-linking in this process will be presented.

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0527 MOLECULES CAUSING MATURATION OF RESTING B CELLS TO ACTIVE IG SECRETION, Charles L. Sidman, The Jackson Laboratory, Bar Harbor, ME 04609.

Molecules which induce the maturation of resting murine B cells to active immunoglobulin (Ig) secretion have been identified. These molecules cause high-rate Ig secretion which is measured by a polyclonal plaque assay, the amount of Ig secreted, or by specific alterations in particular stages of Ig metabolism which are characteristic of secreting B cells. Normal B cells of all thus-far-identified developmental stages, as well as resting B cell-like WEHI-279 tumor B cells, are affected by these molecules. The induction process is direct (ie. requiring no other participating cell types), rapid (maximal in 2-3 days), very efficient (at least half of the target B cells are triggered under optimal culture conditions), and not MHC- or antigen-restricted. At least three distinct molecules, one of which is gamma interferon (IFN-), are active in these assays. The other two, from the cloned helper T cell line S26.5 and the autoimmune mutant mouse called Viable Motheaten, are distinct from IFN- on the basis of not being bound or functionally inhibited by anti-IFN- antibodies, and by their biochemical characteristics. The relationships among these three direct B cell maturing lymphokines, as well as their biological relevance, will be discussed.

0528 B CELL GROWTH AND DIFFERENTIATION FACTORS PURIFIED AND CHARACTERIZED USING TUMOR B CELLS, Susan L. Swain and Richard W. Dutton, University of California, San Diego, La Jolla, California 92093

We have used in vivo derived BCL_1 tumor B cells as well as normal splenic B cells to detect, purify, and characterize B cell growth and differentiation factors. We have found that a growth promoting factor in EL4 supernatants, which we have designated (EL4)$BCGF_{II}$, has an apparent molecular weight of 55K, and an isoelectric point of 5.5, and can also be separated from other lymphokines (in particular, IL2 and $BCGF_I$ of Howard) by DEAE chromatography and phenylsepharose chromatography. (EL4)$BCGF_{II}$ causes rapid proliferation of BCL_1 tumor cells in vitro and can synergize with dextran sulfate in the proliferation of normal B cells. These properties contrast with those of $BCGF_I$, as described by Howard et al. which is 18K, has different chromatographic properties and does not score in the above B cell assays. Differentiation of BCL_1 tumor cells is further promoted by (DL)TRF which can be separated from IL1, IL2, IFN, and both BCGFs.

0529 EVIDENCE FOR A TRANSITIONAL, INTERLEUKIN-SENSITIVE STAGE IN ACTIVATION OF B CELL PROLIFERATION, Gayle D. Wetzel, Susan L. Swain and Richard W. Dutton, Univ. Cal. San Diego, La Jolla, Ca 92093

Activation of B lymphocyte proliferation was examined by cell cycle analysis. Low cell density cultures were used to minimize effects from contaminating cell types and flow cytofluorometry was used to assess cellular size, RNA and DNA contents. B lymphocytes responded to polyclonal activators by increasing their size and RNA content. Some B cells arrested at this early G_1 stage, called G_1A, while others continued through G_1 and initiated DNA replication. Kinetic studies indicated that G_1A cells remained in this stage unless additional proliferation stimuli were present. Hence G_1A appears to represent a transitional stage in B cell activation to proliferation where B cells are responsive to secondary signals. Since additional mitogens or T-cell-derived interleukin sources appeared to enhance the transition from G_1A to S phase, it is likely that B cell growth factors act at distinct points in the cell cycle. Support for this model was obtained from experiments where a B cell tumour, predominantly in G_1A stage, demonstrated clonal proliferation in response to BCGFs in a single cell assay. These results suggest that activation of B cell proliferation proceeds by several distinct stages in which distinct inductive signals are perceived.

0530 CHARACTERIZATION AND PURIFICATION OF B CELL-DERIVED BCDF, Kazuyuki Yoshizaki, Toshimasa Nakagawa, Kenichi Fukunaga, Ling Tsann Tseng and Tadamitsu Kishimoto, Osaka University Osaka, JAPAN.

A subclone of a human B lymphoblastoid cell line, CESS-2, spontaneously secreted a kind of BCDF(B-BCDF) in this culture supernatant without any stimulation. B-BCDF induced IgG and IgM secretions in human B lymphoblastoid cell lines, CESS cells and Cl-4 cells, respectively. BCDF-responsive CESS cells expressed IgG on their surface, while CESS-2, which were able to secrete B-BCDF, did not express surface IgG. B-BCDF could induce Igs-secretion in SAC-stimulated low density peripheral B cells, but did not induce Igs-secretion in non-stimulated B cells. B-BCDF did not show any IL2 BCGF, and γ-interferon activities. B-BCDF was highly purified by gel filtration on AcA-34 column, chromatofocusing and ion exchange chromatography on FPLC system and gel filtiation on HPLC column. Highly purified preparation showed a single protein band in SDS-PAGE analysis. The molecular weight and pH of the factor were 20,000 daltons and pH 5.1-5.2. The minimum protein amount required for Igs-induction in B cell lines was almost 16 ng/ml.

Recognition Elements Mediating T Cell Interactions: MHC, Idiotype, VH, CT, Antigen

0531 TWO SIGNALS INVOLVED IN THE EXPRESSION OF CYTOTOXIC T LYMPHOCYTES, Benjamin Bonavida and Hanna Ostergaard, UCLA School of Medicine, Los Angeles, CA 90024

The mechanism by which CTL recognize and lyse a target cell is not known. It has been shown that Class I and Class II antigens play a role in the initial interaction. However, the subsequent interaction(s) that takes place and leading to lysis has not been identified. To understand this interaction we used a system in which antigen recognition is bypassed following modification of target cells (TC) with sodium periodate (IO_4^-) in a system called oxidative-dependent cellular cytotoxicity (ODCC). The role of the periodate in ODCC may be due to either inter- or intra-crosslinking via Schiff base formation. Treatment of TC with IO_4^- and reduction with borohydride lead to no lysis, even in the presence of soybean agglutinin (SBA) (shown to provide binding but no lysis). However, treatment with IO_4^- at 37° for 1 hr followed by BH_4 or NH_2OH treatment and addition of SBA resulted in target cell lysis. The presence of SBA was necessary and other lectins such as peanut agglutinin and wheatgerm agglutinin also were effective. However, agglutination by poly-L-lysine was not effective. These results suggested that IO_4^- modification of TC may lead to intra-crosslinking and result in target structures that are important for lysis. Such target structures in addition to the agglutinating agent such as SBA are both involved in the expression of cytotoxicity. We conclude from these results that the expression of cytotoxicity is the result of two signals: 1) recognition of specific target cell antigens by specific receptors, and 2) new expression of target antigens important for lysis.

0532 CHARACTERIZATION OF SUPPRESSOR T-CELLS INDUCED BY NEONATAL IMMUNIZATION, Michael P. Cancro and Syamal Raychaudhuri, University of Pennsylvania, Phila., PA 19104

The relative frequency of different B cell clonotypes may be governed by several mechanisms. Genetic control over the initial appearance of primary B cell clonotypes has been demonstrated, but antigen-driven events also play a large role in shaping an individual's ultimate clonal profile. A strong relationship between these two general categories of repertoire modulation was recently demonstrated, using the influenza hemagglutinin (HA) as a model antigen. These studies showed that the HA-specific B cell pool exhibits regular patterns of turnover in clonotype composition during early development, which may be altered by antigen exposure. Specifically, antigen exposure preserves the expression of clonotypes extant at the time of challenge, and suppresses the expression of clonotypes which normally arise subsequently. This induced oligoclonal dominance was shown to be due in part to suppressor T-lymphocytes which prevent primary but not secondary HA-specific humoral responses in vivo.

We have herein examined the properties of these induced T_S populations more closely. The results indicate that: a) The T_S act at the level of T_H cells rather than directly upon primary B cells; and b) the T_S activity is restricted by genetic elements which are linked to but not within the immunoglobulin heavy chain allotype locus.

0533 GENETIC RESTRICTION OF THE ANTIGEN-SPECIFIC ANTIBODY RESPONSE TO IMMUNE COMPLEXES, Michael J. Caulfield, Cleveland Clinic Foundation, Cleveland, OH 44106.

Antigen/antibody complexes were prepared between the TEPC-15 myeloma protein and the cell wall polysaccharide (PnC) extracted from Streptococcus pneumoniae R36a. Complexes prepared in antigen excess, antibody excess, and at equivalence were compared for the ability to stimulate antigen (PnC)-specific plaque-forming cells (PFC) in BALB/c mice. In addition, the number of T15 idiotype-positive cells was determined by plaque-inhibition with a monoclonal antibody (AB1-2) that is specific for a T15 idiotope (Id). The results showed that complexes formed in antibody excess were not immunogenic whereas the response to complexes formed in antigen excess was as high or higher than the response to an optimal dose of free antigen. The PnC-specific response to complexes formed at equivalence was somewhat variable, but it was generally lower than the response to free antigen. The AB1-2 Id was expressed on 94% of PFC from mice (n=20) immunized with immune complexes whereas the AB1-2 Id was was expressed to a lower degree (75%) in mice (n=21) immunized with free PnC. In further experiments, C3H mice, which do not express the AB1-2 Id, were found to be completeley unresponsive to complexes formed at equivalence and were low responders to complexes formed in antigen excess whereas their response to free PnC was equivalent to that of BALB/c mice. On the other hand, C57BL/6 mice which express the AB1-2 Id to a varying degree did respond to TEPC-15/PnC complexes, although their response was lower than that of BALB/c mice. Taken together, these results show that the antigen-specific response to TEPC-15/PnC complexes is genetically restricted and suggest that the response may be dominated by idiotopes expressed in the immune complex.

0534 T CELL RESPONSE TO AN ANTI-IDIOTYPIC MONOCLONAL ANTIBODY IN ALLOGENEIC SJL/J AND SYNGENEIC BALB/c MICE, Timothy J. Gorzynski, Christopher J. Krco, Zuhair Atassi and Chella S. David, Mayo Clinic, Rochester, Minnesota 55905

Previously, we have been successful in producing a number of monoclonal antibodies (MoAb) reactive with hemoglobin. One of these MoAb's, Hb-2d, of B10.D2 origin, is unique in that it reacts specifically with the β chain of human hemoglobin (HuHb) and not with the α chain or with hemoglobin molecules of a variety of other species. To better characterize this MoAb the production of monoclonal anti-idiotypic antibodies was begun. One Moα-IdAb, HId-2a, of BALB/c origin is highly reactive with Hb-2d and appears to detect an idiotype by all criteria thus far tested. Recently, we have begun to use HId-2a in an attempt to manipulate the immune response of SJL/J and BALB/c mice to HuHb. Mice were primed at the base of the tail with 100 μg HId-2a in Freund's complete adjuvant. 7 days later draining lymph node cells were tested for their ability to proliferate in vitro in the presence or absence of HId-2a. Surprisingly, the degree of proliferation observed, as measured by tritiated thymidine incorporation, was both high and specific for HId-2a in both strains of mice suggesting a response to idiotypic determinants. In addition these same lymph node cells were able to proliferate in the presence of HuHb suggesting a possible role for this anti-idiotypic reagent in modulating the response to this antigen. Presently a long term line of SJL/J cells is being maintained. The response of these cells to HId-2a is MHC restricted in that proliferation is observed in the presence of feeder cells from $H\text{-}2^{s}$ but not $H\text{-}2^{d,k,b,p}$ haplotype animals. In addition these cells are strongly Thy-1+ and Lyt-1+ as determined by FACS analysis. The production of cloned cell populations and further characterization is underway.

0535 MAPPING OF Ir GENE THAT GOVERNS T HELPER CELL RESPONSE TO VARIABLE DOMAIN OF LIGHT CHAIN OF MYELOMA PROTEIN 315, Trond Jørgensen and Kristian Hannestad, Institute of Medical Biology, University of Tromsø, Norway

A basic assumption of Jerne's network theory is that the immune system of an individual can recognize its own antigen receptors. It is therefore of interes to study the fine specificity and regulation of immune responses to variable(V) domains of homogeneous immunoglobulins. Previous studies from this laboratory have indicated that T helper cell (Th) responses to the V domains of the isologous myeloma protein 315 are regulated by immune response (Ir) genes. The k haplotype of the H-2 complex conferred high responsiveness to the V domain of the heavy chain (V_H-315) and low responsiveness to V_L-315. Conversely, the d haplotype conferred high responsiveness to V_L-315 and low responsiveness to V_H-315. The V_H Ir gene mapped to the K-A interval of the H-2 complex.(J. exp. Med. 1982, 155:1587). The present experiments were carried out to determine the map position of the V_L Ir gene. Mice of the A.TH strain ($K^{s}A^{s}E^{k}D^{d}$) were high responders to V_L. In contrast, A.TL strain animals ($K^{s}A^{s}E^{k}D^{d}$) were low responders to V_L. These results indicate that the Ir gene governing the Th response to V_L-315 maps to the A subregion of the s haplotype.

0536 A CLONED BALB/c T CELL LINE SPECIFIC FOR GAT + H-$2K^{d}$ CROSSREACTS WITH $Mls^{a,d}$. M.K. Jenkins, C. Waltenbaugh and S.D. Miller, Northwestern Univ. Med. School, Chicago, IL 60611.

A BALB/c T cell line specific for GAT + I-A^{d} was cloned by limiting dilution in microtiter plates containing syngeneic irradiated spleen cells, GAT, and 20% Con A supernatant. Two-three weeks later growth was detected visually in 6 of 96 wells. Clones were expanded and assayed for antigen-specific, H-2 restricted proliferative responses. One of these clones, JTL-2-G12, differs from its sister clones and the parental line as it appears to be restricted by GAT + K^{d} and not GAT + I-A^{d}. This conclusion is based on the observation that B10.LG and B10.GSR spleen cells effectively presented GAT to the JTL-2-G12 clone but not to the parental line. B10.LG and B10.GSR share only the K^{d} allele with BALB/c. Due to the uncertain origin of the LG and GSR strains we are now confirming the K^{d} restriction pattern indicated in the genetic studies by blocking JTL-2-G12 proliferation with anti-K^{d} and anti-Ia^{d} allo- and monoclonal antibodies. JTL-2-G12 is also stimulated in the absence of GAT by irradiated spleen cells carrying the Mls^{a} or Mls^{d} haplotypes. Thus, spleen cells from CBA/J ($H\text{-}2^{k}$, Mls^{d}) stimulate strong JTL-2-G12 proliferative responses while cells from CBA/N ($H\text{-}2^{k}$, Mls^{null}) do not. JTL-2-G12 is a novel cell in that it is reactive to both soluble antigen and Mls and appears to be restricted by Class I determinants. This clone should be a valuable tool in determining:(a) the relationship between the antigen and Mls T cell receptors; (b) the role of Class I restricted cells in the immune response to soluble antigens. (Supported by USPHS NIH Grant AI-18755)

0537 THE ROLE OF REGULATORY T CELLS IN THE GENETIC CONTROL OF IMMUNE RESPONSES TO INSULIN. Peter E. Jensen and Judith A. Kapp, Jewish Hospital of St. Louis, St. Louis, MO 63110.

The immune response to insulin in mice is an all-or-none response that is controlled by H-2 linked Ir genes. Nevertheless, the antibodies stimulated by one variant of insulin are largely cross reactive with other variants including those that fail to stimulate antibodies themselves. Strains bearing the H-2^b haplotype respond to beef and sheep, but not pork, insulin; whereas, H-2^k mice respond to sheep, but not beef or pork, insulin. Previously, we demonstrated that lymph node T cells from pork insulin-primed H-2^b mice could provide help in a secondary antibody response to beef or sheep insulin in adoptive recipients. However, non-responder forms of insulin did not prime helper T cells in H-2^k mice suggesting that nonresponsiveness in different haplotypes may involve interactions among different regulatory T cell subsets. Recently we have confirmed these observations in secondary antibody responses to insulin in vitro. In addition, we have observed that irradiation of pork insulin-primed T cells from H-2^b mice uncovers helper T cells that can respond to pork as well as beef insulin suggesting that unresponsiveness to pork insulin involves a radiosensitive suppressor T cell. In fact, removal of Ly 1^-2^+ T cells from pork insulin-primed T cells also permitted responsiveness to pork insulin and reintroduction of the Ly 1^-2^+ T cells inhibited the response to pork, but not beef insulin. Preliminary studies suggest that specific suppressor T cells may be involved in the maintenance of self-tolerance to insulin.

0538 ROLE OF HELPER AND SUPPRESSOR T CELLS IN THE DEVELOPMENT AND EXPRESSION OF MHC RESTRICTED ANTIBODY RESPONSES TO THE RANDOM TERPOLYMER GAT. Jeffrey P. Lake, Judith A. Kapp and Carl W. Pierce, Jewish Hospital of St. Louis, St. Louis, MO 63110.

Spleen cells from (Responder x Responder;B10 x B10.D2)F_1 mice which have been immunized with L-glutamic $acid^{60}$-L-$alanine^{30}$-L-$tyrosine^{10}$(GAT)-pulsed parental macrophages (MØ) generate in vitro GAT-specific plaque forming cell (PFC) responses when stimulated by the GAT-MØ used for in vivo immunization, but not when stimulated by the other parental GAT-MØ. The lack of response to the other parental GAT-MØ was shown to be due to antigen-specific suppressor T cells which suppressed primary but not secondary responses. Although the observed genetic restriction appeared to be due to the activity of suppressor T cells, it was not clear if the genetic restriction was due solely to suppression or whether helper T cells were also restricted. To examine this question, T cells were separated into subpopulations based on cell surface markers. Radiation resistant, Lyt 1^+, I-J^{b-}, helper T cells from (B10 x B10.D2)F_1 mice immunized with B10 GAT-MØ and cultured with virgin F_1 B cells generated responses when stimulated by B10 GAT-MØ but not by B10.D2 GAT-MØ. By contrast, Lyt 1^+, I-J^{b+}, suppressor inducer T cells suppressed the response of virgin spleen cells stimulated by both B10 and B10.D2 GAT-MØs. These results demonstrate that immunization of (Responder x Responder)F_1 mice with parental GAT-MØ results in the development of antigen specific helper and suppressor T cells. The T helper cells are genetically restricted while the T suppressor cells are not. Nevertheless, because the suppressor T cells suppress primary responses but not secondary responses, they contribute to the genetic restriction observed in the secondary in vitro GAT-specific antibody response.

0539 T CELL RECOGNITION OF Mls PRODUCTS IS RESTRICTED BY CROSS REACTIVE MHC DETERMINANTS. D. H. Lynch, R. E. Gress, S. A. Rosenberg and R. J. Hodes, NIH, Bethesda, MD. 20205

The question of whether Mls-reactive T cells recognize Mls-encoded products in association with MHC determinants was approached by assessing the in vitro proliferative responses of cloned Mls-specific T cell lines to Mls-determinants expressed in the context of various MHC haplotypes. Lyt 1^+2^- T cells of C57BL/6 (H-2^b, Mls^b) origin were stimulated with DBA/2 (H-2^d, Mls^a) lymphoid cells and cloned by limiting dilution. The resulting cloned T cell lines were selected on the basis of positive proliferative reactivity when stimulated by DBA/2 lymphoid cells, but not B10.D2 (H-2^d, Mls^b) lymphoid cells. Potential Mls^a reactive clones were further screened using stimulator cells from the BXD recombinant inbred mouse strains. The resulting Mls-specific T cells lines were found to be optimally stimulated by H-2^d, Mls^a lymphoid cells, were stimulated to a lesser extent by H-2^b, Mls^a lymphoid cells, and were not detectably stimulated by Mls^b lymphoid cells, regardless of their H-2 haplotype. The reactivity pattern of these clones was further explored using stimulator cells from DBA/1 (H-2^q, Mls^a), Dl.C (H-2^d, Mls^a) and Dl.LP (H-2^b, Mls^a) mice. As with the BXD stimulators the level of stimulation by Dl.C cells appeared greater than by Dl.LP cells. DBA/1 cells, however, did not induce proliferative responses in these same cloned T cells. This appears to be a result of MHC-restriction of stimulation since (B10.D2 x DBA/1)F_1 stimulator cells did induce prolifative responses in these cloned T cell lines. Thus, although the frequency and magnitude of responses to Mls products appears similar to those induced by class II MHC determinants, the data presented support the concept that Mls products may be recognized in the context of crossreactive MHC determinants that are shared by some, but not all, mouse strains.

0540 THE ROLE OF Igh-LINKED MOLECULES IN REGULATORY T CELL INTERACTIONS. John G. Monroe, Adam Lowy, Jeffrey A. Drebin and Mark I. Greene. Department of Pathology, Harvard Medical School, Boston, MA.

We have begun studies designed to clarify the nature of immunoglobulin heavy chain allotype-linked restriction of regulatory T cell interactions. The system utilized is the immune response to the azobenzenearsonate (ABA) hapten. The majority of the humoral response to ABA in mice with the Igh-1^e or Igh-1^d genotype express a major crossreactive idiotype (CRI). Antibodies to this idiotype (anti-CRI), when injected intraveneously, are able to suppress the T cell response to ABA as measured by in vivo delayed type hypersensitivity (DTH) and in vitro cellular cytotoxicity (CTL). Recent results demonstrate that i.v. priming of A/J mice with anti-CRI induces an efferent acting, transferrable suppressor cell which, when transferred into antigen primed mice on the day before challenge, are able to suppress the in vivo DTH and subsequent in vitro CTL response. Data indicates that this suppression can be mediated by a soluble factor which suppresses in an antigen nonspecific manner. Interestingly however, while antigen nonspecific this factor is Igh restricted in its suppressor activity. These data suggest that structures on regulatory T cells linked to Igh genes may serve as restriction elements which may not be involved with antigen binding.

0541 CHARACTERIZATION OF T CELL SUBSETS IN (RESPONDER X NONRESPONDER)F_1 MICE WHICH REGULATE RESPONSES TO L-GLUTAMIC $ACID^{60}$-L-$ALANINE^{30}$-L-$TYROSINE^{10}$(GAT). Carl W. Pierce, Craig M. Sorensen and Judith A. Kapp. Jewish Hospital of St. Louis, St. Louis, MO 63110.
After immunization of (Responder x Nonresponder: B10 x B10.Q)F_1 mice with soluble GAT, spleen cells develop PFC responses when stimulated with GAT-pulsed B10, but not B10.Q, macrophages(MØ) This failure to respond is due to a suppressor mechanism involving two distinct T cells. The suppressor inducer cell is a primed, radiosensitive Lyt 1^+2^-, $I\text{-}A^-$, $I\text{-}J^+$, Qa 1^+ cell. The suppressor effector cell need not be primed and is a radiosensitive Lyt $1\text{-}2^+$, $I\text{-}A^-$, $I\text{-}J^+$, Qa 1^+ Cell. When this suppressor mechanism is removed, radioresistant, MHC-restricted, Lyt 1^+2^-, $I\text{-}A^+$, $I\text{-}J^-$, Qa 1^- helper T cells specific for B10 and B10.Q GAT-MØ are demonstrated. These T cell subsets have been characterized further using monoclonal BALB/c anti-C.B20 reagents (1322 A2.1, B3.2, C5.3) reactive only with T cells, and monoclonal reagents reactive with: 1) a single-chain GAT-specific suppressor T cell factor - A4.10; 2) the $I\text{-}J^+$ chain of a two-chain GAT-specific suppressor T cell factor - B3.4; and 3) the antigen-binding chain of a two-chain GAT-specific suppressor T cell factor-B2.2. None of these reagents react with helper T cells. However, suppressor inducer T cells react with A4.10, 1322 A2.1 and B3.2, but not B3.4, B2.2 or 1322 C5.3. Suppressor effector cells fail to react with A4.10 and 1322 A2.1 and B3.2, but react with B3.4 and B2.2 and 1322 C5.3. The 1322 A2.1 and B3.2 reagents react only with the single-chain suppressor factor, whereas 1322 C5.3 reacts only with the antigen-binding chain of the two-chain factor. Thus, A4.10, 1322 A2.1 and B3.2 appear to react only with suppressor inducer T cells and may define a "Tind" marker, whereas B3.4, B2.2 and 1322 C5.3 appear to react only with suppressor effector T cells and may define a "Tsu" marker.

0542 MHC RESTRICTION(S) OF ANTIGEN SPECIFIC INDUCTION OF LYMPHOKINES, PROLIFERATION AND CELL MEDIATED KILLING BY T CELL CLONES, Marianne B. Powell, Barbara S. Conta and Nancy H. Ruddle, Yale University, New Haven, CT 06510.
T cell clones of different Ly phenotypes were compared for their MHC restriction(s) of induction of lymphokine production, proliferation and cell-mediated killing. Ly 1^+ 2^- and Ly 1^- 2^+ murine T cell clones that produce lymphotoxin (LT) and/or gamma interferon (IFN-γ) were isolated by limiting dilution and maintained in T cell growth factor (TCGF). The Ly 1^+ clone was derived from C57BL/6 mice sensitized by subcutaneously injecting the soluble antigen, 4-hydroxy-3 nitrophenyl chicken γ-globulin (NP-CGG) in complete Freund's adjuvant. Both proliferation and production of IFN-γ by the Ly 1^+clone (82F12) are antigen specific (CGG) and MHC restricted. The MHC restriction, mapped to the I-A subregion, was determined using H-2 recombinant mice and monoclonal antibodies (mAb). Proliferation and IFN-γ release are blocked by a mAb to $I\text{-}A^b$ ($A_\alpha A_\beta{}^b$) and by GK1.5, a mAb recognizing L3T4 determinants. The Ly 2^+ clones and Ly 2^+ enriched T cell lines were isolated from draining lymph nodes of CBA/J mice sensitized by skin painting with picryl chloride (PCl). The Ly 2^+ clone and Ly 2^+ enriched T cell lines proliferate and produce LT and IFN-γ in response to trinitrophenyl coupled syngeneic spleen cells (TNP-SC). Their lymphokine production is restricted by class I determinants K^k. The Ly 2^+ lymphokine-producing cells kill MHC-matched TNP-modified fibroblast target cells (A9) in 5 hrs, a time course similar to CTL killing. Culture supernatants from these Ly 2^+ cells stimulated with antigen contain LT, which kills modified or unmodified fibroblasts after 24-48 hrs. These T cell lines and clones should permit clarification of the relationship between LT and CTL-mediated killing.

0543 B CELL DEFICIENT MICE: DEFECTIVE T CELL PROLIFERATIVE RESPONSES RESTORED BY NAIVE B CELL IN VIVO. Yacov Ron, Dept. of Pathology, Yale U. Med. School, New Haven, CT 06510

We have previously shown that mice continuously suppressed from birth with rabbit anti mouse IgM(μ) antibodies are unable to generate antigen specific proliferating T cells when challenged with a variety of antigens emulsified in CFA in the foot pad. Recently, I found this to be true for T cell proliferation induced by picryl chloride skin painting, although the delayed type hypersensitivity response was intact. Others have shown that these B cell deprived animals also lack a subpopulation of helper T cells, namely the helpers for high affinity antibody production and the idiotype specific helpers, as well as contrasuppressor T cells. In the present study I show that the impaired proliferative response is not due to the lack of circulating antibody. Administration of specific antisera using several injection protocols failed to restore the impaired proliferative response. Mixtures of naive splenic and lymph node T cells injected either systemically or locally also failed to restore the proliferative function. On the other hand, a mixture of 10^6 splenic and lymphnode naive B cells (anti thy 1.1 and complement treated cells positively selected on anti μ coated plates) injected locally into the challenged footpad completely restored the proliferative response. Interestingly, the spleen of the reconstituted animals had a very significant rise inthe number of detectable Ig^+ cells. A much larger number ($30\text{-}50x10^6$) B cells administered systemically had no beneficial effect. These results suggest that anti μ treated mice contain T cells that are capable of proliferating but they lack B cells required for the priming of proliferating T cells.

0544 COMPLEX FINE SPECIFICITY OF HUMAN "SB2-SPECIFIC" CYTOTOXIC T CELLS REVEALED BY ANALYSIS WITH DR-NULL MUTANT LYMPHOBLASTOID B CELL LINES. Miguel Sanchez-Perez, William E. Biddison, Robert DeMars, Stephen Shaw, NIH, Bethesda, MD 20205

Ten human cytotoxic T cell (CTL) clones were obtained which appeared to be specific for SB2 when analyzed by traditional approaches: their proliferative responses to panels of unrelated donors correlated with the presence of the SB2 allele and their CTL activity was inhibited only by monoclonal antibodies which bound to SB2-region determinants. However, testing on panels of HLA-mutant cell lines revealed that only six of the CTL clones had the expected specificity -- namely the ability to kill all B cell mutants which retained expression of the SB2 molecule but not to kill SB2-null mutants. One extreme CTL clone (8.3) was able to kill only a subset of the SB2-positive DR-null variants. Which mutants fell in this subset recognized by 8.3 did not correlate with the only known phenotypic variation among them (expression of the DC molecule) nor could it be explained by quantitative differences in their SB2 expression. The other three CTL clones lysed the mutant lines in a manner intermediate between these two extreme groups of CTL; they lysed all SB2-positive mutants (unlike CTL 8.3) but they lysed the 8.3-susceptible subset of mutants better than the other mutants. This heterogeneity of CTL specificity was confirmed by cold target inhibition using the mutant cell lines as blockers. These data suggest that some CTL clones recognize the SB2 molecule in association with other HLA-D region products; biochemical and molecular genetic analyses are being undertaken to identify the nature of these structures.

0545 PRIMED (SJLxPL)F1 T-CELLS FAIL TO RESPOND TO ANTIGEN IN CONTEXT OF THE SJL HAPLOTYPE M.J. Skeen, H.K. Ziegler, C-H.J. Chou, and R.B. Fritz, Emory Univ., Atlanta, Ga.30322

Injection of myelin basic protein (MBP) with appropriate adjuvants into susceptible strains of mice induces experimental allergic encephalomyelitis (EAE), a $Lyt1^+2^-$ T cell mediated autoimmune disease. SJL/J($H\text{-}2^s$) mice respond to an encephalitogenic determinant in the C-terminal half of guinea pig MBP, while the major encephalitogenic determinant for PL/J($H\text{-}2^u$) mice is within the N-terminal 20 amino acids. The C-terminal half of MBP is only weakly encephalitogenic in PL/J mice. Surprisingly, the two specificities are not expressed co-dominantly in (SJLxPL)F1 mice; as in the PL/J parent, the N terminus is the major encephalitogen in F1 mice. Specificity of T cell reactivity was examined <u>in vitro</u> using an assay for genetically-restricted macrophage-dependent IL-2 production. Although primed F1 T cells responded to MBP or MBP peptides in the context of either PL/J or F1 macrophages, they failed to respond if those antigens were presented by SJL/J macrophages. Functional integrity of the SJL/J macrophages was demonstrated by their ability to present antigen to primed SJL/J T cells. These results are consistent with the <u>in vivo</u> specificity of EAE induction. PPD or ovalbumin-primed F1 T cells also failed to respond when cultured with SJL/J macrophages and antigen, however, indicating that this effect is not limited to MBP. Explanations of these findings include a) a failure of priming on the $H\text{-}2^s$ haplotype, b) failure to express $H\text{-}2^s$ I region antigens in F1 cells, c) generation of haplotype-specific suppressor cells or d) lack of expression of the receptor for $I\text{-}A^s$ on the F1 T cell. (Supported by USPHS grant NS10721.)

0546 ORIGIN AND SPECIFICITY OF AUTOREACTIVE HELPER T CELLS IN ANTIGEN-INDUCED POPULATIONS, Maurice Zauderer, David R. Johnson and Denise A. Faherty, Columbia University, New York, N.Y. 10027.

Autoreactive T cells are induced in the course of a normal immune response to randomly chosen antigens. We have previously reported (JMCI 1 #2) that following activation by MHC-syngeneic stimulators clones of such T cells provide a non-specific helper function that serves to enhance proliferation and maturation to immunoglobulin secretion of activated B cells. Optimal induction of autoreactive T cells in vitro is greatly facilitated by an ongoing secondary antigen-specific response. To further investigate the relationship between antigen-specific and autoreactive T cells we have characterized the MHC-specificity of autoreactive T cells induced in secondary cultures of GLϕ primed I-E subregion restricted or KLH primed I-A subregion restricted T cells. Our results indicate that I-E as well as I-A-specific autoreactive T cells are induced following stimulation with either GLϕ or KLH. This suggests that autoreactive T cells are not the immediate progeny of antigen-stimulated precursors.

Congenital and Acquired Immunodeficiency

0547 CORRELATION OF AN X-LINKED GENE WITH THE MURINE IMMUNODEFICIENCY STATE, XID. David I. Cohen, Alfred Steinberg, Stephen Hedrick, William E. Paul, and Mark Davis* Laboratory of Immunology, NIAID, NIH, Bethesda, Md.; Dept. of Med. Micro., Stanford*

A large number of human and murine X-linked mutations have been described which influence lymphocyte development. We are pursuing the isolation of genes which correspond to these mutations. We have isolated a gene, which appears to be a member of an X-linked gene family, with two interesting correlations to the murine X-linked immunodeficiency state, xid. First, utilizing xid-congenic strains of mice, which have inherited only a small percentage of their X chromosome from the xid background, we have located restriction-site polymorphisms which establish that this gene is genetically-linked to the xid trait. Second, utilizing Northern RNA analysis, we have demonstrated that this family is transcribed in presecretory B cell tumors and in plasmacytomas, both of which are mature cells which lack the B cell surface determinant, 14G8, but it is not transcribed in immature B cell tumors, which express the 14G8 determinant. It is also transcribed in certain T cell tumors, but not in liver or kidney cells. Mice bearing the xid defect have an abnormally high percentage of immature B cells expressing the 14G8 determinant; this observation is consistent with the interpretation that activation of a gene on the X chromosome, which is defective in the xid strain, may contribute to terminating expression of the 14G8 determinant on (some) mature B cells. We are currently attempting to define what role this putative gene family might play in lymphocyte development, and whether any member is mutated in xid mice.

0548 AUGMENTATION OF THE IN VIVO GENERATION OF CYTOTOXIC ACTIVITY IN IMMUNOCOMPROMISED MODEL SYSTEMS BY INTERLEUKIN 2. P.J. Conlon, S. Hefeneider, A. Alpert, T. Washkewitz, and S. Gillis.

Interleukin 2 has previously been shown to be obligatory in the generation and prolongation of numerous T lymphocyte effector functions in vitro. Moreover, this important immunoregulatory molecule has been shown to enhance the generation of alloreactive cytotoxic T lymphocytes as well as resident natural killer cell activity in normal murine systems. To further establish the efficacy of this lymphokine, its ability to restore responsiveness in immunoincompetent recipients was investigated. Two immunocompromised model systems investigated the ability of IL-2 to augment the in vivo generation of alloreactive cytotoxic T lymphocytes. In these systems IL-2 derived from both murine and human T-cell lines, as well as recombinant material, was capable of restoring immune function. In the first system, cyclophosphamide treatment concomitant with the allogeneic tumor cell immunization completely ablated the in vivo generation of cytotoxic T lymphocytes unless interleukin 2 therapy was administered. In the second system, corticosteroid pre-treated animals were able to respond to allogeneic tumor cells, but only if IL-2 therapy were included in the regimen. These model systems support the contention that interleukin 2 therapy may be efficacious in overcoming immune dysfunction in immunocompromised patients.

0549 PREFERENTIAL EXPRESSION OF THE "NORMAL" X-CHROMOSOME IN B CELLS OF MICE HETEROZYGOUS FOR THE xid IMMUNE DEFECT, Tommy C. Douglas, Patti E. Dawson and Suzanne M. Cox, Univ. of Texas Grad. Sch. of Biomed. Sci. and Baylor Coll. of Med., Houston, TX 77025

Functional data have suggested that the "recessive" X-linked immunodeficiency gene xid affects predominantly B lymphocytes. As a result of X-chromosome dosage compensation, each individual cell of a female heterozygous for xid should express either the xid-bearing chromosome or its + allele-bearing homolog. We have used an electrophoretic variant of the X-linked isozyme phosphoglycerate kinase (PGK-1) to assess X-chromosome expression in females of the genetic constitution: xid,Pgk-1^b/+,Pgk-1^a. The total nucleated spleen cell populations of these mice expressed approximately 75% PGK-1A and 25% PGK-1B. When purified populations of T cells and B cells were prepared from individual mice by plastic adherence, "panning" on anti-mouse Ig antibody-coated plates, and treatment with anti-Thy-1 antibody plus complement, T cells showed a more balanced expression of the two Pgk-1 alleles (53% PGK-1A), whereas B cells expressed almost exclusively Pgk-1^a (93% PGK-1A). This result indicates that xid: 1)affects B cells differentially, and 2)acts autonomously in individual B cells rather than systemically. Similar results have been reported recently by Nahm et al. (J. Exp. Med. 158:920). We do not yet know whether the biased pattern of X-chromosome expression seen in B cells is due to preferential X-inactivation or to cell selection; it is as pronounced in aged females as in young ones. These results suggest that studies on purified lymphocyte populations may be useful in identifying human females who are heterozygous for X-linked "recessive" immunodeficiency genes.

0550 GROWTH FACTOR DEFICIENCY ASSOCIATED WITH THE T CELL DEFICIENCY IN HODGKIN'S DISEASE, Richard J. Ford, Jerry Tsao, and Shashi Mehta, Department of Pathology, University of Texas, M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77030

Patients with Hodgkin's disease (HD) have long been known to be deficient in their cellular immune responses, which may be responsible for the morbidity and ultimately the mortality associated with HD. This deficiency has been shown to mainly involve T cell responses with anergy to skin test recall antigens, depressed responses to T cell mitogens (PHA and Con A), and deficient mixed lymphocyte culture (MLC) responses. Several types of suppressor mechanisms have been invoked as the cause of the T cell deficiency but their existance remains controversial. The discovery of lymphoid growth factors (interleukins) and their role in the immunoregulation of T cell responses has provided a framework for the dissection of T cell immunodeficiencies. We have studied 14 patients with untreated nodular sclerosing HD to assess their peripheral blood mononuclear cell (MNC) growth factor responses. In patients with skin test anergy, Interleukin 1 (IL-1) production and the response of patients' T cells to exogenous Interleukin 2 (IL-2, TCGF) in vitro were normal when compared with age/sex matched controls. However, the ability of HD patients MNC to generate IL-2 after lectin stimulation was markedly impaired. Identification of an apparent site of growth factor deficiency within the T cell system of HD patients, suggests that an intrinsic cellular functional defect may explain the immunologically hyporesponsive state observed in these patients. If the cause of the IL-2 deficiency can be identified, it may be possible to reverse the deficiency by various strategies of biological response modification. Supported in part by NIH grant CA-31479.

0551 ACQUIRED IMMUNODEFICIENCY IN STREPTOZOTOCIN (STZ)-INDUCED MURINE DIABETES MELLITUS: CORRECTION BY ISLET TRANSPLANTATION. Barry S. Handwerger, Gabriel Fernances, Connie Clark and David M. Brown, Rochester, MN, San Antonio, TX and Minneapolis, MN.

We have investigated immunological function in C57BL/6 mice rendered diabetic by a single i.p. injection of 125-200 mg/kg of STZ. Immunological studies were performed in mice diabetic (blood sugars >300 mg/dl) for a minimum of 4 weeks. Spleen cells from STZ-diabetic mice exhibited markedly diminished plaque forming cell (PFC) responses following in vivo sensitization with the T-dependent antigen, sheep erythrocytes, and the T-independent antigen, pneumococcal polysaccharide SIII, markedly impaired cytotoxic T cell responses following in vivo immunization with allogeneic tumor cells, and diminished delayed hypersensitivity responses following in vivo sensitization with methylated bovine serum albumin. In vitro immunological function of spleen cells from STZ diabetic mice also was abnormal, as manifested by diminished in vitro primary IgM PFC responses to SRBC, defective in vitro generation of cytotoxic cells, and diminished blastogenic responses to the T cell mitogens, phytohemagglutinin and concanavalin A. In contrast, the mitogenic response of diabetic spleen cells to lipopolysaccaride, a B cell mitogen, was normal. To evaluate the role of diabetes in this acquired immunodeficiency, STZ-diabetic mice were transplanted i.p. with syngeneic collagenase-digested neonatal pancreases. In contrast to their non-transplanted diabetic littermates, the transplanted mice (studied 1 month after the onset of normoglycemia) exhibited normal in vivo PFC and cytotoxic cell responses. The abnormalities in immunological function of STZ-diabetic mice, therefore, appear to be a direct consequence of the diabetic state.

0552 RESCUE OF IgM, IgG and IgA PRODUCTION IN COMMON VARIED IMMUNODEFICIENCY (CVI) BY T-CELL INDEPENDENT (TI) STIMULATION WITH EPSTEIN-BARR VIRUS (EBV), Gajin Jeong and Peter Ralph, Sloan-Kettering Institute, Rye, NY 10580

We previously defined categories of B-cell defects in CVI by failure to produce IgG or IgA in response to T-cell-dependent (TD) stimulation by pokeweed mitogen (PWM), Staphylococcus bacteria (Sac) plus PWM, or Sac plus B-cell inducing factor (BIF)(Proc.Natl.Acad.Sci. 79:6008, 1982). Some patients also had defects in TD-production of IgM as well, and some failed even to show B-cell proliferative responses to Sac or anti-Ig antibodies. The present study concerns the response of 21 patients to TI EBV. Most patient B cells showed normal proliferative and IgM responses to EBV. IgG and IgA production was also in the range of that for normal cells in many patients. T suppressor cells of EBV-induced Ig secretion were especially active in some patients, requiring removal of T cells to detect any Ig secretion. Among 12 patients with no TD-production of Ig of any isotype, two showed normal IgM secretion in response to EBV and six others had significant but subnormal responses in all three isotypes. Humoral responses were never detected in the final four patients despite the presence of near normal numbers of B cells, repeated testing, and removal of potentially suppressing T cells and monocytes. These results show that many cases of B-cell detects in CVI involving TD production of IgM, switching to TD production of IgG and IgA, and mitogen responses to anti-Ig and Sac are not absolute defects. The B cells will respond to some stimuli like normals. This raises the hope that suitable therapy may restore humoral immunity in these patients.

0553 *THE V_{kiiib} LIGHT CHAIN SUB-SUBGROUP: REGULATION OF EXPRESSION, Jan A. Moynihan, Julia L. Greenstein, and George N. Abraham, University of Rochester, Rochester, NY 14642*

Many human autoantibodies are preferentially of the V_{kiiib} light chain variable region subgroup. Examples are the IgM cold agglutinins and rheumatoid factors which are cryoglobulins. This rather striking finding of nonrandom association is as yet unexplained. Thus, we are examining the percent of k-containing Ig bearing the V_{kiiib} light chain in normal and disease states; and are studying the mechanisms of regulation of this variable region subgroup.

The percent of k chain-containing Ig bearing V_{kiiib} was determined by ELISA or competitive RIA using a murine monoclonal anti-Vkiiib (%Vkiiib=Vkiiib/k x 100). In normal subjects we find an average of about 10% in IgM, but we can detect no gamma heavy chain associated Vkiiib using methods which can detect less than 0.2% of a V_{kiiib} bearing IgG myeloma. We are currently examining the role of T cell regulation as well as other possible mechanisms for this phenomenon.

In our studies with disease states, we have found the Vkiiib sub-subgroup to be greatly increased in patients with acquired idiopathic hypogammaglobulinemia (70%) as well as some patients with hypogammaglobulinemia resulting from drug-induced immunosuppression (about 40%). This result implies that the V_{kiiib} bearing Ig is regulated in a fashion distinct from other k chain variable region subgroups.

These findings invite further study relating to the regulation of the immune system.

0554 INVESTIGATION INTO THE MOLECULAR BASIS OF THE BARE LYMPHOCYTE SYNDROME. B.Matija Peterlin, John D. Stobo, and Kathleen E. Sullivan, UCSF, San Francisco, CA, 94143.

The bare lymphocyte syndrome is defined by a profound lack of HLA-class I antigens on the surfaces of lymphocytes. The patients are characterized by combined immunodeficiency, frequent infections, and early death. We have described a family, where two sibs have a form of the syndrome. Their peripheral T cells but not their B cells show a complete absence of cell surface class I molecules as determined by immunofluorescence and FACS using anti-HLA-A, B, and C and anti-beta 2 microglobulin monoclonal antibodies. In addition, in these cells, the primary transcripts and mRNAs coding for class I molecules are markedly reduced as compared with those of their parents and unaffected siblings. Their karyotypes are normal. No change in their class I genes is seen on Southern blots. IFN-alpha in various doses does not increase the expression of class I molecules. EBV transformation of peripheral B cells results in EBV-transformed cell lines that after prolonged culture express normal amounts of class I antigens. These antigens are electrophoretically indistinguishable from those of their unaffected family members. In our family, this syndrome seems to represent primarily a defect of class I molecules expression in T cells. Moreover, the events involved in this defect occur at a pretranslational level.

0555 ABROGATION OF SPECIFIC T-CELL FUNCTION BY HTLV INFECTION. Mikulas Popovic, Dean Mann, Niel Flomenberg*, David Volkmann, Bo Dupont*, Anthony Fauci, Robert Gallo. National Institutes Health, Bethesda, MD; Sloan-Kettering Institute, New York, NY

Two functionally different types of T-cells, one clone specific to a soluible antigen (Kyehole Limpet Hemocyanine, [KLH]) with helper/inducer function, and two others with specific cytotoxic activity directed against HLA Class II antigens were infected with different HTLV isolates. Upon infection with HTLV, both helper/inducer as well as cytotoxic T-cells lost their specific function. In the case of the HTLV infected helper/inducer T-cell clone, no response to KLH was found either in presence or absence of antigen presenting cells. Similarly, both HTLV infected cytotoxic T-cell clones showed a reverse correlation between the percentage of HTLV positive cells and cytotoxic activity. A ten-fold decrease or complete loss of cytotoxic activity was found when the percentage of HTLV positive cells was over 70%. The results from these studies suggest one of the possible mechanisms which could be involved in induction of an immunodeficiency by HTLV.

What Is the Nature of the MHC/Ag Corecognition Receptor On mu T Cells?

0556 MOLECULAR CHARACTERIZATION OF THE PUTATIVE MURINE T CELL ANTIGEN RECEPTOR, James P. Allison, Tom St. John, Bradley W. McIntyre, University of Texas Science Park, Smithville, TX 78957, and Stanford University Medical Center, Stanford, CA 94307

Numerous studies have demonstrated that the T cell antigen specific receptor is a disulfide-linked heterodimer with subunits of 40-48 kD. We have produced a series of antibodies to epitopes carried by the molecule, including monoclonal antibodies to clonotypic epitopes specific to individual cell lines as well as antisera and monoclonal xenoantibodies to constant region epitopes which cross react with the heterodimers of different T cell lines. Using these reagents, we have isolated the putative receptor from different T cells for comparison of primary structure via two-dimensional peptide mapping. The results indicate a) that the peptide maps of α and β subunits are extremely different, suggesting that the chains are encoded by different genes, and b) that both subunits contain both constant and variable regions, and c) different functional subclasses of T cells may express receptors with isotypic structures. The antibodies have proven useful in the screening of expression vector cDNA libraries in an approach to the cloning of the genes encoding the subunits. (Supported by PHS CA 26321 awarded by the National Cancer Institute.)

0557 A T CELL MEMBRANE HETERODIMER (p43, p39) PROTEIN INVOLVED IN ANTIGEN RECOGNITION OR T CELL ACTIVATION. Reinhard Burger, Konrad Reske, Ursula Mauer, Dierk von Steldern and Matthias Husmann, Inst. of Immunology, Univ. of Heidelberg, FRG.

A monoclonal T cell specific antibody (Ab 188) was obtained which recognized the majority (90%) of peripheral guinea pig T lymphocytes and a minor population of thymocytes. In the continuous presence of Ab 188 in cultures of primed T cells with antigen- or mitogen-pulsed syngeneic macrophages or with allogeneic macrophages a marked, dose-dependent inhibitory effect of Ab 188 on T cell proliferation was observed whereas other T cell specific antibodies had no inhibitory effect. Time course experiments revealed that the corresponding antigen is involved in the initial phase of T cell activation; addition of Ab 188 to the cultures after 16 - 24 h had no longer an inhibitory effect. In the presence of Ab 188, Il 2 activity in the culture supernatants was markedly reduced. Soluble or immobilized Ab 188 had no mitogenic activity for T cells. Two-dimensional (2D) SDS-PAGE of immunoprecipitates obtained from NP 40 lysates of internally ^{35}S-methionine labeled polyclonal Con A-induced T cell blasts revealed that Ab 188 precipitated a two chain molecule. Both subunits, with a mol. wt. of 43.000 and 39.000, exhibited a marked charge heterogeneity in isoelectric focussing (1. D.). On human or murine T cell clones functionally and structurally similar proteins were recently identified. These heterodimers expressed clonotypic determinants which are involved in T cell recognition and obviously represent at least part of the T cell receptor for antigen. Ab 188 might recognize the analogous protein of the guinea pig via a kind of conserved or framework determinant expressed independent of T cell specificity. Therefore, Ab 188 is used for comparison of the structural properties of the molecules derived from alloreactive T cell clones of distinct specificity. In addition, heteroantisera and additional monoclonal antibodies are produced for further functional and structural analysis against material purified by an Ab 188-immunoabsorbed column.

0558 ANTIBODIES TO THE T CELL RECEPTOR ON AN INSULIN SPECIFIC IA RESTRICTED T CELL HYBRIDOMA, Arun Fotedar, Wallace Smart, Eugene W. Holowachuk, Thomas G. Wegmann and Bhagirath Singh, Department of Immunology & MRC Group on Immunoregulation, University of Alberta, Edmonton, Alberta Canada, T6G 2H7.

A novel approach was utilized to generate anti T cell receptor antibodies to a beef insulin specific and IA^d restricted T cell hybridoma. Sprague Dawley rats were neonatally tolerized with a closely related pork insulin specific and IA^d restricted T cell hybridoma. Tolerized rats were subsequently immunized to generate sera and B cell hybridomas. The sera generated from such tolerized and immunized animals were shown to inhibit IL2 release by the beef insulin reactive T cell hybridoma, and not by other T cell hybridomas. The inhibitory activity could be absorbed out by the relevant T hybrid and not by Balb/c spleen cells or other T cell hybrids. Relevant sera from tolerized and subsequently immunized rats were used to immunoprecipitate proteins from I^{125} labelled T cells and a band at ~43K was immunoprecipitated from the relevant hybrids. Monoclonal antibodies with similar characteristics have been generated. Functional and biochemical data generated from these probes to understand T cell restricted recognition will be discussed.

0559 GENOMIC REARRANGEMENTS INVOLVING A POSSIBLE T CELL RECEPTOR GENE, Nicholas R.J. Gascoigne, Yueh-hsiu Chien, Nadine E. Lee, Stephen M. Hedrick and Mark M. Davis, Stanford University, Stanford, CA 94305

A series of eleven distinct cDNA clones were isolated from an antigen specific T_h hybridoma line that were both T-cell specific and on membrane bound polysomal RNA (Hedrick et al. in preparation). One of these clones, TM-86, was shown by southern blot analysis to be rearranged in a variety of T_h hybrids (8/8) and T-lymphomas (3/3) but not in a B-lymphoma (op. cit.). In addition, the sequences of a variety of of thymus and spleen derived cDNA clones indicate the existence of variable, constant and joining regions.

In order to characterise these rearrangements further, genomic clones corresponding to TM-86 were isolated from phage libraries prepared from liver DNA and the T_h hybridoma 2B4 respectively. These clones have now been characterised by heteroduplex analysis and restriction mapping. These data show a clear rearrangement between the liver and tumour derived genes. DNA sequencing is now in progress to investigate this further.

0560 THE ROLE OF LFA-1 IN THE BINDING AND ACTIVATION OF THE MHC RESTRICTED, ANTIGEN SPECIFIC RECEPTOR ON T-CELLS. William T. Golde, John W. Kappler, and Philippa Marrack. National Jewish Hospital, Denver, Co. 80206.

Previous experiments reported by our laboratory describe the contribution of the L3T4 protein on T-cells to the overall binding avidity of MHC restricted, antigen specific T-cells for their targets. In similar experiments, we have shown a differential sensitivity of a panel of antigen reactive, cloned T-cell hybrids to monoclonal antibodies directed against LFA-1. Though this sensitivity was seen in response to antigen, no effect was observed with any antibody tested on the Con A response of these hybrids. Neither of these observations had any correlation to the density of LFA-1 on the cell surface. They do however, correlate directly with the rating of these hybrid's response to low doses of antigen.

It is our hypothesis that the LFA-1 molecule also functions to increase the overall avidity of the reaction between T-cells and antigen presenting cells. It appears that this protein functions to increase the binding capacity of low affinity receptor and does not have any effect when the receptor is of high affinity.

0561 BIOCHEMICAL CHARACTERIZATION OF THE T CELL RECEPTOR, Charles Hannum, Philippa Marrack, and John Kappler, National Jewish Hospital, Denver, CO 80206

We are attempting to purify and biochemically characterize the polypeptide chains of the membrane-bound antigen receptor found on the surfaces of T cells. Monoclonal antibodies specific for certain human and mouse T cell receptors, when attached to Sepharose 4B, enable the rapid purification of such receptors from NP-40 lysates of the appropriate T cell tumors or hybridomas. Extensive washing of the affinity beads before elution of the receptor removes most but not all contaminating proteins, and thus two-dimensional (non-reduced followed by reduced) SDS-PAGE is required to isolate the chains of the disulfide-linked heterodimer. In the case of the human T cell receptor the alpha and beta chains are actually separated during SDS-PAGE because of their size difference (46 and 40Kd, respectively). The alpha and beta chains of the mouse T cell receptor, however, are nearly identical in apparent molecular weight (43 Kd), and thus another technique is required for their separation. Hydroxylapatite chromatography is now being tested because of its ability to separate SDS-solubilized polypeptides. We now show that the alpha and beta chains of a human T cell (HPB-MLT) receptor can be completely resolved on hydroxylapatite with no detectable loss of protein. Currently this technique is being tested for its effectiveness in separating the chains of a mouse T cell receptor.

0562 BIOSYNTHETIC CHARACTERIZATION OF THE ANTIGEN-SPECIFIC MHC-RESTRICTED RECEPTOR ON MURINE T CELLS, Kathryn Haskins, Ralph Kubo, Philippa Marrack and John Kappler, National Jewish Hospital and Research Center, Denver, CO 80206

The antigen-specific MHC-restricted T cell receptor in the mouse has been identified by clone-specific antibodies and we have shown through immunoprecipitation studies of ^{125}I surface labeled membrane proteins that this molecule is an 80,000 MW disulfide-linked heterodimer consisting of two subunits of approximately 43,000 MW each. NEPHGE analysis indicates that the two subunits can be separated on the basis of their pIs: an acidic chain (about pH 5) and a basic chain (about pH 7). We have recently carried out comparative analyses of receptor material on the I-region restricted, antigen-specific T cell hybridoma, DO-11.10, labeled biosynthetically. Two dimensional SDS PAGE showed that the specifically precipitated product was a disulfide-bonded protein with subunits at about 43,000 daltons. Unlike the single diffuse band at 43,000 MW observed in surface-labeled material, the major intrinsically labeled product appeared to consist of two bands, distinguishable by slight differences in size. In addition, a second product of approximately 75 kd composed of 38 kd subunits was observed. This may be the precursor form of the expressed receptor product. By NEPHGE analysis, the processing of the receptor molecule is indicated by the complexity of the intrinsically labeled products as compared to the surface labeled receptors. The biosynthetically labeled material resolves as clusters of spots with possible overlapping pIs, most probably due to differences in the degree of glycosylation. The assembly of the two subunits appears to occur early in the synthesis of the receptor.

0563 CHARACTERIZATION OF THE ANTIGEN RECEPTOR FROM A MURINE CLONED HELPER T CELL LINE, Jonathan Kaye and Charles A. Janeway, Jr., Yale University, New Haven, CT 06510.

We have generated a monoclonal antibody against a murine cloned helper T cell line which recognizes conalbumin in the context of $I\text{-}A^k$ and is also alloreactive to $I\text{-}A^b$. This monoclonal antibody does not react with other helper T cell clones nor normal T cells. Immunoprecipitation analysis demonstrates that this antibody reacts with a disulfide linked heterodimer on the helper T cell clone surface, consisting of an acidic and a basic subunit each with an apparent MW of 42,000.

At concentrations as low as 1ng/ml, this monoclonal antibody will induce the production of IL-2 from the cloned helper T cells. In the presence of accessory cells or an IL-1-rich supernatant, the monoclonal antibody also induces proliferation of these cells. A Fab fragment prepared from this monoclonal antibody will block the response to antigen and self-Ia, and to allogeneic Ia, suggesting that a single molecule recognizes both of these moieties.

The clonotypic nature of the determinant recognized by this antibody, the ability of this antibody to mimic antigen and Ia in the activation of these cells, and the ability of the Fab fragment to block specific activation suggests that this antibody identifies the antigen/Ia receptor on these cloned helper T cells.

0564 MONOCLONAL ANTIBODIES AGAINST THE ANTIGEN RECEPTORS OF ABA-TYR REACTIVE T CELL CLONES
Craig T. Morita, Joel W. Goodman and George K. Lewis, University of California, San Francisco, CA (ACS FRA-264 and NIH AI05664)

To determine the function and structure of antigen receptors on p-azobenzenearsonate-L-tyrosine (ABA-Tyr) reactive T cell clones from A/J mice, we have immunized semi-congenic A.BY mice with an ABA-Tyr reactive T cell clone, 16-F2. From the spleen cells of one immunized A.BY mouse, we produced hybridomas whose monoclonal antibodies inhibit the antigen induced proliferation of the 16-F2 clone. Four monoclonal antibodies showed specificity for the immunizing clone. These antibodies inhibited the antigen induced proliferation of five other ABA-Tyr reactive T cell clones while not affecting the IL-2 induced proliferation of these clones or the proliferation of a seventh ABA-Tyr reactive clone or a number of KLH reactive clones. Monoclonal antibodies against the major cross-reactive idiotype of A/J anti-ABA antibodies did not inhibit the proliferation of any of these ABA-Tyr reactive clones. When assessed by indirect immunoflourescence and FACS analysis, the four antibodies bound to the immunizing 16-F2 clone and 50% of the T cells in the original ABA-Tyr reactive line. The antibodies bound to less than 5% of the T cells, however, from a second ABA-Tyr reactive line. When radioiodinated T cell clone extracts were immunoprecipitated and analyzed by SDS-PAGE, a dimeric molecule of 80-85 kD was found which migrates at 37-42 kD upon reduction. This molecule was found on the two clones tested whose proliferation was inhibited but not on the ABA-Tyr reactive clone whose proliferation was not inhibited. We conclude that these monoclonal antibodies react with the antigen receptors of a subpopulation of ABA-Tyr reactive T cells.

0565 MONOCLONAL ANTIBODY ANALYSIS OF THE ANTIGEN RECEPTOR ON A CLONED T CELL HYBRID
Lawrence E. Samelson and Ronald H. Schwartz, Laboratory of Immunology, NIAID, NIH, Bethesda, MD 20205

Monoclonal antibodies specifically binding the antigen receptor of a pigeon cytochrome c specific and Ia molecule restricted T cell hybrid were generated. The two antibodies that were isolated inhibited IL 2 release only from 2B4 the immunizing clone. Immunoprecipitation and SDS-PAGE analysis of detergent lysates from surface labeled hybrids revealed a molecule with an apparent molecular weight of 82-86kd under non-reducing conditions. In the presence of reducing agents, two bands at 42-44 and 46-48kd were observed. The structure of the molecule was further examined with two dimensional (non-reduced--reduced) electrophoresis. This study confirmed that the precipitated receptor contained interchain disulfide bonds. Interestingly, the two bands that migrated below the diagonal in this study were not vertically aligned implying that they were not linked together prior to reduction. Two dimensional IEF--SDS-PAGE demonstrated that the 46kd band resolved only into a basic band while the 42kd material resolved into both acidic and basic components. The data are consistent with the interpretation that the antigen receptor on the 2B4 hybrid exists in at least two different forms on the cell surface, a 42kdβ-42kdα heterodimer and a heterodimer composed of a 46kdβ chain and either the observed 42kdα chain or a nonlabeled α chain. These hypotheses are currently under investigation.

0566 GENES ENCODING LYMPHOCYTE CELL-SURFACE RECEPTOR MOLECULES.
Thomas P. St. John, James P. Allison, Michael W. Gallatin and Irving L. Weissman, Stanford University Stanford CA 94305.

We have isolated a number of specific phage lambda cDNA clones by the immunodetection of antigenic determinants expressed on β-galactosidase-cDNA fusion proteins. Monoclonal antibodies and sera directed against both clonotypic and framework determinants of the T cell antigen receptor have allowed the isolation of a number of distinct DNA sequences. A monoclonal antibody directed against a lymphocyte surface protein implicated in specific homing to peripheral lymph nodes has resulted in the repeated isolation of a gene with an unusual sequence organization. Other DNA sequences isolated encode antigenic determinants present on B220 (two different monoclonals), transferrin receptor, the glucocorticoid receptor p53, and Lyt 1.

0567 Ig V_H Genes and Antigen Receptors of T Lymphocytes
David S. Ucker, Center for Cancer Research, M.I.T., Cambridge, MA 02139

The presenceof idiotypic determinants mapping to the immunoglobulin (Ig) heavy chain locus on some thymus-derived (T) lymphocytes suggests that genes related to heavy chain Ig variable region (V_H) genes may play a role in encoding the antigen receptors of T lymphocytes. We have screened RNA from clonal lines of T lymphocytes with a battery of V_H probes and have found cross-hybridizing mRNA species. In particular, a cytolytic T lymphocyte (CTL) line with specificity for fluorescein (FL) in the context of the H2-D^d haplotype on the surface of target cells exhibits a unique mRNA species hybridizing with a V_H probe derived from a nitrophenol(NP) specific hybridoma. A full length cDNA copy of the mRNA species has been cloned and analyzed. It represents the functional transcript of an unrearranged V_H gene; the open reading frame extends slightly beyond the normal Ig gene rearrangement site. Polyclonal and monoclonal antibodies against a synthetic peptide corresponding to the carboxyl terminus of the protein predicted by the nucleotide sequence have been prepared and reveal the presence of that V_H protein on the CTL cell surface. Intriguingly, this CTL lyses H2-D^d targets derivatized with NP as well as FL. Preliminary experiments suggest that the anti-V_H peptide antibodies specifically block target lysis by this CTL clone. The ability of antibodies to block γ-interferon production in response to appropriate targets currently is being tested. We also are screening CTL populations of identical and related target specificities for expression of this and similar V_H proteins. Finally, we are testing whether the target specificity of unrelated CTL lines can be converted to FL or NP specificity upon gene transfer of this V_H cDNA clone.

Dialogue Between T and Macrophage Subsets

0568 ACTIVATION OF T_h CELLS REQUIRES ASIALO-IA ON ANTIGEN-PRESENTING CELLS, Carol Cowing Michael Frohman and Joan Chapdelaine, Univ. of Penn. Med. School, Phila., PA 19104

Adherent antigen-presenting cells (APC) and resting B cells express Ia molecules, but these molecules are glycosylated differently in the two cell types. The chains of I-A and I-E molecules from resting B cells contain terminal sialic acid while those of APC are asialo. The two cell types also differ in their capacity to activate T_h cells: APC can present both MHC alloantigens and nominal antigens, inducing T cells to divide and differentiate; small resting B cells fail to activate T cells even with added IL-1.

To assess the role of asialo-Ia in the activation of T_h cells, small resting B cells were treated briefly with neuraminidase to remove sialic acid from membrane glycoproteins. Desialylated resting B cells were capable of activating T_h cells to both alloantigen and nominal antigens if exogenous IL-1 was present. Neuraminidase treatment of T cells did not result in their activation, nor did neuraminidase treatment of APC increase their capacity to stimulate T cells.

Activated (LPS treated) B cells can also function as APC with added IL-1. The acquisition of the capacity to present antigen is an early event of B cell activation, corresponding with the increase in membrane expression of Ia. Evidence suggests that the newly synthesized Ia in activated B cells is of the asialo form, as neuraminidase treatment of activated B cells had no effect on their capacity to present antigens.

Taken together, the data are consistent with a model that APC function is dependent upon the expression of asialo-Ia and imply that T_h cells recognize the non-polymorphic carbohydrate side chains on Ia.

0569 REGULATION OF IL1-RELEASE: ROLE OF THE IA GLYCOPROTEINS, Scott K. Durum, Richard K. Gershon and Carl Higuchi, Yale University, New Haven, CT 06510

Interleukin 1 (IL1) is released from activated macrophages. This activation can be achieved by a variety of stimuli, including activated T cells and microbial products. We describe a method of preparing activated Lyl T cells that can subsequently stimulate release of large quantities of IL1 from macrophages; this interaction between T cells and macrophages is H-2-restricted and blocked by anti-IA monoclonal antibodies (MABs). When concanavalin A is included during the period of their interaction, no H-2-restriction is observed between T cells and macrophages, and yet, anti-IA MABs block this unrestricted interaction as well. Finally, when lipopolysaccharide is used to stimulate macrophages (in the absence of T cells) anti-IA MABs also inhibit IL1 release. We suggest therefore that IA glycoproteins may serve an active - possibly receptor-like - role on cell membranes; thus, in macrophage membranes, IA molecules may transduce signals resulting in release of IL1.

0570 MACROPHAGE Ia-RESTRICTED PRESENTATION OF PROCESSED SHED ALLOANTIGENS DURING INDUCTION OF ALLOSPECIFIC CTL. Hana Golding and Alfred Singer, NCI, NIH, Bethesda, MD 20205

Macrophages are potent stimulators of allogeneic T cells. Their function depends on expression of MHC and production of soluble mediators which initiate cellular interactions leading to generation of cytotoxic T cells (CTL). In this study we asked whether, during the induction of allospecific CTL, macrophages can function as classical antigen-presenting cells; pick up shed allogeneic MHC molecules, process and present them to syngeneic T cells. We approached this question using macrophage depleted stimulator and responder populations which, in themselves, cannot generate CTL. Their responsiveness could be reconstituted by stimulator macrophages or macrophages of responder origin. In the latter case pretreatment of responder macrophages with chloroquine (which disrupts lysosomal function) completely abrogated their ability to reconstitute the CTL response of syngeneic T cells, indicating that antigen processing was involved. Similarly treated stimulator macrophages remained functional. The processed determinants were derived from the stimulator cells since paraformaldehyde fixed stimulator cells were incapable of stimulating allo-CTL in the presence of responder macrophages, unless exogenous IL-2 was aded. These findings indicate that in this system, shedding of stimulator cells surface molecules is required for activation of T_H (and IL-2 production) by syngeneic macrophages. This MØ-T_H interaction could be completely blocked by monoclonal αIa Ab, indicating that the processed shed alloantigens were represented by syngeneic macrophages in the context of self Ia. Such presentation of shed alloantigens by syngeneic macrophages may play an important role in transplant rejection of MHC-incompatible organs seemingly devoid of endogenous macrophage like cells.

0571 MURINE IFN-γ: A PLEIOTROPIC EFFECTOR OF THE IMMUNE SYSTEM. Patrick W. Gray, Department of Molecular Biology, Genentech, Inc., South San Francisco, CA 94080

The single gene for murine IFN-γ (immune interferon) codes for a 16,000 mw protein which is glycosylated and secreted from stimulated lymphocytes. This gene has been cloned, characterized, and engineered for expression in mammalian cells and E. coli. In collaboration with other investigators, recombinant murine IFN-γ has been shown to possess many biological activities. While this molecule was first isolated on the basis of its potent antiviral activity, IFN-γ also is an active effector of macrophage function. IFN-γ activates macrophages to become cytotoxic towards tumor cells in vitro and causes macrophage differentiation and proliferation. IFN-γ also activates Natural Killer cells to become cytotoxic. This activation of specific cellular populations may be related to the observed antitumor effect in vivo. IFN-γ by itself has an antiproliferative activity on certain tumor cell lines in vitro. This protein is also involved in B cell regulation; it induces B cells to mature and secrete immunoglobulin and induces major histocompatability complex antigens. IFN-γ is involved in a complex regulation of other lymphokines, most notably interleukin 1, interleukin 2, and lymphotoxin.

0572 DIFFERENT MACROPHAGE ACTIVATING FACTORS ARE RELEASED BY DIFFERENT T CELL CLONES, Peter H. Krammer, Claire Kubelka and Diethard Gemsa, Inst. of Immunol., Univ. of Heidelberg and Inst. of Immunol. and Genetics, DKFZ, Heidelberg, F.R.G.

We have studied macrophage activating factors (MAFs) from two long term T cell clones, clone 96 and 7.1.2E8. Incubation of resident murine macrophages with MAF 7.1.2E8 increased RNA-, protein-, and glycoprotein-synthesis, hexosemonophosphate shunt (HMPS) activity, release of oxygen metabolites (O_2,H_2O_2), pinocytosis, phagocytosis and tumor cytostasis whereas no effect on prostaglandin E (PGE) release, schistosomula killing and tumor cytolysis could be observed. In contrast, MAF 96 increased glycoprotein synthesis, HMPS activity, release of oxygen metabolites and PGE, schistosomula killing, tumor cytostasis and cytolysis whereas RNA- and protein-synthesis and pinocytosis were decreased and phagocytosis remained unaffected. These MAFs may help to clarify the mechanisms of macrophage activation, and the relevance of macrophages for infectious and neoplastic diseases.

0573 BIOLOGICAL SIGNIFICANCE OF Ia ANTIGENS SHED FROM MACROPHAGES.
Sho Tone Lee, Frixos Paraskevas and Chella David. University of Manitoba, Winnipeg, Canada and Mayo Clinic, Rochester, Minn. U.S.A.

Mouse T cells cultured in vitro loose their Ia antigens which can be reconstituted with Ia antigens shed from macrophages. Supernates from 3 hour macrophage culture contain Ia antigens which bind to proper anti-Ia immunoadsorbents. T cells from Balb/c, C_3H/He, B10·K but not B10·S or SJL/J mice could take up Ia antigens shed from macrophages of certain strains. Using a large number of congenic and non-congenic strains it was shown that only supernates of macrophages from I-E^k strains could reconstitute the T cell Ia antigens. With a monoclonal anti-Ia·7 antibody, it was shown that the macrophage-T cell exchange involves Ia·7. Complexes of Ig and antigen are present in the serum of mice 6 hours after immunization which are strongly cytophilic for T cells and markedly enhance the IgG response. Ia antigens shed from macrophages inhibit the uptake of these complexes by T cells or at the same time block the IgG enhancement. Among the Ia antigens shed from macrophages, determinants coded by the I-E subregions (Ia·7) are important in both functions. Thus Ia·7 determinants on the surface of T cells act as receptors for the complexes, while in a 'soluble' form, block their function.

(This work was supported by grants from NCI and MRC of Canada).

0574 PURIFICATION OF A MURINE T CELL DERIVED CSF ACTIVITY TO HOMOGENEITY,
Diane Mochizuki, David L. Urdal, Carl J. March, James D. Watson†, Steven Gillis and Paul J. Conlon. Immunex Corp., Seattle, WA 98101. †University of Auckland, Auckland, New Zealand.

Previously, we reported the partial purification and characterization of 3 CSF species derived from the culture supernatants of a myeloid cell line WEHI-3B and T cell line LBRM-33. The 3 species designated CSF-2α, CSF-2β and CSF-2γ were separable by both biochemical and biological criteria. Two species, CSF-2α and CSF-2β, were produced by both WEHI-3B and LBRM-33 cells. The third species, CSF-2γ was produced only by the LBRM-33 cell line. The three bioassays used to measure the CSF activities were as follows: 1) the bone marrow proliferation assay (MBMA); 2) Bone marrow colony forming assay (CFA); and 3) the FDC-P2 proliferation assay. The MBMA and the CFA were capable of detecting all 3 CSF species, whereas the FDC-P2 assay detects only CSF-2α and CSF-2β. Further characterization of the biological role of these CSFs and their relationship to other lymphokines we felt, could only be realized using homogeneous CSF. LBRM-33 CSF-2α was the initial species selected for purification and sequence determination because the LBRM-33 cells produce relatively abundant quantities of CSF-2α. In addition, the FDC-P2 proliferation assay for CSF-2α is conducive to monitoring purification steps. Finally, there is evidence that CSF-2α acts on multiple cellular targets, indicating a primary role in regulating hemotopoiesis. We will describe the purification scheme used to purify CSF-2α to homogeneity and present partial N-terminal amino acid sequence information.

0575 CHARACTERIZATION OF THE DEFECTIVE AMLR IN RHEUMATOID ARTHRITIS.
Richard M. Pope, Linda McChesney, Norman Talal and Michael Fischbach, University of Texas Health Science Center, Audie L. Murphy Memorial Veterans Administration Hospital, San Antonio, TX 78284

Cellular communication, represented in part by the autologous mixed lymphocyte response (AMLR), is defective in patients with rheumatoid arthritis (RA). These studies were performed in order to further characterize this defect and to define its relationship to disease activity. T lymphocytes were stimulated with either a mitocycin C treated B lymphocyte enriched (B cell) or a macrophage enriched (MØ) population. Employing cells isolated from the peripheral blood (PB) of patients with active RA, a significant ($p<0.001$) reduction of ^3H incorporation of T lymphocytes stimulated by both B cells and MØ was observed. The B lymphocytes were significantly ($p<0.001$) less stimulatory than MØ in patients with active RA compared to the controls. MØ in higher concentrations were normally capable of suppressing the B lymphocyte-T cell AMLR. MØ from patients with RA were somewhat less suppressive than normal MØ. No excessive suppression was observed. Significant ($p<0.05$-0.001) associations between disease activity as measured by both ESR and the joint count and the AMLR, employing either B cells or MØ, were observed. Defects of the AMLR were due to diminished production of interleukin-2 (IL-2) as measured by an IL-2 dependent cell line. Defects in proliferation could be partially restored by the addition of IL-2 to the AMLR. These data indicate that the defective AMLR observed in patients with RA is associated with disease activity, is more prominant when B lymphocyte enriched populations are employed as the stimulator cells and is associated with a defective production of IL-2. These abnormalities of cellular communication may in part be responsible for the altered immunoregulation observed in patients with RA.

0576 HETEROGENEITY IN THE RESPONSE OF T CELLS TO ANTIGEN PRESENTED BY DIFFERENT ACCESSORY CELLS, Michael J. H. Ratcliffe *°, Michael H. Julius* and K. Jin Kim*.
* Basel Institute for Immunology, Basel, Switzerland and ° ICRF Tumour Immunology Unit Department of Zoology, University College London, UK

Antigen specific BALB/c T cell lines were generated from in vivo primed lymph node T cells. Viable T cells were harvested every 5 days and restimulated with fresh antigen and irradiated syngeneic spleen cells. After 4 in vitro stimulations, purified T cells from such lines failed to respond to antigen in the absence of an exogenous source of antigen presenting cells (APC). Addition of irradiated splenic or peritoneal APC restored antigen specific H-2 restricted T cell proliferation. A range of B cell lymphomas also supported specific T cell proliferation. Presentation of both soluble and particulate antigen was demonstrated.

After 12 in vitro stimulations in the presence of irradiated syngeneic spleen cells and antigen, the T cell lines responded, as before, to antigen in the presence of exogenous splenic APC. The B cell lymphomas however were completely unable to support antigen specific T cell proliferation. Lack of responsiveness was not restored by the addition of exogenous IL-1, IL-2, IL-3 or factors produced by an ongoing T-APC interaction.

These results demonstrate that T cells have the capacity to distinguish between antigen presented by B lymphoma cells and splenic APC and hence there are qualitative differences in the presentation of antigen by distinct APC types.

0577 A NOVEL LYMPHOKINE ACTIVITY INVOLVED IN T CELL ACTIVATION, David H. Raulet. Massachusetts Institute of Technology, Cambridge, MA 02139

We have found evidence for a novel lymphokine activity in T cell activation. The factor is produced by T cell hybridomas which also produce small amounts of Interleukin-2 (IL-2), but can be separated from IL-2 by depleting the latter factor by absorption with a clone IL-2 addicted CTL clone. The novel lymphokine activity, which we call "T cell activating factor (TAF)", stimulates production of IL-2 by enriched T cell populations in conjunction with Concanavalin A or alloantigen. Furthermore, the factor synergizes with IL-2 preparations in atimulating activation (^{3}H-Thymidine incorporation) of T cell populations. We propose that TAF is a T cell-derived lymphokine which regulates production of other T cell derived factors, such as IL-2.

0578 ACCESSORY MECHANISMS IN HUMAN T CELL ACTIVATION, Lanny J. Rosenwasser, Dominic E. Picarella, Thomas E. Brown, and Mark R. Windt. Tufts-New England Medical Center, Boston, MA 02111

Our laboratory is interested in defining the precise parameters of accessory functions in human T cell antigen recognition. In order to study the mechanisms of antigen presentation in humans, we have begun to study the cellular and molecular characteristics of human immune reactivity to insulin in vitro. The insulin specific proliferative response of periperhal blood mononuclear cells (PBMC) from 19 subjects with insulin dependent diabetes mellitus (IDDM) was examined. We have found that at least 4 of those subjects made significant in vitro blastogenic responses to heterologous (beef or pork insulin). These PBMC responses are T cell and monocyte dependent as assessed by standard methods of PBMC fractionation as well as elimination of cellular subpopulations by monoclonal antibodies. In addition, optimal T cell recognition of autologous monocyte associated insulin is also dependent on partially purified interleukin-1 (IL-1), a mediator also produced by monocytes. Autologous monocytes, frozen in dimethylsulfoxide and fetal calf serum, and then thawed after 2-4 weeks at -70°C, were also capable of supporting accessory functions in insulin specific T cell activation. In order to more fully explore the mechanism of specific antigenic epitope presentation to immune T cells in humans, we are establishing long term T cell lines reactive to insulin, with the idea of eventually developing insulin specific T cell clones or T-T hybridomas. We are also involved in studying other accessory cells such as human vascular endothelial cells (HVEC). We have found that HVEC are capable of producing an IL-1 like activity and providing accessory functions for T cell activation.

0579 AGE-DEPENDENT DEFICIT IN T CELL RESPONSE: DEFECTIVE ANTIGEN PRESENTING CELLS, Stephen A. Stohlman, Glenn Matsushima, and Nancy Casteel, University of Southern California School of Medicine, Los Angeles, CA 90033

Young SJL mice have a unique age-dependent functional T-helper cell deficiency that is overcome by aging. We have found that 6 week old SJL but not C57Bl/6 mice are unable to mount a DTH-type response to SRBC, vesicular stomatitis virus or mouse hepatitis virus. In SJL this response reaches maximal adult levels by 12 weeks of age. Intravenous transfer of as few as $5x10^4$ thioglycolate elicited peritoneal exudate cells from 12 week donors reconstitutes the response of 6 week recipients. The cell mediating this reconstitution is a radiation resistant (2000R), Sephadex G-10 adherent cell, expressing Ia but not Thy-1.2 on its cell surface. Transfer of cells from recombinant inbred strains clearly shows that expression of Ia is required, suggesting that SJL have a unique maturational defect in antigen presenting cells that inhibits their ability to respond to antigen.

0580 LANGERHANS CELLS PRESENT HAPTENS IN IMMUNOGENIC FORM IN THE FACE OF COMPETING DOWN-REGULATING SIGNALS FROM OTHER EPIDERMAL ELEMENTS, Sabra Sullivan, J.Wayne Streilein, and Paul R.Bergstresser, UT Health Science Center Dallas, Dallas, TX. 75235

We have observed that skin which is deficient in normal numbers of functioning Langerhans cells (LC) fails to sustain the induction of contact hypersensitivity (CH) to simple haptens. Moreover, immunizing through LC deficient skin results in a state of specific unresponsiveness which can be adoptively transferred with T cells. To assess the role of LC in these processes, we have taken advantage of our ability to enrich routinely hamster epidermal cell suspensions (EC) for LC (38-44%). Hamster EC enriched for LC, when haptenated in vitro with TNBS and inoculated intravenously into recipient hamsters, induce intense CH with no evidence of accompanying down regulation; TNP-EC depleted of LC induce unresponsiveness when inoculated intravenously. In mice, inoculation with TNP-EC containing up to 15% LC induces CH of moderate intensity, but also evokes a significant component of down regulation. We conclude that LC compete with other epidermal cells in their capacity to present antigen in an obligatorily immunogenic manner. More recently, we have utilized ficoll gradients and FACS techniques to develop a protocol that allows us to enrich for, and deplete mouse EC of LC. EC prepared in this manner contain 97% Ia+ LC; the cohort EC depleted of Ia+ cells contains virtually no LC. With these enriched cell suspensions it now becomes possible to determine in mice whether Langerhans cells also deliver an obligatory immunogenic signal and what role they, and other epidermal cell populations, play in evoking the down regulatory component.

0581 DISTINCT ROLE OF ADULT AND NEONATAL MONOCYTES IN THE ANTIGEN-INDUCED PLAQUE-FORMING CELL RESPONSE IN MAN. Maarten J.D. van Tol, Jitske Zijlstra, Ben J.M. Zegers and Rudy E. Ballieux, University Children's Hospital "Het Wilhelmina Kinderziekenhuis", Utrecht, The Netherlands.

Human cord blood mononuclear cells (CBMC) are able to mount an antigen-specific IgM plaque-forming cell (PFC)-response after in vitro stimulation with the T cell-dependent antigen ovalbumin (OA). The antigen dose requirement for an optimal induction of PFCs in cultures of CBMC differs from that obtained for adult peripheral blood mononuclear cells (PBMC), i.e. 0.03 µg OA/ml for CBMC vs. 3.0 µg OA/ml for adult PBMC. Studies on semi-allogeneic combinations of neonatal or parental lymphocytes and monocytes (adherent cells) respectively revealed that adherent cells (AC) play a pivotal role in the determination of the optimal antigen dose. It emerged from antigen pulsing experiments that neonatal AC require a hundredfold less amount of OA to function effectively as antigen presenting cells. By culturing CBMC in the presence of the prostaglandin synthetase inhibitor indomethacin the antigen dose response curve shifted to that obtained for adult PBMC. Antigen pulsing experiments suggested that the action of indomethacin is located at the site of the AC. These results are suggestive for a role of PGE_2 in the modulation of the antigen handling properties of monocytes. However, data concerning the PGE_2 secretion by adult and neonatal AC and the observation that Organon 7258, also an inhibitor of the cycloxygenase pathway, did not affect the antigen dose-response relationship for CBMC indicate that indomethacin acts on the antigen handling by neonatal monocytes along another mechanism than the inhibition of PGE_2 production.

0582 ANTIGEN PRESENTATION AND IL-1 PRODUCTION BY HLA-DR COMPATIBLE ENDOTHELIAL CELLS. Cynthia Wagner, Denis R. Burger, Elaine McCall, R. Mark Vetto, VA Medical Center, Portland, OR 97201

We have investigated the mechanism of antigen presentation by vascular endothelial cells (VE). Human umbilical vein VE were propagated in long term culture by supplementation with ECGF and fibronectin. Subcultured VE (passages 3,5,6,7,9,17,18) were used to present antigen to MO-depleted human T cells and activation assesed by ^{3}H-thymidine uptake. Ia (HLA-DR in man) compatible VE were effective in reconstituting MO-depleted T-cell proliferation whereas fibroblasts and smooth muscle cells cultured from the same umbilical veins were not. The VE reconstituted responses were antigen-specific and blocked by monoclonal antibodies to endothelial antigens but not to MO antigens. Although it has been difficult to demonstrate Ia on the surfaces of VE, these responses were HLA-DR restricted and blocked by monoclonal antibodies to HLA-DR framework structures. Whether VE synthesize and express surface Ia antigens only after intereaction with lymphocytes was suggested using immunoprecipitation of internally radiolabelled VE membrane proteins. Additional experiments demonstrate that endothelial cells secrete a factor that has IL-1-like activity. Thus VE appear to express the conventional requirements of antigen-presenting cells; Ia and IL-1.

0583 IMMUNOSUPPRESSIVE MACROPHAGES ASSOCIATED WITH TERMINATION OF THE L5178Y LYMPHOMA TUMOR DORMANT STATE IN DBA/2 MICE, E. Frederick Wheelock, Michael K. Robinson, Takeshi Okayasu. Hahnemann University, Philadelphia, PA 19102

In the L5178Y lymphoma tumor dormant state, small numbers of lethal tumor cells persist in the peritoneal cavity of clinically normal DBA/2 mice for many months before growing out to produce ascitic tumors. During the 30 day period before developement of ascitic tumors, macrophages with increased capacity to suppress the in vitro generation of secondary anti-L5178Y cell cytotoxic T lymphocyte (CTL) response appear in the peritoneal cavity. These macrophages, when transferred to the peritoneal cavity of L5178Y cell-immunized mice, subvert their resistance to L5178Y cell transplantation. Immunosuppressive macrophages cultured in vitro for 24 hours produce a factor which also suppresses the in vitro generation of CTL. This immunoregulatory factor is being characterized and identified. We believe that immunosuppressive macrophages disrupt the immunoregulatory circuits that control the tumor dormant state, by preventing the generation of cytotoxic T lymphocytes in the peritoneal cavity. As a consequence, L5178Y cells in a tumor dormant state can proliferate progressively to form an ascitic tumor.

Cognate and Non-Cognate T-B Cell Interaction: Physiological Relevance

0584 CAN THE FINE SPECIFICITY OF T CELLS DICTATE THE FINE SPECIFICITY OF ACTIVATED B CELLS? F.Celada, F.Manca, E.Sercarz°, A.Kunkl and D.Fenoglio, Dept. of Immunology, University of Genoa, 16132 Italy and Dept. of Microbiology, UCLA, Los Angeles, CA, U.S.A. 90024.

If one considers natural (non derivatized) protein antigens bearing a number of different sites recognized by T and/or B cells, the question is whether T cells with one single specificity can help all the possible B cells available in the antigen specific repertoire. We used an in vitro helper assay in which T cells were primed with E.coli B-galactosidase (GZ) (MW125 Kdx4) or with cyanogen bromide peptides. The B cells were specific for the whole molecule. Antibodies were quantitated by ELISA. The fine specificity was analyzed by the capacity of some but not all binding antibodies to restore the enzymatic activity of a panel of different GZ preparations obtained from mutant strains of E.coli (activating antibodies). Optimal production of binding antibodies was observed when T cells had been primed with GZ and CB18. Activating antibodies were obtained when T cells had been primed with CB20 and CB21. In contrast with these data, Krzych and Sercarz have recently reported that in an hapten-carrier system help is only provided by CB2 and CB10 primed T cells. These results indicate that T cells' fine specificity can dictate B cell fine specificity. Whether this restriction is due to a particular steric orientation of the bridging antigen or to the requirement for T and B epitopes to sit on the same "Processing unit" still has to be established.

0585 THREE CLASSES OF SIGNALLING MOLECULES ON B CELL MEMBRANES, R.B. Corley, N.J. LoCascio, T. Kuhara, L.W. Arnold, P.S. Pillai, D.W. Scott and G. Haughton. Duke Medical Center, Durham, NC 27710 and University of North Carolina, Chapel Hill, NC 27514

The question of whether surface immunoglobulin (sIg) and Ia molecules have a signalling function in helper T (Th) cell-dependent activation of resting B cells is being evaluated. Two sources of B cells have been used, one a purified population of hapten-binding B cells, the other a B cell lymphoma, CH12, with known antigen specificity. CH12 has the activation properties of a resting B cell. The requirement for antigen in B cell activation was studied using Th cells selected such that they interacted with B cells in the absence of the sIg-antigen binding. B cells were not activated when normal ratios of Th cells and B cells were used unless the B cell antigen was also present in culture, demonstrating that sIg-ligand interactions contribute to B cell activation. The requirement for sIg-ligand binding could be overcome using higher multiplicities of T cell help. However, Th cell recognition of B cell Ia was still essential. Preliminary evidence that Th cell-Ia interactions result in the transmission of an activation signal is provided from the study of CH12. Only Th cells that interact with I-E molecules on CH12 activate the lymphoma, even though CH12 express both I-A and I-E molecules. Whether only one Ia molecule is functional on normal resting B cells is unknown and currently under investigation. Taken together, these data indicate that both sIg and Ia molecules function as signalling receptors in B cell activation, possibly by inducing the expression of a third class of membrane receptors, in particular those that bind the factors that promote growth and differentiation of responding B cells.

0586 POLYCLONAL STIMULATION OF RESTING B LYMPHOCYTES BY ANTIGEN-SPECIFIC T LYMPHOCYTES, Anthony L. DeFranco, Jonathan D. Ashwell, Ronald H. Schwartz and William E. Paul, Department of Microbiology & Immunology, University of California, San Francisco, CA 94143 and Laboratory of Immunology, NIAID, NIH, Bethesda, MD 20205

Polyclonal B lymphocyte activation, proliferation, and antibody secretion were stimulated by a long term cloned T lymphocyte line (11.4) in the presence of its antigen, GAT. These B cell responses were observed in cells from both normal and Xid mice and were not restricted by antigens of the major histocompatability complex (MHC) provided that optimal numbers of T cells, optimal numbers of syngeneic antigen presenting cells, and optimal concentrations of GAT were all present. The ability to stimulate this MHC-unrestricted pathway of B cell activation and proliferation was not unique to 11.4 cells, but was also a property of the majority of cloned T cell lines and interleukin-2 secreting T cell hybridomas tested. The polyclonal unrestricted nature of the B cell response strongly suggests the existence of a novel T-cell derived factor or factors that can cause resting B cells to enter G_1 phase of the cell cycle. Virtually all resting B cells enlarged in response to this putative B cell activating factor released by antigen-stimulated 11.4 cells. Anti-immunoglobulin reagents can also stimulate this event, suggesting that parallel pathways exist for B cell activation. These activating factors appeared to be limiting for B cell proliferation as evidenced by a marked synergy between anti-μ and suboptimal numbers of antigen-stimulated 11.4 cells.

0587 ACTIVATION OF B CELLS BY AUTOREACTIVE T CELLS, Alison Finnegan and Richard J. Hodes, Immunology Branch, NCI, NIH, Bethesda, MD 20205

Studies were carried out to characterize the relationship of autoreactive T cells to antigen specific MHC-restricted T cells in their ability to induce B cells to synthesize immunoglobulin. A number of autoreactive T cell lines and clones were isolated from antigen primed spleen and lymph node cell populations. Autoreactive T cells were found to be activated by direct recognition of either syngeneic I-A or I-E subregion encoded antigens in the absence of any apparent foreign antigen. Cloned autoreactive T cells when stimulated were capable of inducing unprimed B cells to synthesize IgM antibodies. The activation of B cells to produce IgM required the autoreactive T cells to interact with snygeneic antigen presenting cells, but did not require the addition of exogenous antigen. Once activated these T cells induced both syngeneic and allogeneic B cells to produce antibody polyclonally. Cloned autoreactive T cells were also able to induce the production of IgG by B cells. In contrast to the circumstance required for the synthesis of IgM antibodies, the induction of IgG antibodies by the same cloned T cells required primed B cells and stimulation with specific antigen. The activation of B cells to produce IgG required the direct recognition by the autoreactive T cell of self Ia determinants expressed on the B cell surface. These results indicate that autoreactive T cells resemble antigen-specific MHC-restricted T cells in their ability to function as T helper cells through distinct MHC-restricted and MHC-unrestricted pathways.

0588 MHC RESTRICTED ANTIBODIES. Pavol Ivanyi, Jacqueline Limpens, Tine Leupers. Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Plesmanlaan 125, 1066 CX Amsterdam.

C57B1/6 ($H\text{-}2^b$) mice were immunized with Sendai virus coated syngeneic cells. Cytotoxic antibodies were tested on normal and Sendai virus coated lymph node cells of syngeneic and allogeneic H-2 congenic mouse strains. A selected example of two sera after 12 weeks of immunization appear as illustrative.

C57B1/6		B10		B10.D2		B10.BR		B10.M		B10.Y		B10.Q		B10.S		B10.RIII	
b		b		d		k		f		p		q		s		r	
V-	V+	V-	V+	V-	V+	V-	V+	V-	V+	V-	V+	V-	V+	V-	V+	V-	V+
0	22	0	15	0	7	0	4	0	24	0	8	0	9	0	0	0	0
0	24	1	17	0	15	3	20	0	9	5	24	0	16	0	0	0	8

(V- and V+ for normal and Sendai coated cells, cytotoxicity is expressed by the Cytotoxicity Scoring Grade which roughly corresponds to titer).

These data show that injections of Sendai virus coated syngeneic cells could induce cytotoxic antibodies whose resction pattern is dependent on two factors, 1) presence of virus, 2) the H-2 haplotype of the target cells. These findings are similar to data obtained with MHC restricted CTL's but we do not know whether we are dealing with the same phenomenon. Certainly, H-2 public but not H-2 private determinants appear as restriction elements.

0589 HELPER T CELL EPITOPE SPECIFICITY REGULATES B CELL (ANTIBODY) SPECIFICITY, H. Kawamura, I. Berkower, C. Glover and J. Berzofsky, NCI, NIH, Bethesda, MD 20205

We have studied the role of helper T cell epitope specificity in determining the specificity of antibodies made to different parts of a globular protein antigen, myoglobin, in which each epitope has a unique location in relation to every other epitope. Splenic B lymphocytes from mice immunized with native myoglobin were cocultured with helper T lymphocytes from mice immunized with cleavage peptide fragments of myoglobin, in the presence of native myoglobin in the culture. Antibodies secreted into the culture fluid were assessed for fine specificity by binding to fragments of myoglobin in a radioimmunoassay. When the T cells were from mice immune to fragment (1-55), the antibodies made against whole myoglobin usually bound preferentially to fragment (1-55), more than to fragment (132-153). In contrast, when the T cells were immune to fragment (132-153), the antibodies produced by the same B cell preparation preferentially bound fragment (132-153). Similarly, help by T cell clones specific for fragment 132-153 resulted in a higher proportion of antibody specific for fragment 132-153 than did help by polyclonal myoglobin-specific T cells. Also, myoglobin-specific B10.A(3R) cells, which, because of Ir gene control, do not make anti-fragment (132-153) when helped by myoglobin-specific T cells, do make antibodies with this fine specificity when helped by fowl gammaglobulin specific T cells cultured with myoglobin coupled to this carrier. We conclude that the helper T cell preferentially helps B cells which bind epitopes with certain steric relationships to the site bound by the T cell. One such steric constraint may be proximity. These findings explain how Ir genes for myoglobin, which control T cell epitope specificity can simultaneously control the fine specificity of antibodies made.

0590 IS MOPC-315 A MODEL FOR ANTIGEN SPECIFIC MODULATION OF THE $LYB3^+,5^+,7^+$ B CELL SUBSET? J.D. Kemp and J.W. Rohrer, Univ. of Iowa Coll. of Med., Iowa City, IA 52242 and Univ. of S. Alabama Coll. of Med., Mobile, AL 36688.

Recent studies have indicated that MOPC-315, an IgA, λ_2, anti-TNP secreting mouse plasmacytoma, is $Lyb3^+$(1). Further studies indicate that clone growth control requires macrophages and suggest that an H-2 restricted interaction only occurs between the T cells and macrophages (present evidence suggests that, for clone growth control, an H-2 restricted interaction exists neither between MOPC-315 and the macrophage nor between MOPC-315 and the T cell). These findings closely resemble those of Singer and Hodes with regard to the $Lyb5^+$ B cell subset (2) and are compatible with the model of Howard and Paul regarding the roles of monocyte and T cell derived factors in the control of $Lyb5^+$ B cell proliferation (3).

Interestingly antibody secretory control of MOPC-315 is apparently macrophage independent and is controlled by idiotype specific T cells (4). Soluble effector factors secreted by these cells may be the same (or related to) the TRF-like factors which influence B cell differentiation (3).

(1) J.D. Kemp, J.W. Rohrer, and B.T. Huber (1982) Immunol Rev 69:127.
(2) A. Singer and R.J. Hodes (1983) Ann Rev Immunol 1:211.
(3) M. Howard and W.E. Paul (1983) Ann Rev Immunol 1:307.
(4) J.W. Rohrer, R,K, Gershon, R.G. Lynch, and J.D. Kemp (1983) J Mol Cell Immunol 1:50.

0591 COMPARATIVE CHROMATOGRAPHIC PROPERTIES OF HUMAN B CELL GROWTH FACTOR AND GAMMA INTERFERON, Jean-Pierre Kolb, Juana Wietzerbin, Lili der Stepani and Rebecca Falcoff, Institut Curie, Paris, France.

The presence of B cell-growth factor (and other T cell-derived lymphokines) was tested in supernatants of human lymphocytes stimulated with PHA. In the course of the various steps of fractionation used for the enrichment of gamma interferon, both activities copurified on several matrix. For instance, they could be absorbed and then eluted from silicic acid beads, columns of Cibacron Blue Sepharose, Procion Red Agarose and Decyl Agarose. However, successful separation according to their differences in molecular weight was achieved by elution from polyacrylamide slab gels.

Thus, both molecules share a number of biochemical characteristics and these results stress the need for caution when ascribing physiological properties to a given lymphokine.

Besides, BCGF could be separated from human T cell replacing factor (TRF) by its chromatographic behaviour on Decyl Agarose, and from interleukin 2(Il-2) by the technique of gel elution

In conclusion, those four lymphokines, (IL-2, TRF IFNγ and BCGF) although displaying some common physico-chemical properties, could be readily dissociated on appropriate matrix.

0592 ROLE OF ANTIGEN-SPECIFIC B CELLS IN THE INDUCTION OF SRBC-SPECIFIC T CELLS, Barbara A Malynn and Henry H. Wortis, Tufts University School of Medicine, Boston, MA 02111.

In this study, we address the question of antigen presentation by antigen-activated B cells. The data indicate that optimal in vitro proliferation of T cells from mice primed with SRBC or HoRBC requires the presence of B cells primed to the relevant antigen. B cells prepared from lymph nodes of mice primed with irrelevant antigens are not effective antigen presenting cells for SRBC-specific T cell proliferation. This is true even when both SRBC and the antigen to which the B cells are primed are included in the culture. In contrast, B cells specific for a hapten determinant coupled to SRBC are able to induce proliferation of T cells specific for SRBC determinants. The requirement for specific B cells is retained in the presence of optimal concentrations of exogenous IL-1. Proliferation induced by B cells is blocked by addition of monoclonal anti-Ia antibodies. We conclude from these data that antigen specific B cells play a role in the induction of T cell proliferative responses to SRBC and HoRBC antigens. Two models are proposed: either B cells, upon antigen interaction with surface antibody, are able to act as accessory cells to induce Ia-dependent proliferation of immune T cells; or B cells augment the T cell proliferative response by secretion of antibody, leading to opsonization of the antigen for macrophage uptake and presentation.

0593 MHC-RESTRICTED COGNATE RECOGNITION OF ANTIGEN IS REQUIRED FOR T CELL ACTIVATION OF LYB5$^+$ CELLS, D. E. Mosier and A. J. Feeney, Fox Chase Cancer Center, Phila., PA 19111

One model of T cell-dependent antibody formation proposes that two distinct forms of T-B cell interaction occur and that they are determined by the phenotype of the B cell subset being activated. The Lyb5$^-$ B cell subset (present in CBA/N xid mice and normal mice) is postulated to be induced to antibody formation by MHC-restricted T-B interaction. The interaction between T cells and Lyb5$^+$ B cells (absent in CBA/N mice) is suggested to be MHC-unrestricted and factor-mediated.

We have tested the general applicability of this model using the in vitro antibody response to phosphorylcholine-keyhole limpet hemocyanin (PC-KLH). Lyb5$^-$ B cells from CBA/N mice do not make a primary IgM response to PC-KLH, so the anti-PC IgM response derives mainly (if not exclusively) from the Lyb5$^+$ B cell subset. KLH-specific T cell lines were shown to induce unprimed B cells from normal (but not CBA/N) mice to produce PC-KLH responses. These responses required hapten-carrier linkage and T cell requirement could not be replaced by T cell-derived factors. The interaction between T cells and B cells (as well as antigen-presenting cells) was dependent upon sharing the same MHC locus. The same KLH-specific T cell lines could help CBA/N B cells produce anti-TNP antibody when stimulated with TNP-KLH. These results show that Lyb5$^+$ B cells have the same requirements for MHC-restricted T-B interaction as Lyb5$^-$ B cells and, therefore, that the above model is not generally correct.

0594 MHC-RESTRICTED HELPER SIGNAL IN THE PFC RESPONSE OF ANTI-Ig-ACTIVATED B CELLS, Hans-Peter Tony and David C. Parker, University of Massachusetts Medical School, Worcester, MA 01605

In order to study B cell-T cell cooperation more closely, we set up a system of polyclonal B cell activation via rabbit-anti-Ig and cloned T helper cells reactive against the $F(ab')_2$ of normal rabbit Ig. We established a set of T cell hybridomas which produce IL-2 and induce B cell proliferation and Ig secretion in an antigen-specific and MHC-restricted way. These T cell hybrids help small B cells to polyclonal Ig secretion under conditions in which antigen nonspecific helper factors are insufficient: 1) upon stimulation with soluble $F(ab')_2$-αIgD, and 2) with low doses of $F(ab')_2$ αIgM. This system enables us to study T cell activation in T cell-B cell cooperation as well as different stages of B cell activation in low density cultures. Our results indicate that 1) anti-Ig and the T cell hybrids can provide all necessary signals for a small, resting B cell to proliferate and mature to high-rate Ig secretion, and 2) the response is proportional to the number of B cells and the amount of anti-Ig, and 3) the response is fully MHC-restricted at the T-B cell level for B cell proliferation as well as maturation to Ig secretion.

0595 MHC AND IgH RESTRICTION REQUIREMENTS BETWEEN T HELPER CELLS AND LYB-5$^+$ AND LYB-5$^-$ MEMORY B CELLS IN RESPONSE TO PHOSPHOCHOLINE (PC), Linda S. Wicker, Alfred Singer, and James J. Kenny, Merck Sharp & Dohme Research Laboratories, Rahway, NJ 07065, Immunology Branch, NCI, NIH, and the Uniformed Services University of the Health Sciences, Bethesda, MD 20814

The immune response to PC in normal mice is composed of antibodies derived from the two major B cell subsets, Lyb-5$^+$ and Lyb-5$^-$. Lyb-5$^+$ B cells produce T15$^+$ PC-specific antibodies primarily of the IgM and IgG3 classes whereas Lyb-5$^-$ B cells produce T15$^-$ antibodies which are predominantly IgG1 and IgG2(a + b). In order to study genetic restrictions in T helper cell activation of Lyb-5$^+$ and Lyb-5$^-$ B cells, we have utilized an in vivo adoptive transfer protocol. Keyhole Limpet Hemocyanin (KLH)-primed T helper cells and normal or xid B cells primed with PC-Hy (PC conjugated Limulus polyphemus hemocyanin) were transferred to irradiated recipients along with PC-KLH. IgM, IgG3, IgG1, and IgG2 anti-PC plaque-forming cell responses were analyzed seven days later. We have found that T helper cells and B cells need not possess identical genes at the IgH locus; such IgH-mismatched cells generate normal secondary anti-PC antibody responses in respect to idiotype and isotype. However, adoptive transfer of PC' $H\text{-}2^b$ and $H\text{-}2^k$ B cells and b x k --> k KLH' chimeric T cells revealed MHC restriction in the activation of IgG3, IgG1, and IgG2 memory B cells. Both Lyb-5$^+$ and Lyb-5$^-$ B cells producing these isotypes required an MHC-restricted T helper cell signal. In contrast, a large T15 predominant IgM response could be elicited in an MHC-unrestricted manner from PC' Lyb-5$^-$ B cells.

0596 ACTIVATION OF ANTIGEN-SPECIFIC B CELLS ISOLATED FROM UNPRIMED AND TNP-PRIMED MICE, Eitan Yefenof, E. Charles Snow, J.W. Uhr and E.S. Vitetta, University of Texas Health Science Center, Dallas, Texas 75235

Antigen (TNP)-binding cells from normal (ABC) and TNP-primed (PABC) mice were enriched and analyzed. 70-80% of the enriched cells bound TNP. Both cell populations expressed sIgM and sIgD. Following activation by thymus-dependent (TD) or thymus-independent (TI) antigens, ABC differentiated into IgM-secreting cells while PABC produced mostly IgG. Differentiation of both ABC and PABC in response to TI-TNP was dependent upon the addition of T cell-derived cytokines. However, optimal IgM responses in ABC required higher concentrations of antigen and cytokines than those required to induce IgG responses in PABC. In the presence of cytokines, anti-Ig antibodies could induce ABC to proliferate but not to differentiate into antibody-secreting cells. Under the same circumstances, PABC proliferated and differentiated into IgG-secreting cells. When TD-TNP was employed, ABC were activated to secrete anti-TNP IgM only under conditions of cognate recognition, i.e. in the presence of primed T cells and the appropriate carrier. On the other hand, when TD-TNP was employed, differentiation of PABC into IgG-secreting cells was accomplished by the addition of cytokines to the culture. The ability of PABC to differentiate in response to anti-Ig and TI-TNP may be attributed either to residual antigen-primed T cells "contaminating" the B cell cultures (and perhaps secreting high levels of cytokines) or to inherent differences in activation requirements of memory vs virgin B cells.

Subsets of Inducer Cells Stimulating B Cells, Ts Or Tc

0597 HELPER T CELLS FOR NATURAL KILLER (NK) ACTIVITY ARE PRESENT IN MOUSE PEYER's PATCHES (PP), Saverio Alberti*, M. Allegrucci, S.F. Spreafico and F. Colotta, Istituto Mario Negri, Milan, Italy (*at present: Stanford University, Stanford CA 94305)

NK activity regulation has been widely investigated. However, very little is known on NK helper cells. The present report evidences the presence of NK activity T helper cells in mouse PP. PP and intraepithelial large granular lymphocytes (IE-LGL) cells, demonstrated to be NK effectors, were obtained from C3H/HEN mouse gut. Input PP cell suspension, PP cells eluted from a nylon wool column and PP cells treated with carbonyl iron were added to IE-LGL in a 4 hr NK assay in PP/IE-LGL ratios from 0.1-100%. Carbonyl iron treated PP cells enhanced IE-LGL NK activity from 2- to 4-fold. Nylon wool eluted PP cells had no significant effect from input PP population reduced up to 50% IE-LGL cytotoxicity. Significant enhancements were evident even at carbonyl iron-treated PP/IE-LGL ratio of 0.1%. Freezing and thawing of PP cells abrogated NK enhancement. FACS sorting of Thy-1.2$^+$ and Thy-1.2$^-$ PP cells demonstrated that helper activity resided in Thy-1.2$^+$ cells. These results seem to indicate a presence of T cells in PP which can help NK activity once adherent phagocytic inhibitory cells are removed. Moreover, a NK regulatory system with both enhancing and depressing properties has been evidenced in mouse gut.

0598 IMMUNOREGULATORY T CELL INTERACTIONS IN MAN. Nitin K. Damle, Nahid Mohagheghpour and Edgar G. Engleman. Stanford University Medical Center, Stanford, Ca 94305, USA.

The human immune response is regulated by two nonoverlapping populations of mature T cells- the helper/inducer subset that expresses Leu 3/ T4 marker and the cytotoxic/suppressor subset that expresses Leu 2/ T8 marker. Recently we have used combinations of monoclonal antibodies to isolate subsets within each of the two lineages of T cells and study their interactions in the generation of antigen-specific suppressor T cells in alloantigen- or PPD-stimulated cultures. Antigen-primed Leu 3+ inducer T cells activated autologous Leu 2+ cells to differentiate into antigen-specific suppressor cells in the absence of priming antigen. Moreover, only Leu 2+ cells that lacked the 9.3 marker, an antigen expressed by the majority of T cells including precursors of cytotoxic T cells, differentiated into suppressor T cells. To analyze the inducer population, fresh Leu 3+ cells were separated into Leu 3+,8- and Leu 3+,8+ subpopulations using anti-Leu 8 monoclonal antibody. Although both Leu 3+,8- and Leu 3+,8+ cells proliferated in response to allogeneic nonT cells or PPD, and upon activation expressed comparable amounts of HLA-DR (Ia) antigens, the Leu 3+,8+ cells alone induced autologous Leu 2+ cells to differentiate into suppressor-effector cells. However, once activated, Leu 2+ suppressor T cells inhibited the antigen-induced response of both Leu 3+,8- and Leu 3+,8+ cells without altering kinetics of the response. These results indicate that autologous T-T interactions among phenotypically distinct subpopulations of T cells are required to generate antigen-specific suppressor T cells. (supported by NIH grants CA-24607, HL-13108, and grants from the Department of Health Services, State of California)

0599 EFFECT OF ANTI-LYT-2 AND ANTI-L3T4 MONOCLONAL ANTIBODIES ON THE INDUCTION OF ANTI-SELF + TNP AND ALLOSPECIFIC CYTOTOXIC T LYMPHOCYTES, Annick Guimezanes, Anne-Marie Schmitt-Verhulst and Michel Pierres, Centre d'Immunologie INSERM-CNRS de Marseille-Luminy, Case 906, 13288 Marseille cédex 09, France.

The respective role of Lyt-2 and L3T4 positive cells in the in vitro induction of cytotoxic T lymphocytes (CTL) was investigated using monoclonal antibodies (mAb) directed against these molecules. When present during the 5 days sensitization culture of T cells, anti-Lyt-2 mAb inhibited both the induction of anti-self + TNP and alloreactive CTL (whether the stimulating cells shared I region or not with the responding cells). By contrast, anti-L3T4 mAb inhibited only the induction of anti-self + TNP CTL and not the induction of allospecific CTL, whether these CTL were induced by stimulating cells differing for the whole haplotype or only for class I antigens (recombinant or mutant mice).

When present during the 3 days of mixed lymphocyte cultures, both anti-Lyt-2 and anti-L3T4 inhibited the proliferation of responding cells. The fact that the anti-L3T4 inhibition of proliferation influenced the induction of anti-self + TNP CTL but not that of allospecific CTL might indicate that the induction of CTL requires different pathways depending on the type of stimulation used. Experiments are in progress to analyse the respective roles of class I and class II antigens during the induction of allospecific (stimulated by KID- or K-different cells) and anti-self + TNP CTL.

0600 STIMULATION OF A SUBPOPULATION OF CELLS OBTAINED FROM THE SYNGENEIC MIXED LYMPHOCYTE REACTION, Elizabeth D. Johnson and Chella S. David, Mayo Clinic, Rochester, MN 55905

Long term bulk cultures of the syngeneic mixed lymphocyte reaction respond to Ia antigens expressed on syngeneic spleen cells. After limiting dilution, a subpopulation of cells are obtained that are stimulated by syngeneic spleen cells, syngeneic CON A/PHA lymph node blasts, and relatively high concentrations of autoreactive cells. They do not respond to unstimulated lymph node cells or allogeneic cells. Genetic analysis of the Con A/PHA response mapped the restriction to the I region. This stimulation is not due to the presence of residual Con A since the mitogen alone does not stimulate the cells. The stimulation of the cells with syngeneic spleen cells was inhibited with various monoclonal I-A and I-E antiserum (17-227, 10-2-16, 13-4 and 17-3-3) as has been shown for the bulk cultures. The response was also inhibited with an allo-anti-I-J serum. The response to the autoreactive cells was not significantly inhibited with any of the above monoclonal antiserum. When allo I region antiserum were tested, the stimulation by autoreactive cells was not inhibited with the anti-I-J serum, but was significantly inhibited with an anti-I-A and an anti-I-E serum. Current investigations are underway to try and separate the splenic and autoreactive stimulations and to characterize the phenotype of the respective responding populations.

0601 IMMUNOREGULATION BY LONG-TERM Th AND Ts CELL LINES, Judith D. Levich, William O. Weigle and D. Elliot Parks, Scripps Clinic and Research Foundation, La Jolla, CA 92037

Regulatory interactions involved in the initiation and termination of immune responses can be defined using long-term T cell lines having suppressor and helper function. Murine suppressor (Ts) and helper (Th) cell lines have been established which regulate the lymph node cell proliferative response (LNP) and the plaque-forming cell response to the protein antigen human gamma globulin (HGG). Experiments done to determine the genetic restriction of suppression have revealed that long-term Ts and their targets must be identical in the I-region to the left of I-C in order for suppression to occur. In addition, it is likely that suppression occurs by an interaction between cultured Ts and an inducer T cell, possibly a Th, based on two types of experiments. First, long-term Ts suppress such disparate functions as antibody secretion and LNP, indicating that a cell type common to both immune functions may be the target for suppression. Second, suppression of LNP is maximal when Ts are added at the initiation of the assay, suggesting that cultured Ts interact with an early-acting inducer T cell. Both these hypotheses are being tested using Th and Ts cell lines. Also being determined are the requirements for suppression of Th cell lines by Ts cell lines and the requirements for rendering Th tolerant to stimulation by HGG.

0602 EFFECTS OF LYMPHOKINES ON THE SPECIFICITY OF CYTOTOXIC T LYMPHOCYTE RESPONSES, Daniela Männel, Werner Falk, and Wulf Dröge, Institute for Immunology and Genetics, DKFZ, 6900 Heidelberg, F.R.G.

The specificity of cytotoxic T lymphocyte (CTL) responses was studied in cultures with limited helper potential. Thymic responder cells (10^5 to 10^6 per culture) or Nylon wool-purified spleen cells (10^5) were cultured with allogeneic or TNP-haptenated syngeneic or without stimulator cells for 5 days. The stimulator cells were either gamma irradiated or glutaraldehyde fixed. After 5 days of culture the cytotoxic response was measured in a 5 hr ^{51}Cr-release test on different target cells. Co-culture of thymic responders with irradiated splenic stimulator cells in the presence of semi-purified interleukin 2 (IL2) and T cell cytotoxicity inducing factor 2 (TCF2) preparations led to preferential cytolysis of targets that expressed the same H-2 as the stimulator cells. This preference was lost when preparations of T cell cytotoxicity inducing factor 1 (TCF1) were added to those cultures. Concomitantly, the requirement for stimulator cells was lost. This polyclonal activation was not due to contaminating mitogen. These data suggest the possibility that contact of the antigen receptor with antigen may not be a necessary signal for the activation of CTL precursor cells. The combined action of several soluble mediators appears to be sufficient for activation. If one or more of these mediators are limiting, the stimulator cell may be required to provide the(se) mediator(s) in close proximity, thus introducing specificity to the system.

0603 A HUMAN T CELL CLONE OF HELPER/INDUCER PHENOTYPE THAT SELECTIVELY ACTIVATES SUPPRESSOR CELLS, Nahid Mohagheghpour, Nitin K. Damle, Dilip K. Moonka, Candace P. Terrell and Edgar G. Engleman, Stanford University Medical Center, Stanford, CA 94305

Once activated by antigen, Leu 3+ (T4+) T lymphocytes provide help for the differentiation of B lymphocytes into immunoglobulin (Ig) secreting plasma cells and induce the generation of cytotoxic (Tc) and suppressor (Ts) cells from Leu 2+ (T8+) precursor cells. Recent studies of fresh T cells by us and others indicate that each of these inductive influences of Leu 3+ cells is mediated by a distinct Leu 3+ subpopulation. To define these regulatory activities at the clonal level, alloactivated Leu 3+,8+ T lymphoblasts were isolated after a 7-day mixed lymphocyte culture, cloned at 1 cell/well, and the clones expanded with IL-2 in the presence of the priming allogeneic cells. After one month, several clones were tested functionally and found to proliferate specifically to the original stimulator cells. One of these clones (SP-21) when cultured with fresh autologous Leu 2+ cells, induced the latter to suppress MLC between fresh autologous Leu 3+ cells and the original allogeneic stimulator cells. However, the SP-21 clone failed to provide help for the generation of Ig secreting B cell or allospecific Leu 2+ Tc cells. Finally, clone SP-21 exhibited no detectable allospecific cytotoxic activity and did not suppress the proliferative response of autologous Leu 3+ cells to the original stimulator allogeneic non-T cells. This functional profile has not changed during eight months of continuous culture, suggesting that clone SP-21 is a stable suppressor-inducer T cell clone.

0604 HELP FOR B AND T CELL RESPONSES IN VIVO MEDIATED BY AN ALLOSPECIFIC T-HELPER CLONE Trevor Owens, Nicholas R. J. Gascoigne and I. Nicholas Crispe, ICRF Tumour Immunology Unit, Department of Zoology, University College London, UK

Both B lymphocytes and cytotoxic T lymphocytes respond to signals from the T helper (Th) compartment. It is not known whether these activities are mediated by distinct Th clones, or whether individual Th cells can mediate several helper functions. We have addressed this question using in vivo systems for the assay of one Th clone (MTH-1). This clone carries the cell surface markers Thy 1.2 and L3T4a, but lacks Lyt 2. It recognizes a minor alloantigen shared by DBA/2,B10.D2 and NZB spleen cells, and such recognition is restricted by $H\text{-}2E^d$. Recognition of antigen in vitro is accompanied by secretion of IL-2 and interferon. In vivo, both primary and secondary CTL responses to multiple minor alloantigens are enhanced by small numbers ($>10^4$) of MTH-1 cells. Recognition of alloantigen in a T-depleted B cell population results in the polyclonal activation and maturation of the B cells to secrete immunoglobulin; also, antigen-primed B cells are augmented in their in vivo synthesis of specific antibody to the Thy 1.1 alloantigen by around 10^5 MTH-1 cells. Taken together, these results suggest that a single Th clone can help both B cells and T cells.

0605 ANTIGEN SPECIFIC HELPER ACTIVITIES AND T CELL SUBSET CIRCUITRY, Janet M.D. Plate, Rush Presbyterian St. Luke's Medical center, Chicago, Il. 60612.

Antigen specific helper T-cell products can support the generation of cytotoxic T-lymphocytes in exogenous helper-dependent assay systems. A main question that we have approached in the present study, is the relationship between our antigen-specific helper factors and nonspecific factors, especially interleukin-2, IL-2. First, we have demonstrated that allografts can prime T cells for the production of antigen specific helper factors before Il-2 is ever secreted/detected. Secondly, we have found that the antigen specific factor together with antigen can trigger the induction of IL-2 synthesis/release, in vitro. Thus, antigen specific soluble helper factors for Cytotoxic T-lymphocyte generation are involved in the initiation of the response, and as a consequence of such events, IL-2 is secreted. T cell hybridomas that do not secrete IL-2, even upon Con-A stimulation, but do secrete a factor(s) in a constitutive manner that results in the induction of IL-2 secretion in an antigen specific response have been isolated and are currently under study.

Immunoregulatory Dysfunction: Role In Autoimmune Disease

0606 DIABETIC AUTOANTIBODIES IN THE BB-RAT AND MAN RECOGNISE AN ISLET CELL PROTEIN OF M_r 64000, Steinunn Bækkeskov, Thomas Dyrberg, and Åke Lernmark, Hagedorn Research Laboratory, Gentofte, Denmark.

Alterations in both humoral and cellular immune response implicate immune factors in the etiology of the pancreatic β-cell destruction leading to insulin dependent diabetes mellitus in the BB-rat and man. We have previously demonstrated that sera from newly diagnosed IDDM patients containing islet cell surface antibodies (ICSA) to β-cells have autoantibodies against a M_r 64000 human islet cell protein. This protein is highly hydrophobic and therefore probably an intrinsic membrane component. Antibodies to this protein were present in a non-concordant twin already two years before he became diabetic. ICSA are present in the majority of BB-rats showing either morphologic or metabolic evidence of the diabetic syndrome. We have tested whether sera from diabetic BB-rats can immunoprecipitate rat islet cell proteins. Lysates of ^{35}S methionine labelled rat islet cells were subjected to immunoprecipitation with sera from 26 diabetes susceptible BB-rats (33-114 days old), 19 of which developed diabetes (71-109 days of age), 7 had impaired glucose tolerance and/or insulitis. Control sera were from 26 diabetes non-susceptible BB-rats and 8 normal Wistar rats. Sera from 25 susceptible BB-rats immunoprecipitated a M_r 64000 rat islet cell protein. In 6 animals followed prospectively antibodies to this protein appeared up to 8 weeks before the clinical onset of IDDM. In the control group sera from 6 non-susceptible BB-rats but none of the normal Wistar rats immunoprecipitated the M_r 64000 component. The results indicate that the M_r 64000 islet cell protein is a major target antigen for diabetic autoantibodies in both the BB-rat and man.

0607 MURINE MYASTHENIA GRAVIS: ROLE OF I-A MOLECULES, Premkumar Christadoss*, Sam Munro*, John Lindstrom†, Roger Melvold‡ and Norman Talal*, Clinical Immunology Section, Audie L. Murphy Memorial Veterans Hospital and Department of Medicine, UT Health Science Center, San Antonio, TX 78284, †The Salk Institute, La Jolla, CA 92212, ‡North Western University, Chicago, IL 60611.

The I-A subregion gene(s) influence susceptibility to experimental autoimmune myasthenia gravis (EAMG). In order to determine the importance of the immune response gene product, the Ia antigen, in EAMG pathogenesis, we studied the degree of EAMG susceptibility of an Ia mutant strain B6.C-H-2^{bm12} (bm12). The bm12 mutation has been localized to the beta I-A polypeptide chain. The bm12 lacks the Ia.8 and Ia.39 determinant and expression of I-A molecules as compared to parent B6. The bm12 and B6 were immunized twice with acetylcholine receptors (AChR) in complete Freund's adjuvant and assayed for lymphocyte proliferation, autoantibody titer and carcass muscle AChR content. As shown in the table, mutation of the I-A molecule can convert an EAMG susceptible strain into a relatively resistant strain. The relative resistance of EAMG induction in the bm12 is due to lack of Ia.8 and /or Ia.39 determinants and/or quantitative expression of I-A antigen.

Strain	Lymphocyte Proliferation (S.I.± SEM)	Autoantibody ($\times 10^{-10}$ ±SEM	% Muscle AChR loss (mean)	Clinical EAMG
B6/Kh	17.2±1.5	18.1±4.8	41.9	3/11
bm12	10.2±1.8	5.1±3.1	15.9	0/10
	($p<0.01$)	($p<0.01$)		

0608 T-CELLS AND THE REGULATION OF AN INDUCED AUTOIMMUNE STATE, Anne Cooke, Paddy Hutchings, Anne-Marie Varey and Brian R. Champion, Middlesex Hospital Medical School, London W1P 9PG, England.

We have studied the induction and regulation of induced erythrocyte autoantibodies in mice. Normal mice make erythrocyte autoantibodies when challenged with high doses of rat RBC. These mice also develop suppressor T-cells which are capable of suppressing the induction of autoantibodies in naive recipients. We have shown that such T-cells do not act as suppressor effectors but as inducers of suppression in the recipients.

We have recently investigated the regulation of induced erythrocyte autoantibodies in SJL mice, a strain known to be highly susceptible to the induction of both thyroiditis and a relapsing form of experimental allergic encephalitis, and also to be defective in tolerance induction. We find that this strain develops high levels of autoantibody and also is incapable of generating effective suppression of induced autoantibodies on transfer to naive SJL recipients. In order to further investigate the role of T-cells in induction and regulation of the induced autoimmune state we have isolated T-cell lines from normal and SJL mice immunised with rat RBC. The properties of these cell lines will be discussed.

0609 GENETIC CROSSES BETWEEN AUTOIMMUNE (NZB) AND NORMAL (SWR) MICE PRODUCE NEPHRITOGENIC ANTI-DNA AUTOANTIBODY IDIOTYPES THAT ARE INHERITED FROM THE NORMAL PARENTS. Syamal K. Datta, Jerrie Gavalchin, Janice Nicklas, and Robert S. Schwartz. Tufts University School of Medicine, Boston, MA 02111.

The major manifestation of autoimmune disease in NZB mice is hemolytic anemia; the incidence of nephritis in this strain is low and delayed. In marked contrast, when NZB mice are crossed with normal SWR mice, 100% of the F_1 hybrids rapidly develop a lethally severe glomerulonephritis. We have analyzed and compared 70 monoclonal anti-DNA antibodies derived from the F_1 hybrids and their NZB parents. One set of F_1-derived autoantibodies bearing the allotype of the normal SWR parent, are IgG_2b in isotype and have distinctive antigen binding specificity and isoelectric focussing patterns with cationic pI. Although they were derived from different F_1 individual animals, they share a crossreactive idiotype. Thus anti-DNA antibodies encoded by genes of the normal SWR parent are expressed due to some cellular and genetic deregulation upon crossing with the NZB strain and contribute to the high incidence of severe nephritis in the F_1 hybrids.

0610 IMBALANCE OF LYT-2^+ CELL SUBSETS IN AUTOIMMUNITY-PRONE MOUSE STRAINS, Francis J. Dumont, Merck Sharp & Dohme Research Laboratories, Rahway, NJ 07065

The Lyt-2^+ cell population plays a crucial role in immunoregulation. Functional Lyt-2^+ subsets can be defined on the basis of Lyt-1 expression by cytotoxicity but not by flow cytofluorometry (FCF) analysis. We have produced a xenogeneic monoclonal antibody (9F3) detecting an 85,000 Mr cell surface protein that clearly distinguishes two subsets of Lyt-2^+ cells by means of two-color FCF analysis. Evidence was obtained that these subsets do not correspond to the classical Lyt-1^+ 2^+ and Lyt-1^- 2^+ subsets. In immunologically normal strains, the proportions of the 9F3 subsets among the Lyt-2^+ population (60% $9F3^-$, 40% $9F3^+$) as well as their absolute numbers in peripheral lymphoid tissues remained constant throughout aging (2-14 months). In contrast, in mice with genetically determined propensity to develop systemic autoimmunity, we observed a shift in the relative proportions of these subsets ($9F3^+$ cell augmentation, up to 90%) that was similar in all autoimmune strains examined although it reflected different numerical evolutions depending on the strain. In mice of the lpr/lpr genotype, there was an expansion of the $9F3^+$ cells. In BXSB males and NZB x NZW females, there was both a limited expansion of $9F3^+$ cells and a decrease of $9F3^-$ cells. In aging MRL +/+ females, there was only an absolute decrease of $9F3^-$ cells. The net effect of these alterations is an imbalance of 9F3-defined Lyt-2^+ subsets. Since this abnormality is common to autoimmune strains, it may be related to immunoregulatory defects contributing to autoimmunization. Functional characterization of 9F3-defined Lyt-2^+ subsets is underway to test this hypothesis.

0611 MONOCLONAL ANTIBODIES AGAINST THE ACETYLCHOLINE RECEPTOR (AChR) PLUS AChR ENHANCE THE ACTIVATION OF AChR-SPECIFIC T CELL LINES, Donard S. Dwyer, Wolfgang E.F. Klinkert and Berthold C.G. Schalke, Max-Planck-Gesellschaft, Klinische Forschungsgruppe für Multiple Sklerose, D-8700 Würzburg, Federal Republic of Germany.

Rat T cell lines have been generated which recognize AChR from Torpedo californica in the context of self Ia antigen. These T cells bear the $W3/25^+$ inducer phenotype and can provide help for in vitro production of antibody against AChR. Normally, the T cell lines are stimulated by optimal concentrations of AChR presented by irradiated syngeneic thymocytes. Suboptimal concentrations of antigen which alone produce little activation of the T cell line can stimulate proliferation in the presence of certain anti-AChR monoclonal antibodies (mAbs) The enhanced activation depends on the isotype of the mAb that is used: IgG 2b is effective while IgG 2a does not facilitate stimulation. Isotype preference and the ability to enhance activation are properties of the antigen presenting cells (dendritic cells). The observed phenomenon is antigen specific, and in fact, the complexes of mAb plus AChR may actually suppress the proliferative response of irrelevant T cells to some extent.

These findings may be relevant to T cell-dependent antibody responses in general and to the development of autoimmune diseases, especially myasthenia gravis. Thus, low levels of self-antigen together with small amounts of autoantibody (produced as a consequence of idiotypic networks or polyclonal activation of B cells?) may combine to initiate an autoimmune response by T lymphocytes, particularly in susceptible individuals.

0612 ABNORMAL T-B LYMPHOCYTE COLLABORATIONS IN SYSTEMIC LUPUS ERYTHEMATOSUS, M.K.Harikumar, R.A.Knight and M.L.Snaith, Univ. College London, London, ENGLAND

Abnormal collaborations between T lymphocytes and B lymphocytes in the elicitation of autoantibodies has been suggested in the case of autoimmune disease (P.A.Bretcher,1973 Cell Immunol. 6,1).Recent evidence has shown that allogeneic T lymphocytes induce autoantibodies similar to that found in Systemic Lupus Erythematosus, from normal mice following chronic Graft versus Host disease(Rolink & Gleichmann 1983, J.Exp.Med. 158,546).We have looked at the ability of T lymphocytes from SLE patients to collaborate with allogeneic ER- cells in an antigen specific antibody prod.system.We have used the Influenza virus A/X31 (H3N2) as antigen and used microELISAs to assay for Flu specific antibody(Zanders et al J. Immunol Meth. 1981, 47, 333) and to assay for anti s.sDNA (Pietsky&Peters, J. Immunol. Meth.1981,41 187).We have found that 11/27 SLE patients T lymphocytes collaborate with normal ER- cells to elicit anti Flu antibody. None of the 20 normal allogeneic collaborations elicited anti Flu antibody nor did they produce any anti DNA antibody. However low dose X-irradiation normal allo T lymphocytes were able to elicit anti Flu antibody but not anti DNA antibody from normal ER- cells. Examination of supernatants from cultures of 5 allogeneic collaboration, with SLE ER- cells as responders,showed the presence of anti ssDNA antibody.This evidence suggests that abnormal T-B interaction may be involved in autoantibody production in Systemic Lupus Erythematosus.

0613 LY-1 B: A FUNCTIONALLY DISTINCT B CELL SUBPOPULATION, Kyoko Hayakawa, Richard R. Hardy Alfred D. Steinberg and Leonore A. Herzenberg, Stanford University, Stanford, CA 94305

We recently demonstrated that the pan-T cell surface antigen, Ly-1, is found on a subpopulation of B cells, termed Ly-1 B. Further characterization showed that Ly-1 B express B lineage cell surface antigens, but do not express other T cell antigens. The population is found in spleen but not in lymph nodes or bone marrow and appears early during development so that Ly-1 B constitutes a significant fraction of the B cells in newborn (3-5 days) mice but decreases to a minor population in adults. Certain autoimmune mouse strains (NZB) have increased levels of Ly-1 B (particularly increasing with age),and this enlarged population is unusual in that Ly-1 B from these mice secrete large amounts of IgM into the supernatant when cultured in the absence of exogenous antigen. Moreover, such secreted IgM contains autoantibody, binding to thymocytes or ssDNA. Most strikingly, we have found that Ly-1 B are exclusively responsible for a particular type of autoantibody that lyses bromelain treated mouse erythrocytes. In contrast, the response to most exogenous antigens such as SRBC, DNP-KLH and TNP-Ficoll does not come from Ly-1 B. Furthermore, Ly-1 B are significantly enriched in peritoneal exudate compared with spleen. Analysis of Ly-1 B in PEC reveals frequency differences among various strains of mice: NZB mice have the highest levels; most mice have an intermediate level; the SJL strain shows a distinctively low level; and xid mice totally lack PEC Ly-1 B. The finding of high levels of this unusual B cell population in peritoneal exudate and the strain differences of the PEC Ly-1 B level suggests a key role for Ly-1 B in the context of antigen presentation and in the induction of regulatory cells.

0614 LIMITING DILUTION ANALYSIS OF INTERLEUKIN 2 AND COLONY STIMULATING FACTOR PRODUCER CELLS IN NORMAL AND AUTOIMMUNE MICE, Steven H. Hefeneider, Paul J. Conlon, Steven K. Dower, Alan R. Alpert, Christopher S. Henney and Steven Gillis.

MRL/MP lpr-lpr (MRL-lpr) mice spontaneously develop an age related disease characteristic of human systemic lupus erythematosus. Old MLR-lpr mice (4 months of age) develop antibodies to nucleic acids, display immune complex glomerulonephritis, and have a massive T cell associated lymphadenopathy. Several investigators have observed an age related loss of interleukin 2 (IL-2) production by mitogen stimulated lymphoid cells from MRL-lpr mice which develops in concert with the onset of autoimmune disease. The loss of IL-2 production has been suggested to be involved in the development of autoimmune disease seen in these animals. In this report we examined the frequency of both IL-2 and colony stimulating factor (CSF) producer T cells in the MRL-lpr mouse using a limiting dilution analysis assay. Our results showed that the total number of IL-2 and CSF producer cells present in autoimmune animals was similar if not greater than the number of identical lymphokine producer cells found in normal control mice. In addition, IL-2 and CSF producer T cells from autoimmune MRL-lpr mice produce similar levels of lymphokine activity as did producer T cells from normal mice. These data argue forceably against the previously hypothesized role that a paucity of IL-2 production may be involved in the etiology of autoimmune disease. To the contrary, it might appear that IL-2 production and/or use may contribute to the development of experimental autoimmune phenomena.

0615 COLLAGEN TYPE II SPECIFIC T CELLS MEDIATING SYNOVITIS IN DBA/1 MICE, Rikard Holmdahl, Lars Klareskog, Kristofer Rubin and Hans Wigzell, Dept of Immunology, BMC, S 75123 Uppsala, Sweden

Collagen type II, the major component of joint cartilage, has been shown to be arthritogenic (D. Trentham, J. exp. med. 146:857, 1977). Immunization in incomplete Freunds adjuvans induces polyarthritis in rats and mice.
We have shown that this autoimmune arthritis depends on the presence of functional T-lymphocytes since nude, thymusdeficient, rats did not develop collagen induced arthritis.
Furthermore, with the help of immunohistochemical staining techniques we could show that the cell infiltration in the arthritic joints of rats is mostly composed of macrophages and T helper cells (Clin exp Immunol 51:117, 1983).
In order to more precisely investigate the role of T cells in collagen induced arthritis we have established long term cultures of T helper cells specific for collagen type II from the high responder DBA/1 mouse strain.
None of our T cell lines are crossreactive with collagen type I or synthetic collagen polypeptides; they recognise other determinants than the proline-glycine dipeptide moiety. One of the cell lines, analysed for specificity, contains cells recognising collagen type II from several species (bovine, chick, rat, mouse). Clones from this line distinguish between rat and mouse collagen. When $5x10^6$ or more line cells are injected intraperitoneally or subcutaneus in irradiated (500 rad) DBA/1 mice the recipients develop synovitis with lymphocyte infiltration after 7 days.

0616 PARTICIPATION OF CLASS II ALLOANTIGEN STIMULATED CELLS IN REGULATION OF CLASS I ANTIGEN DISPARATE THYROID GRAFT REJECTION, Noah Isakov and Fritz H. Bach, University of Minnesota, Minneapolis, MN 55455

Primary in vitro alloactivation of T lymphocytes by class I and class II alloantigens activate cytotoxic T cells and helper T cells respectively. Collaboration between these two subpopulations lead, in vitro, to an efficient class I alloantigen specific cytolytic response. Using a sensitive organ transplantation system in mice, we tested whether in vivo alloactivation of Th cells by class II alloantigens may potentiate the specific immune response against class I antigen disparate thyroid grafts. We found that (B6xA.TL)F_1 mice reject only 25% of grafts from class I antigen disparate bml or (bmlxA.TL)F_1 donor mice. In contrast class I-II antigen disparate (bmlxA.TH)F_1 thyroid grafts were rejected by more than 80%. When (B6xA.TL) F_1 mice were transplanted with bml thyroid grafts under the left kidney capsule, and (bmlxA.TH) F_1 under the right kidney capsule, all bml thyroid grafts were rejected. Similar experiments in other combinations of strains indicated that presentation of the class I alloantigen in association with class II alloantigen of one allele, but not of a different allele, will induce class I antigen disparate thyroid graft rejection. The results demonstrated that: 1) thyroid grafts express immunogenic class I and II molecules; 2) presentation of class II alloantigens in association with class I alloantigens may potentiate in vivo rejection of class I antigen disparate grafts; and 3) class II molecules may have a regulatory role in determining response intensity against class I alloantigens.

0617 IDIOTOPE MEDIATED REGULATION OF A CROSSREACTIVE IDIOTYPE IN MRL/1 MICE. A. Marshak-Rothstein, D. Parker and T. Rothstein, Department of Microbiology, Boston University School of Medicine, Boston, MA 02118.

Monoclonal anti-idiotope antibodies (anti-Ids) directed against the major crossreactive idiotype characteristic of the A/J anti-azophenylarsonate response have been shown to be potent immunosuppressive reagents. In order to clarify the physiological mechanisms responsible for this type of suppression, we have examined the regulatory capacity of our anti-Ids in a variety of immunocompromised situations. MRL/1 mice develop an accelerated SLE-like syndrome associated with lymph node enlargement and the production of a variety of autoantibodies. The capacity of anti-Ids to suppress Id expression in the context of the MRL/1 disease state was determined by producing backcross (BC) mice which were phenotypically MRL/1 and carried the A/J allotype. Administration of anti-Ids to these BC mice failed to suppress Id expression. The ineffectiveness of these reagents may be explained by our finding that mice with the MRL/1 phenotype show an accelerated clearance of anti-Id from the circulation when compared to littermate controls. This rapid clearance cannot be attributed to complexing of the anti-Id to preformed Id present in the sera as a result of polyclonal B cell activation; examination of preimmune sera failed to detect serum Id levels in an assay sensitive to 10ng/ml. Other explanations for the accelerated clearance of anti-Id are under investigation.
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0618 VARIANTS OF HLA-DR-ASSOCIATED Ia MOLECULES EXPRESSED IN PATIENTS WITH HLA-DR4-ASSOCIATED DISEASE, Gerald T. Nepom, Barbara S. Nepom, Jane G. Schaller, and John A. Hansen, Fred Hutchinson Cancer Research Center, Puget Sound Blood Center, Genetic Systems Corporation, and the University of Washington School of Medicine, Seattle, WA 98104

The expression of specific Ia molecules present on HLA-DR4-associated haplotypes was investigated on cells from patients with juvenile rheumatoid arthritis, a HLA-DR4-associated disease. The hypothesis that MHC molecules may function directly as Ir genes for immune dysfunction in this disease was tested by a structural analysis of the major Ia (DR and DS/DC) products, in normal individuals and in patients with JRA.

Using 2-D gel electrophoresis, distinct structural variations were found for the products of both DR and DS loci among homozygous DR4 cell lines. Thus, HLA-DR4 is not a single haplotype. Among 17 normal lines, 5 DR variants and 3 DS variants were observed. In contrast, among 9 patients with JRA homozygous for DR4, all gave strikingly similar patterns. Further characterization identified a rare and subtle expression of a particular HLA-encoded specificity in 7/9 of the JRA patients. These data suggest that the known association of HLA-DR4 for JRA may indeed be due to an Ir gene-type effect contributed by particular distinct Ia molecules, and not to the DR4 specificity itself.

0619 THE CONVERSION OF A TOLEROGENIC TO AN IMMUNOGENIC SIGNAL BY A LYMPHOID DENDRITIC CELL-LIKE TUMOR LINE, Richard P. Phipps, P.S. Pillai and David W. Scott, Immunology Unit, University of Rochester Cancer Center, Rochester, N.Y. 14642

The P388AD.2 lymphoid dendritic cell-like tumor line can present the tolerogen, fluoresceinated-sheep gamma globulin (FL-SGG), in an immunogenic fashion to B cells. Using an in vitro culture system, we demonstrated that tolerogen-pulsed P388AD.2 cells, but not the macrophage-like P388D1 cells, could specifically enhance (up to 400%) a plaque forming cell (PFC) response to challenge with a FL-thymic independent (TI) antigen. The increased responsiveness was specific for the FL-hapten and occurred only when P388AD.2 cells were pulsed with FL-immunoglobulins but not with synthetic FL-tolerogens or other FL-antigens. The augmentation required the presence of histocompatible T-cells. In addition, if cultures were depleted of $Lyt1^+$ but not $Lyt2^+$ cells, no augmentation occurred. We have also attempted to convert a tolerogenic signal into an immunogenic one in an in vivo system using tolerogen-pulsed P388AD.2 cells. Normal mice were injected intravenously with culture medium, soluble FL-SGG, FL-SGG pulsed or unpulsed P388AD.2 cells. Four days later the spleens were removed and spleen cells were challenged with FL-TI antigen. In complete agreement with results from our in vitro system, we found that tolerogen-pulsed P388AD.2 cells were able to specifically increase the PFC response 2 to 4 fold over control values. These data imply that if tolerogen is presented by the appropriate accessory cell, it may be converted into an immunogen. We postulate that the presentation of tolerogen or autoantigen by cells possessing the characteristics of lymphoid dendritic cells may initiate a course of autoimmune disease. Supported by an Arthritis Foundation Fellowship (RPP) and NIH Grant AI-20757 (DWS).

0620 HLA-DR BEARING T-LYMPHOCYTES IN DISORDERS OF IMMUNOREGULATION, S. H. Pincus, D.O. Clegg, and J.R. Ward, Univ.of Utah School of Medicine, Salt Lake City, Utah, 84132.

It has been demonstrated that T-cells bearing HLA-DR antigens arise in response to immune stimulation. In vitro this is seen when T-cells are incubated with mitogens. In vivo, a rise in circulating T-cells bearing this marker is found following a booster immunization, after an infection, and in diseases involving the immune system. We have found that in normal individuals fewer than 5% of circulating T-cells express HLA-DR antigens visible by indirect flourescent microscopy. In rheumatoid arthritis, gluten sensitive enteropathy, dermatitis herpetiformis, and normal individuals of the HLA B8/DR3 haplotype, the number of these T-cells is greater than 10%. Using thymidine incorporation we have demonstrated that HLA-DR+ T-cells are not proliferating at a higher rate than HLA-DR- T-cells. RNA hybridization studies using an HLA-DR alpha chain gene probe indicate that these cells synthesize, rather than adsorb, the DR antigen. Double immunoflourescence studies indicate that HLA-DR+ T-cells invariably express the following cell surface antigens: TAC-1 (the interleukin-2 receptor), SB (a class II MHC antigen), T3, and J2 (a 26K glycoprotein found on T-cell blasts). Studies with anti-T4 and T8 revealed that HLA-DR+ T-cells are predominantly of the T4 phenotype. But, in several patients a significant proportion of DR+ T-cells expressed both T4 and T8 antigens. The T4+/T8+ T-cells also expressed the T-6 (thymocyte) antigen. This population of T-cells may represent an immature set of T-cells in transit from the thymus to the active site of inflammation.

0621 IDIOTYPIC RELATIONSHIPS OF A MONOCLONAL ANTI-Sm ANTIBODY, David S. Pisetsky, Durham Veterans Administration Hospital, Durham, NC 27705

The expression of antibodies to the Sm antigen, a small nuclear ribonucleoprotein particle, is characteristic of systemic lupus erythematosus (SLE). To elucidate genetic mechanisms determining this response, the idiotypic relationships of a monoclonal anti-Sm antibody derived from an autoimmune MRL-lpr/lpr mouse have been studied. Y2, an IgG2aK anti-Sm antibody, bears a common idiotype that is present in sera of MRL-lpr/lpr mice independent of the presence of anti-Sm. This idiotype is also spontaneously present in sera of several normal mouse strains with absorption experiments demonstrating that the specificity present in normal mice is the same as that present in the MRL-lpr/lpr mice. Survey of other monoclonal antibodies has demonstrated a shared idiotype between Y2 and another MRL monoclonal antibody, 4K1, which is IgG2bK. 4K1, however, binds to a perinuclear antigen distinct from Sm. These results suggest that antibodies to Sm are derived from an idiotype family widely found among mouse strains and expressed normally. Despite this pattern of expression, this idiotype family may, however, in the setting of autoimmunity, preferentially give rise to antibodies with autoantibody activity. These results further suggest that if idiotype interactions are involved in regulating the anti-Sm response, they occur by determinants other than the common Y2 idiotype.

0622 **HAPTEN-SPECIFIC T CELL RESPONSES IN THE MRL/Mp-lpr/lpr MOUSE, Charles F. Scott, Jr., Makoto Tsurufuji, Christopher Lu and Man-Sun Sy, Department of Pathology, Harvard Medical School, Boston, MA 02115**

We have studied T-cell mediated responses to the simple haptens trinitrophenyl (TNP) and azobenzenearsonate (ABA) in the autoimmune MRL/Mp-lpr/lpr (MRL-l) mouse and in the congenic MRL/Mp-+/+ (MRL-n) mouse. We found that MRL-l mice, when compared to age-matched MRL-n mice, were deficient in their ability to mount cytolytic T lymphocyte (CTL), proliferating T cell (Tp) and delayed-type hypersensitivity cell (T_{DH}) mediated responses to these haptens. Furthermore, we observed that hapten-specific CTL responses in the MRL-l mouse could be boosted to levels seen in the MRL-n mouse by the in vitro addition of Interleukin-2 (IL-2). However, no such restoration of Tp responses by in vitro IL-2 was observed in the MRL-l mouse, nor could the in vivo administration of IL-2 restore T_{DH} responses in these mice. Our results implied a difference between the CTL on the one hand, and the T_{DH} and Tp on the other, in their ability to utilize exogenous IL-2 in the MRL-l mouse.

0623 ABNORMAL SURFACE PHENOTYPE OF T CELLS OF LYMPHOPROLIFERATIVE MRL/Mp-lpr/lpr MICE, Fumio Takei, Pathology, University of British Columbia, and the Terry Fox Laboratory, B.C. Cancer Research Centre, Vancouver, B.C. V5Z 1L3, Canada.

The MRL-Mp-lpr/lpr (MRL/l) mouse develops T cell lymphoproliferative syndrome which is associated with a systemic lupus erythrematosus-like autoimmune disease. The surface antigen profile of proliferating T cells was characterized by rat monoclonal antibodies recently produced in this laboratory. They include anti transferrin receptor (YE1/9.9), YE3/19.1 which reacts with an antigen (m.w. 14,000-18,000) on activated T and B cells, YE1/7.1 which reacts with a subpopulation of fetal thymocytes as well as in vitro activated immature thymocytes, and YE1/19.1 which defines a PC-1-like antigen (m.w. 115,000 dimer) expressed on EL-4 and NS-1. The majority of lymphocytes from the enlarged lymph nodes of MRL/l mice reacted with YE1/7.1 and YE1/19.1. In contrast, lymphocytes from the congeneic MRL/n mice, which lack the lpr gene and which do not develop the lymphoproliferation syndrome, did not react with these antibodies. This Lyt-1^+ YE1/7.1^+ YE1/19.1^+ surface phenotype is expressed by EL-4 but not other cell lines or any normal lymphocyte populations so far tested. Moreover, the majority of the MRL/l lymph node cells did not express the transferrin receptor or YE3/19.1 antigen which are strongly expressed on activated and proliferating normal lymphocytes. These results suggest that the cells in the enlarged MRL/l lymph nodes are different from normal T cells and they may represent a unique T cell subpopulation. Since they seem to be non-proliferating, the enlargement of the lymph nodes may be due to the excessive production of these cells from their precursors.

0624 ANTI-IA ANTISERA PROTECTS AGAINST THE INDUCTION OF TYPE II COLLAGEN ARTHRITIS, Paul H. Wooley, Harvinder S. Luthra, William P. Lafuse, John M. Stuart and Chella S. David, Department of Immunology, Mayo Clinic, Rochester, Minnesota 55905

The intradermal injection of native Type II collagen in adjuvant into mice induces an experimental mode of arthritis. Susceptibility to collagen-induced arthritis (CIA) is regulated by the MHC genes and the species source of collagen. In mice of the H-2^q haplotype, the CIA susceptibility gene has been mapped to the I-A region. These mice are susceptible to arthritis induced by chick, bovine, rat and human Type II collagen, while mice bearing H-2^r I region genes are only susceptible to Bovine Type II collagen. The injection of alloantisera or monoclonal antibody directed against I region gene products prior to immunization with Type II collagen reduced the incidence of CIA in both B10.Q (H-2^q) and B10.RIII (H-2^r) mice compared to controls. Monoclonal antibody 25-9-17 and alloantiserum MI 11 (anti-Ia.5) pretreated B10.Q mice had a CIA incidence of 10/31 and 5/16 respectively, a significantly lowered disease incidence compared to untreated B10.Q mice (19-29) or mice pretreated with monoclonal antibody MKD6 (4/6). Alloantiserum MI 112 (anti-Ia.1,2,3,7) and monoclonal H10-93.2 significantly reduced the CIA incidence in B10.RIII (4/14 and 0/5 respectively) compared to untreated controls (13/17). The DTH response to Type II collagen was not reduced b anti-Ia treatment, however the antibody response to Type II collagen was delayed, being significantly reduced at 14, but not 28 days after immunization.

0625 INDUCTION OF AUTOREACTIVITY BY ANTI-IDIOTYPIC ANTIBODIES, M. Zanetti, J. Rogers and D. H. Katz, Medical Biology Institute, La Jolla, CA 92037.

Anti-idiotypic (anti-Id) antibodies (Abs) have been shown to be potent modulators of the immune response. Anti-Id can substitute the antigen and induce Abs which share the idiotype (Id') and/or the antigen binding specificity (Ab1') with the antibody (Ab1) against which they had been originally generated. Whether similar rules apply to regulation of the immune response to self antigens has not been verified yet. To test this hypothesis, we immunized naive mice and rats with purified rabbit Abs against the Id of a mouse monoclonal Ab (mAb) which recognizes a highly conserved, hormonogenic site, on a classical autoantigen, thyroglobulin (Tg). By competitive inhibition of the binding of mAb62 to homologous anti-Id, 92% of anti-Id62 manipulated BALB/c mice were found to produce Id' (inhibition from 25 to 88%). About 50% also produced autoantibodies to Tg. In contrast, control mice challenged with non-anti-Id62 Ig, failed to produce Id' and autoantibodies to Tg. Similarly, immunization with anti-Id62 induced Id' and autoantibodies to Tg in 5/5 rats of the BUF strain. To better understand the relationship between autoantibodies to Tg and Id' elicited in normal animals by anti-Id62, an idiotypic analysis was carried out on 3 Tg-specific mAbs derived from an anti-Id62 manipulated mouse. Competitive inhibition studies showed that anti-Id62-induced autoantibodies were idiotypically very similar to the original mAb62 and suggest that these autoantibodies are likely to be of the Ab1' type. Our results suggest that anti-idiotypic responses can play a relevant role in termination of natural tolerance to self antigens as well as in the prevention of reconstitution of tolerance once this has been broken by other means. Supported by a grant-in-aid from QUIDEL.