T AND B LYMPHOCYTES: RECOGNITION AND FUNCTION

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Membrane Structure and Transduction of Signals Across Membranes

594 ROLE OF THE STRUCTURE OF THE PLASMA MEMBRANE'S CYTOPLASMIC FACE IN LYMPHOCYTE FUNCTION. Michael J. Crumpton, Alan P. Johnstone and Michael J. Owen, National Institute for Medical Research, Mill Hill, London NW7 1AA, U.K.

Regulation of lymphocyte behaviour by the cell surface structure may be regarded as comprising three stages, namely interaction, transmission and modulation. The initial regulatory event is the interaction of antigen, mitogen and/or "soluble factors" with a specific receptor(s) exposed on the cell surface. The information generated by this interaction(s) is next transmitted across the surface membrane into the cell interior. The third stage, modulation, corresponds to the redistribution ("patching" and "capping") of surface components induced by multivalent ligands; this redistribution is most probably mediated by intracellular contractile proteins. In respect of information transfer and modulation, transmembrane glycoproteins are especially favoured candidates. Thus, since they are in contact with both the extra- and intra-cellular compartments, they provide a potential channel by which interactions with ligands at the outer surface and with contractile proteins at the inner surface can be directly expressed as structural changes at the inner and outer surfaces respectively. If the above analysis is correct, then the delineation of how the lymphocyte surface regulates lymphocyte behaviour requires knowledge of the orientation of the receptors within the membrane and of the structure of the membrane's cytoplasmic face.

Studies of the orientation of lymphocyte membrane glycoproteins and of the structure of the inner membrane surface were based on the availability of sealed, "inside-out", plasma membrane vesicles. Transmembrane glycoproteins were identified by comparing the patterns of polypeptides labeled by lactoperoxidase-catalyzed iodination of the outer and inner membrane surfaces, and by immune precipitation of deoxycholate-solubilised, ^{125}I-labeled inside-out vesicles. Positive evidence was obtained for human histocompatibility (HLA) A, B and DRw antigens having a transmembrane orientation whereas in contrast, membrane-bound human IgM as well as mouse IgM, IgD and Thy-1 antigen were not labeled on the inner membrane surface. Antisera raised by immunizing with the inside-out membrane vesicles contained antibodies against several non-glycosylated membrane-associated proteins, including actin and albumin, as judged by immunofluorescence, immunoprecipitation and crossed immunoelectrophoresis. Evidence will be presented in support of albumin being located on the inner surface of pig mesenteric lymph node lymphocytes and human B lymphoblastoid cells.

595 IMMUNE RESPONSES TO MODIFIED CELL SURFACE ANTIGENS, R. W. Baldwin[1] and V. S. Byers[2] Cancer Research Campaign Laboratories, University of Nottingham, U.K.[1] and Dept. of Dermatology, University of California, San Francisco[2]

One of the components in the immunological repertoire mediating rejection of neoplastic cells involves T lymphocyte recognition of tumor associated antigens. The finding, therefore, that most naturally occurring (spontaneous) tumors do not express tumor rejection antigens, at least at levels sufficient to elicit significant responses (1), implies that alternative methods have to be sought to increase T cell recognition of tumor cells. Firstly, it has been proposed that the failure of tumor antigens, such as oncofetal antigens, to initiate tumor rejection responses may be related to their membrane instability leading to shedding (2). Consequently one approach to increasing immunogenicity has been to modify tumor cells so that plasma membrane turnover is inhibited. The second approach has been to introduce new cell surface determinants which may increase the recognition of weak tumor antigens. This includes the introduction of determinants such as PPD to enhance T cell recognition or modification of existing cell membrane antigens. In addition to chemical modification, new receptors may be introduced by virus infection of tumor cells. The latter approach has proved effective with a number of tumors, but it has the inherent disadvantage that viral products are introduced. Therefore different approaches to modifying the cell surface antigens have been evaluated. These include insertion and/or deletion of cell surface antigens by treating tumor cells with mutagens and carcinogens. For example, treatment of rat mammary carcinoma cells in culture with 3-methylcholanthrene under conditions allowing metabolic activation induces changes in alloantigen expression. An alternative approach of modifying cell membrane structures by the insertion of lipophilic molecules has been suggested by associated studies showing that alkylcatechols such as pentadecylcatechol (PDC), as well as analogs with unsaturated side chains, can be inserted into lymphocyte membranes where they function as haptens in generating blastogenic responses with peripheral blood lymphocytes from naturally sensitized human donors. These compounds are highly lipophilic and model membrane studies have established that PDC is incorporated into, and interacts with, dipalmityl lecithin vesicles firstly by increasing the transitional temperature and secondly decreasing the fluidity of the mobile phase above the transitional temperature. The effects of these compounds will be illustrated by considering their capacity to modify cell surface antigens and to introduce haptenic determinants into normal and neoplastic cells.

1. Baldwin, R. W., Embleton, M. J. and Pimm, M. V. (1978) In: Antiviral Mechanisms in the Control of Neoplasia, Plenum Press.
2. Price, M. R. and Baldwin, R. W. (1977) Cell Surface Rev. 3, 423.

Triggering and Tolerance of Cells Via Receptor Interactions

596 RESTRAINTS ON CURRENT CONCEPTS OF SELF-TOLERANCE, Erwin Diener and Coreen A. Waters, Department of Immunology and MRC Group on Immunoregulation, University of Alberta, Edmonton, Alberta T6G 2H7.

Most models of self-tolerance are based on extrapolations of data gained from autoimmune phenomena or from experiments with adult or neonatal animals with antigens which were deliberately selected for their tolerogenic effect. Furthermore, theoretical considerations in concert with often all too circumstanial evidence have divided immunologists into those who are faithful to the concept of either clonal deletion or active suppression as the cause of self-tolerance. Current thinking on mechanisms of self-tolerance therefore largely disregards the potential importance of the molecular structure of self antigens or their mode of presentation to the relatively undifferentiated immune system during ontogeny. In view of these considerations, we felt it appropriate to utilize a self-tolerance model which permits access of various test antigens to the fetus well before immunocompetent cells arise. The tolerogenic potency of human gamma globulin (HGG), bovine serum albumin (BSA) and a synthetic haptenated peptide of defined geometry [Glu Tyr Lys (TNP) (Glu Tyr Ala)$_5$]$_n$ (TNP-18) administered transplacentally *in utero* was assessed in Balb/cCr mice. Contrary to expectation, only deaggregated HGG but not BSA or TNP-18 was able to induce tolerance *in utero* even though all three antigens were shown to cross the placenta. As for HGG, unresponsiveness in the offspring was specific and complete for both T and B cells and waned for both cell classes in parallel with loss of antigen from the circulation by 15 to 20 weeks of postnatal life. Foster-nursing experiments demonstrated that a four day period of *in utero* exposure to HGG was sufficient to induce tolerance in the offspring. Experiments to monitor HGG specific suppressor cells in tolerant offspring showed that during but not before the postnatal period of tolerance breakdown, suppressor cells could regularly be found, in contrast to the observations made when tolerance to HGG is induced in adult animals. We conclude that self-tolerance depends on the molecular structure of self-antigens or their mode of presentation, and that it operates via clonal deletion rather than active suppression.

597 BIOCHEMISTRY OF LYMPHOCYTE COMMUNICATION: TRIGGERING AND CELLULAR RECEPTORS. James Watson, Department of Microbiology, University of California, Irvine.

Humoral factors secreted by T lymphocytes and macrophages appear to play a role in cell communication leading to the triggering of immune responses. A factor has been purified from the culture supernatants of Concanavalin A-activated murine spleen cells with helper T cell-replacing activity in three assay systems: (i) stimulation of antibody responses to erythrocyte antigens in T cell-depleted cultures, (ii) amplification of production of cytotoxic T cells in thymocyte cultures, and (iii) stimulation of mitogenic responses to Con A in thymocyte cultures where the cell density is too low to support responses to Con A alone. The biologic activity has been purified to homogeneity by salt precipitation, gel filtration, ion exchange chromatography, isoelectric focusing (IEF), and polyacrylamide gel electrophoresis. TRF activity is found in protein-containing molecules with a Stokes radius corresponding to a globular protein of 30-35,000 daltons molecular weight, and a pI ranging from 4-5. When IEF-purified TRF is radiolabeled and electrophoresed under reducing conditions, a single peak is observed indicating TRF is composed of a single polypeptide chain. Quantitative assays reveal this material is active at concentrations of less than 10^{-9}M in each lymphocyte response system used. The production of TRF requires the presence of T cells, and limiting dilution analyses reveal one in 20,000 spleen cells are capable of producing TRF. Partially-purified TRF has been used to establish continuous cultures of T lymphocytes. These cells contain antigen-specific helper T cells, and have been maintained for 30 weeks in culture. Experiments are directed toward two issues: (i) are the factors which are required for the growth of T lymphocytes in continuous culture similar to the TRF active in the three helper assays described above, and (ii) will the antigen-specific helper T cells release factors that possess T cell-replacing activity. A source of factor from cloned cell lines is required to determine if TRF interacts with specific receptors on lymphocytic or monocytic cells, or whether TRF possess antigen-specificity.

Watson, J., Aarden, L.A., and Lefkovits, I. (1978) The Purification and Quantitation of Helper T Cell-replacing Factors Secreted by Murine Spleen Cells Activated by Concanavalin A. J. Immunol. In Press.

Cell-Cell Interactions: Genetics and Mediators

598 MULTIPLE MHC LOCI CONTROLLING LYMPHOCYTE INTERACTIONS, Tomio Tada, Masaru Taniguchi, and Ko Okumura, Department of Immunology, Faculty of Medicine, University of Tokyo, and Laboratories for Immunology, School of Medicine, Chiba University, Chiba, Japan.

Regulation of the antibody response is performed via a complex series of cellular interactions involving phenotypically different subsets of T cells. Certain domains of this network has been known to be regulated by the products of major histocompatibility complex (MHC). For example, the suppression of specific antibody response is initiated by the antigen-specific suppressive T cell factor which is controlled by a gene in I-J subregion of MHC. The factor has both antigen-specificity and an ability to recognize the second cell type to interact with. The second cell type being Lyt-$1^+,2^+,3^+$ subclass has the determinant also coded for by a gene in I-J subregion, and activates the third cell type of Lyt-$2^+,3^+$ subclass to induce an antigen-nonspecific suppressor. In our own experimental system, such a series of interacting cells, which we call 'cognitive domain', are interconnected by the products of I-J subregion genes. In addition, we have recently reported that some but not all helper T cells have a determinant coded for by a gene mapped in the same I-J subregion. The effect of this helper T cell of Lyt-1^+ subclass, designated as Th_2, is directly suppressible by I-J^+ suppressor factor from the same haplotype strain. Thus, the suppressive cell interactions are governed by the products of I-J subregion.

A crucial question to be asked is whether these I-J subregion products expressed on functionally different subsets of T cells are the same or different, or more specifically, whether these I-J subregion gene products are controlled by the same or different loci. Two approaches were made to answer this question. An anti-I-J antiserum was absorbed with Lyt-1^+ or Lyt-$2^+,3^+$ splenic T cells, and the residual activity to remove suppressor and Th_2 cells was assessed. The second approach was to make T cell hybridoma cell lines expressing homogeneous products encoded by I-J subregion genes. Results from both approaches indicated that there are closely linked multiple I-J subregion loci controlling determinants expressed on different subsets of T cells, and that these products are playing important roles in the suppressive cell interactions. In addition, some of the hybridoma cell lines were shown to be transplantable to histocompatible F_1 mice to produce a large quantity of ascites having antigen-specific suppressor activity. The examination of the activity of such homogeneous I-J subregion products enabled us to confirm the previously determined antigen-specificity, molecular weight, and genetic restriction in the suppressive cell interactions. We conclude that I-J subregion has multiple loci which control the cell surface molecules involed in the whole process of cell interactions in the suppressor domain (Supported by grants from the Ministry of Education, Culture and Science, Japan).

599 ANTIGEN SPECIFIC MEDIATORS OF CELL INTERACTION, Marc Feldmann, Peter Erb and Sirkka Kontiainen, Tumour Immunology Unit, Zoology Dept., University College, London WC1; Institute of Microbiology, University of Basel; Dept. Bacteriology & Serology, University of Helsinki.

Antigen specific mediators have been described at several levels of immune regulation, such as macrophage factors involved in the induction of T cell help (abbreviated GRF), antigen specific helper factors released by activated T cells (abbreviated HF) and antigen specific suppressor factors (abbreviated SF). These are isolated from cultured cells, and usually assayed in vitro, although there are also multiple reports of in vivo effects of such molecules. Structural analyses of these entities has been hampered by the restricted quantities present, and so various approaches have been used to characterize these molecules. Serological approaches have revealed that all three entities express I region associated antigens, and that HF and SF have a common basic pattern, a 'constant' region, bearing a factor specific determinant, the binding site for its target, and the effector function; and a 'variable' region, which binds to antigen and contains idiotypic markers. However, despite this serological characterization, there are many unknown aspects, such as the valency of these factors the number of chains and the nature of the Ia determinants expressed: whether they are peptide or carbohydrate in nature.

The relationship of antigen specific factors to MHC linked Ir gene control is unclear. Antigen specific suppressor factors may be elicited readily from both responder and non-responder T cells in vitro, and thus are not intimately related to Ir gene function in the systems studied. This is in contrast to the situation with the Ia containing genetically related macrophage factor, GRF, which is only released by responder macrophages. Since in most of the systems analyzed, the antigen presenting macrophages are the site of Ir gene expression it thus appears that GRF is a soluble Ir gene product. The relationship of HF to Ir gene expressing is not clear, and there is no evidence that the Ia antigen on HF is an Ir gene product. The relationship of specific factors to the function of the cell of origin, and interrelationships with nonspecific factors will be discussed.

600 WHAT IS THE NATURE OF THE ANTIGENIC COMPLEX RECOGNIZED BY T LYMPHOCYTES?
Ethan M. Shevach, Laboratory of Immunology, NIAID, NIH, Bethesda, Maryland 20014

Macrophages modified by the trinitrophenyl (TNP) hapten have been used to clarify the nature of the antigenic determinant(s) recognized by the antigen specific guinea pig T lymphocyte. We have previously demonstrated that the genetic restriction on the interaction of the TNP-modified macrophage and primed T lymphocyte was regulated by the Ia antigens of the macrophage used for initial sensitization and that following removal of alloreactive cells T cells could be sensitized to TNP-modified allogeneic macrophages. In the present experiments, evidence for an association between TNP-conjugated membrane components and Ia antigens was obtained from studies in which TNP-modified macrophages were briefly treated with anti-TNP serum or anti-Ia serum prior to addition to primed T lymphocytes. Thus, a marked reduction in the stimulatory capacity of freshly modified cells could be achieved by brief treatment with anti-TNP serum, while the stimulatory capacity of macrophages which had been TNP-modified and then "aged" for 24 hours prior to addition to primed T cells was unaffected by similar treatment. This result suggests that macrophage presentation of the TNP determinant is not simply a surface display phenomenon and that the macrophage must process membrane conjugated TNP in a manner so that it is inaccessible to anti-TNP antibody to create the relevant immunogen recognized by T cells. In contrast to these results, treatment of TNP-modified macrophages with anti-Ia serum either immediately after or 24 hours after TNP modification resulted in a markedly deficient antigen presenting cell. As brief treatment of TNP-modified macrophages with anti-Ia serum did not inhibit Ia antigen synthesis, it is therefore likely that the susceptibility of the "aged" TNP-modified macrophage to the inhibitory effects of anti-Ia serum resulted from removal of TNP determinants which had become associated with Ia antigens on the macrophage surface. In order to directly investigate the relationship between the TNP moiety and macrophage Ia antigens, macrophages were TNP-modified, radio-iodinated, and lysed in detergent. When TNP-derivatized proteins were isolated using an anti-TNP immunoabsorbent and the presence of TNP-derivatized antigens in the eluted proteins determined by immunoprecipitation techniques, no hapten modified Ia antigens were detected. Furthermore, when Ia antigens from TNP-modified cells were eluted from an anti-Ia immunoabsorbent, no protein other than Ia antigens was detectable. Thus, although the functional studies with anti-Ia serum are strongly in favor of the model that TNP-conjugated membrane proteins are complexed with Ia antigens on the surface of the macrophage, we have not been able to prove the existence of this complex by the biochemical techniques employed. Nevertheless, a complex maintained by the integrity of an intact cell membrane might very well exist.

601 GENE COMPLEMENTATION IN THE MURINE T-LYMPHOCYTE PROLIFERATIVE RESPONSE.
Ronald H. Schwartz, Akihiko Yano, Alan M. Solinger, Michiel E. Ultee, Emanuel Margoliash and William E. Paul. Laboratory of Immunology, NIH, Bethesda, MD 20014 and Department of Biochemistry and Molecular Biology, Northwestern University, Evanston IL 60201.

The immune responses to poly $(Glu^{55}Lys^{36}Phe^{9})n$ [GLϕ] and pigeon cytochrome c are both controlled by two MHC-linked Ir genes, one mapping in the I-A subregion, the other in the I-E/C subregion. In the case of pigeon cytochrome c, the major antigenic determinant has been localized to the carboxy-terminal end of the molecule and involves the recognition of two amino acid substitutions relative to the mouse sequence. Cross-stimulation with cytochromes c sharing only one of the two critical amino acid substitutions demonstrated that one family of T cell clones recognizes both substitutions. Thus, two complementing Ir genes control the response to a single antigenic determinant.

The cellular sites of expression of these complementing Ir genes has been examined in several ways. Antibodies directed against the gene products of either the I-A or the I-E/C subregion were capable of inhibiting the T-lymphocyte proliferative response to GLϕ, suggesting that both products are expressed at the cell surface. Reconstitution of lethally irradiated high responder F_1 mice with a mixture of bone marrow cells from both low responder parental strains failed to generate chimeras which could respond to GLϕ. This suggested that at least one cell type involved in the immune response had to express both gene products. That the antigen-presenting cell (APC) was one such cell type was demonstrated by presenting GLϕ to primed F_1 T cells on nonimmune spleen cells. Only high responder F_1 spleen cells could present; low responder parental spleen cells, possessing only one of the two gene products, failed to present, even when both parental types were added together. The histocompatibility requirement for antigen presentation of GLϕ was also examined. Identity at the I-A subregion between T cells and APCs was required but identity at I-E/C was not. B10.A(5R) responder T cells could be stimulated by F_1 progeny of B10 crossed with B10.A, B10.BR, B10.D2, B10.P and B10.RIII but not B10.Q, B10.M or B10.BSVS. This pattern correlates with the data of others on the serological persence of Ia.7 and the biochemical presence of an I-E/C subregion gene product in these strains. The data suggest that the Ia.7 bearing, I-E/C gene products of the complementing strains are all similar if not identical and that this molecule is critically involved in the presentation of GLϕ. Overall, the experiments support the conclusion that both complementing Ir gene products must be expressed in the same APC in order to generate a T cell proliferative response.

Virus-Lymphocyte Interrelationship

602 ON THE ROLE OF MHC AND THYMUS ON T CELL RESTRICTION AND RESPONSIVENESS, Rolf M. Zinkernagel, Department of Immunopathology, Scripps Clinic and Research Foundation,

Thymus derived lymphocytes (T cells) are generally specific for both a foreign antigenic determinant and a self determinant that is a major transplantation antigen. Differentiation of T cells' specificity for self is influenced more by the major histocompatibility complex (MHC) type of the thymus than by the genome of the maturing lymphohemopoietic stem cells. However, the latter function importantly in at least two ways: a) T cells reach full immunocompetence only if thymic selection is followed by "post-thymic" maturation, which depends on histocompatibility between the thymic MHC and some of the lymphohemopoietic cells; b) Only antigen presented on cells of lymphohemopoietic origin is immunogenic and capable of triggering effector T cells; therefore, antigen-presenting cells and thymus must be histocompatible for T cells to become sensitized. Whether the influence of lymphohemopoietic cells in T cell maturation reflects their role in generating the diversity of receptors specific for widely differing foreign antigens or the need for expansion of small numbers of committed T cells is unresolved.

While selecting the T cells' self-restricted range of specificity, the thymic MHC seems also to select the capacity of T cells to respond to antigens that are controlled by MHC-coded immune response (Ir) genes. Because the MHC regions that code for the restricting elements of specificity also code for Ir genes that regulate the responsiveness of these T cells, these restricting genes and Ir coding genes seem to be intimately linked or may be identical.

These results have important theoretical implications for understanding T cell differentiation and recognition as well as associations between the MHC and disease. The additional practical consequences are applicable clinically in attempts to reconstitute immunodeficient patients.

603 EXPRESSION OF MOUSE LEUKEMIA VIRUSES IN LYMPHOCYTES, Robert C. Nowinski, Fred Hutchinson Cancer Research Center, Seattle, WA 98104.

The mouse leukemia viruses (MuLV) are a highly polymorphic group of agents that occur as endogenous infections in inbred and feral mice. All mice contain some genetic equivalents of MuLV, although considerable variation occurs in the particular viruses that are inherited by one mouse strain or another. In addition, host genetic factors influence the extent to which these viruses are expressed, which in turn, determines the leukemia susceptibility of a particular strain.

Although mice of the high leukemic AKR strain produce endogenous MuLV throughout their lifespan, leukemias do not arise until after 6 mos of age. This may relate to the fact that the MuLVs of AKR mice are biologically heterogeneous, and in the induction of leukemia, there is a requirement for both the expression of several different endogenous MuLV, as well as for the *de novo* generation of recombinant viruses. With monoclonal antibodies against the proteins of MuLV we have identified four distinct groups of virus in leukemia cells. These include ecotropic and xenotropic endogenous MuLV, and two classes of recombinant viruses that have ecotropic or polytropic host range properties. In oncogenicity assays only the recombinant viruses were found to accelerate the occurrence of leukemia.

MuLV genomes contain three genes: the *pol* gene codes for viral DNA polymerase, the *env* gene codes for the envelope proteins gp70 and p15(E), and the *gag* gene codes for the p30, p15, p12, and p10 core proteins. Thymus cells of preleukemic and leukemic AKR mice express on their cell surface elevated levels of antigens associated with gp70, p15(E), and p30. The p30 antigenicity is contained in two polypeptides of 85,000 and 95,000 daltons that correspond to glycosylated forms of the primary *gag* gene translation product. These glycosylated *gag* proteins are found only on the cell surface of thymocytes of preleukemic and leukemic mice.

In fact, the expression of these viral proteins on the surface of thymocytes varies both quantitatively with the age of the mouse and qualitatively with the cell populations that express these antigens. Four discrete stages in the leukemic pathway can be identified: (1) Low numbers of thymus cells from young (2 mos) mice express p30 and gp70 antigens. Expression of gp70 is restricted to subcapsular thymocytes. (2) Thymuses of 6 mos old mice show a selective depletion of cortical cells, with a concomitant increase of medullary cells. These medullary cells express high levels of viral antigens. (3) Thymus of some 8 mos old mice demonstrate selective hypertrophy of a single lobe. The enlarged lobe contains a population of blast cells that express very high levels of viral antigens on their cell surface. These cells are not transformed, however, since transplantation does not lead to rapid leukemia induction. (4) Thymuses of leukemic mice contain almost exclusively large blast cells. These cells express very high levels of viral antigen, and upon transplantation, they rapidly induce leukemias.

The increased expression of viral antigens on the cell surface of thymocytes is correlated with an increased production of infectious viruses. At the time of amplified gene expression there also is an elevation in the number of thymocytes producing infectious ecotropic, xenotropic, and polytropic MuLV.

Lymphocyte Recognition and Function: Future Problems

604 MHC AND ANTIBODY GENES: STRUCTURE, REGULATION AND EVOLUTION. Hood, Leroy, E. Division of Biology, California Institute of Technology, Pasadena, CA 91125.

Recent analyses of organization of antibody genes have provided new insights into mechanisms for antibody diversity, regulation and evolution. First, analyses at the protein and nucleic acid levels suggest that the genes coding for antibody light chains are comprised of three distinct segments - V, J (or joining) and C (1,2). Moreover, the existence of multiple J segments and the fact that they apparently extend from the C region into the third hypervariable region, suggests that the combinatorial joining of multiple V and J segments may be an important mechanism for generating antibody diversity (1). Second, DNA rearrangements occur during the differentiation of antibody-producing cells to join the V and J segments (2). These DNA rearrangements may play a fundamental role in committing the antibody-producing cell to the expression of a single immunoglobulin. Finally, the presence of intervening DNA sequences between each of the homology units or domains of the α heavy chain gene raises intriguing possibilities about mechanisms for the evolution of antibody genes (3). These data will be summarized and their implications discussed.

Structural work on the gene products encoded by the MHC will be summarized. The significance of these data for models of MHC gene organization and evolution will be discussed.

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605 GENETICS OF Ia ANTIGENS--EVOLUTIONARY IMPLICATIONS, D. H. Sachs, J. K. Lunney, D. L. Mann, K. Ozato, and N. Shinohara, Immunology Branch, NCI, NIH, Bethesda, MD 20014.
The detection of serologic crossreactions between the products of the H-2K and H-2D regions provided the first clue that these two regions may have been derived from common primordial genes, an hypothesis which has recently been substantiated in several laboratories by the demonstration of marked sequence homologies between the K and D molecules. Such serologic crossreactions can thus provide a useful probe for studying the evolution of polymorphic antigen systems. In an attempt to use such probes to discern evolutionary relationships between Ia antigens within and between species, we have developed a variety of crossreacting anti-Ia antisera and have compared their reactivities both by serology and by immunoprecipitation techniques.

Alloantisera prepared between various strains of mice have revealed numerous public Ia specificities shared by different haplotypes, as well as certain private or haplotype-specific Ia specificities. Certain mouse anti-Ia alloantisera were found to react with Ia-like antigens on lymphocytes of numerous other species, including rats, pigs, and human beings. Although these sera detected haplotype-specific alloantigens in mice, they generally reacted much more broadly among members of other species. If the Ia antigenic determinants reside in the polypeptide chains comprising these antigens, then these interstrain and interspecies crossreactions probably imply the conservation of certain series of alternative amino acid sequences during the evolution of these polymorphic systems, and thus also imply the origin of the relevant genes from a common progenitor.

Although mouse Ia antigens determined by the I-A and I-E/C subregions appear to be of similar molecular weight, we have not yet found any examples of public Ia antigenic specificities determined by both subregions. We have also failed to detect any Ia antigens determined by the I-E/C subregions of certain haplotypes, notably $H\text{-}2^b$ and $H\text{-}2^s$, despite the use of genetically appropriate alloantisera and highly crossreactive xenoantisera. However, both murine subregion products demonstrate interspecies crossreactions. One of the Ia antigenic determinants shared between the $H\text{-}2^k$ haplotype of mice and rat Ia antigens has been localized to the I-A subregion, while the major crossreaction between this murine haplotype and human Ia antigens was found to be determined by the I-E/C subregion. These observations are consistent with independent evolution of the different I subregions over a relatively long evolutionary period, with a possible loss of subregions during the derivation of certain species and strains.

606 MACROPHAGE-LYMPHOCYTE INTERACTIONS: CONTROL MECHANISMS, M. Feldman, S. Segal, E. Tzehoval and M. Fridkin, The Weizmann Institute of Science, Rehovot, Israel

Studies were carried out on the positive and negative signals which control the immunogenic effects of macrophages. We demonstrated that a tetrapeptide, tuftsin (Thr-Lys-Pro-Arg), associated with the Fc portion of the Ig heavy chain, when applied to macrophages simultaneous with antigen, activated macrophage dependent education of T-lymphocytes. Studies of structural analogs of tuftsin indicated that the dominant sequences in the activation of the immunogenic effect of macrophages, is the Proline-Arginine dipeptide. Since Pro-Arg sequences appear in many peptide hormones, this may represent a more general principle associated with the generation of the intracellular signals activating programmed cells.

Studies on the negative control indicated that spleen cells from high zone tolerant animals prevented the macrophage dependent generation of initiator T-cells. Suppressor cells from spleens of HGG tolerant animals, when seeded on macrophages fed simultaneously with HGG and KLH, prevented also the macrophages from signalling an anti-KLH response. Such suppressor cells were found to act as cylotoxic cells on the tolerogen presenting macrophages. We propose that they function as anti-modified self killers, acting on macrophages possesing self antigen "modified" by the tolerogen.

Structure of the Molecular Products of the MHC

607 STRUCTURAL STUDIES ON THE MURINE Ia ALLOANTIGENS, Richard G. Cook, Mark H. Siegelman, Ellen S. Vitetta, J. Donald Capra and Jonathan W. Uhr, The University of Texas Southwestern Medical School, Dallas, Texas 75235

Our laboratories are currently investigating the nature and extent of primary structural variation of the alloantigens encoded by the I-A and I-E/C subregions. Normal murine splenocytes were radiolabeled with ^{3}H or ^{14}C amino acids and the Ia α and β subunits isolated by immunoprecipitation and SDS-polyacrylamide gel electrophoresis. Amino-terminal sequence analysis of both A (k and b haplotypes) and E/C (k and r haplotypes) encoded products has demonstrated that only the β polypeptides display haplotype associated differences in the NH_2-terminal 20 amino acids. The E/C (Ia.7) encoded products from 4 haplotypes (k, r, p, and d) were also examined by comparative tryptic peptide mapping. By this technique, the α subunits from these haplotypes show about 90% coincidence of peptides, while the β polypeptides only about 50% homology. Studies examining I-A subregion molecules by peptide mapping are currently in progress. Thus, from the limited data available the major structural variability of Ia antigens appears to reside in the β polypeptide. Finally, preliminary data toward the determination of the complete structure of the β chain of the $I\text{-}A^{k}$ subregion will be presented. The β polypeptide was prepared from splenocytes labeled with mixtures of ^{3}H-amino acids; both tryptic and chymotryptic peptides were subsequently isolated by preparative ion-exchange chromatography. Amino acid composition and sequence analysis of these peptides will be presented.

608 PARTIAL N-TERMINAL AMINO ACID SEQUENCES OF MOUSE TRANSPLANTATION ANTIGENS. R.M.Maizels, J.A.Frelinger*, and L.Hood. Division of Biology, California Institute of Technology, Pasadena, California 91125, and *Department of Microbiology, University of Southern California Medical School, Los Angeles, California 90033.

The H-2 histocompatibility antigens of the mouse display a remarkable polymorphism both serologically and structurally. To characterize more fully the extent and pattern of variation among alleles of H-2, partial N-terminal amino acid sequences of the K and D gene products of the $H\text{-}2^{q}$ and $H\text{-}2^{s}$ haplotypes were analyzed. These data, presented below, bring the total number of alleles so characterized to nine, and clearly reflect the complexity of the transplantation antigens. Although the K and D loci are separated by 0.5 map units, no distinguishing characteristic of one or other set of gene products has been detected. For example, K^{s} appears to be more similar to the D^{d} molecule described elsewhere, then to any other K-end molecule. The degree of variability between gene products is surprisingly extensive (up to 30% of residues compared) especially in view of the comparatively recent divergence of the mouse from other rodent species. These amino acid sequence patterns suggest that either or both the evolutionary history or the genetic organization of the major histocompatibility antigens is more complex than at first suspected.

H-2 allele	Position: 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
	K^{q}				Leu	Arg	Tyr	Phe	-		Ala	Val		Arg		Leu				Arg	Phe	Ile			Val		Tyr	Val
	K^{s}				Leu	Arg	Tyr	Phe	Val		Ala	Val		Arg		Phe				Arg	Tyr						Tyr	Val
	D^{q}		Pro		-	Arg	Tyr	Phe	-		Ala	Val		Arg	Pro	Leu				Arg	Tyr	Ile			Val		Tyr	Val
	D^{s}		Arg		Leu	Arg	Tyr	Phe	Val		Ala	Val		Arg		Leu				Arg	Tyr				Val		Tyr	Val

609 STRUCTURAL STUDIES ON THE FOURTH COMPONENT OF COMPLEMENT, Michael C. Carroll and J. Donald Capra, The University of Texas Health Science Center at Dallas, Dallas, Texas 75235

The complement system is composed of at least 20 serum proteins which when activated mediate an important effector function of the immune response. Several of the early components of this system, i.e., C2, C4 and Factor B, have been linked to the major histocompatibility complex (MHC) in various mammalian species, including man, mouse and guinea pig.

In contrast to the extensive polymorphism described for other molecules controlled by the MHC, i.e, histocompatibility and immune response region associated antigens, only limited structural variation has been reported for these MHC linked complement components.

C4 from various species representing at least four different mammalian orders has been isolated in order to compare the subunit size of each chain of these three chain molecules. In addition, an SDS polyacrylamide gel peptide analysis technique has been adapted to examine each chain for structural variation. This technique has certain advantages over previously used serological and electrophoretic techniques as it localizes differences to individual polypeptide chains as well as provides information on the extent of structural variation.

610 BIOCHEMICAL AND STRUCTURAL CHARACTERIZATION OF MAJOR HISTOCOMPATIBILITY ANTIGENS OF THE RABBIT, Edward S. Kimball, John E. Coligan and Thomas J. Kindt, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Laboratory of Immunogenetics, Bethesda, MD 20014

The isolation, biochemical characterization and N-terminal amino acid sequence of the major products of the rabbit histocompatibility complex (RL-A) is presented. The material was isolated from a rabbit lymph node tumor (RL-A^n) cell line grown in culture with tritium-labelled amino acids. Cells were lysed by detergent solubilization and a glycoprotein fraction was obtained by affinity chromatography over a lentil lectin-Sepharose 4B column. Products of the rabbit major histocompatibility complex were further purified from that pool by affinity chromatography using purified sheep anti-rabbit beta-2-microglobulin (B_2m). Glycoprotein with a single chain of 43,000 daltons non-covalently associated with B_2m was obtained. Separation of B_2m from the putative RL-A was accomplished by gel exclusion chromatography over Sephadex G-100 in 1M formic acid. The RL-A was obtained in aggregated and monomeric forms, the first material eluting in the void. Isoelectric focussing in a sucrose gradient indicated that the aggregated RL-A had pI's of 5.3 and 6.1 whereas monomeric RL-A yielded a single peak with a pI of 6.1. N-terminal amino acid sequence analysis was performed by using radiosequencing techniques on the first 35 residues using 11 labelled amino acids. A 91% homology was shown to exist between RL-A^n and HLA-A2 or H2-K^b. Sequence analysis to date has not given indication of multiple products, although these are suggested by isoelectrofocussing studies.

611 STRUCTURAL AND SEROLOGICAL COMPLEXITY OF THE MURINE MHC H-2D^d AND H-2L^d GENE PRODUCTS. Duane W. Sears*, Pamela H. Wilson*, Catherine M. Polizzi* and Stanley G. Nathenson°. *UCSB, Santa Barbara, CA, and °Albert Einstein College of Medicine, Bronx, NY.

Tryptic peptide map studies by high resolution, cation-exchange chromatography reveal that the H-2 antigen precipitates by the anti-H-2.28 family alloantiserum, D28b (B10.Br x LP.III anti-B10.A(2R)), are structurally complex. D28b and anti-H-2.4 (B6 x C3H anti-B10.A) both precipitate glycoproteins extracted by detergent from RADA-1 (H-2^a) tumor cells. Paired-label tryptic peptide maps reveal that the major proteins in these two precipitates are identical, or nearly identical. However, after immunoadsorbent removal of the H-2.4 antigen, D28b weakly precipitates another protein which is structurally distinct from the H-2.4 antigen. Approximately 25% of the arginine peptides are shared between the H-2L^d product and the H-2.4 antigen indicating that the two antigens are no more similar to one another in sequence than are any other two H-2 antigens. This finding, being the first evidence that the sequences of the two proteins are different, supports the proposal by others that, in addition to the H-2D^d region (which codes for the H-2.4 antigen), there is at least one other region, H-2L^d, encoding for another unique antigen. Because D28b cross-reacts with the H-2.4 antigen, its reactivity with the BALB/c-H-2^{db} mutant was re-examined since others have found that lymphocytes from the mutant lack a detectable H-2L^d product but express a structurally unaltered H-2.4 antigen while being resistant to cytotoxic killing by D28b. Confirming the resistance to cytotoxic killing by D28b, we find that D28b does precipitate a 45000 dalton glycoprotein from mutant lymphocyte extracts suggesting that D28b can precipitate the H-2.4 antigen but cannot mediate cytotoxicity against this antigen.

612 SEQUENCE STUDIES OF HLA-A AND -B ANTIGENS, H.T. Orr, R.J. Robb, H. Bilofsky, T. Wu, E. Kabat and J.L. Strominger, Harvard University, Cambridge, MA, Columbia University College of Physicians and Surgeons, New York, NY and NIH, Bethesda, MD

Previous structural studies on human histocompatibility antigens (HLA-A and -B) have shown similarities between these membrane glycoproteins and immunoglobulins (Ig). To further understand the nature and extent of this relationship, sequencing studies of the heavy chains of HLA-A and -B antigens were undertaken. The sequence of a portion of HLA-B7 containing one of the two disulfide bridges (residues 165-260) has been completed. The number of residues between the two cysteines in this fragment of HLA-B7 is 54, similar to that found in Ig constant domains. Using the PROPHET computer program and the Dayhoff file, the sequence of this region of HLA-B7 was compared with regions of Ig and non-Ig proteins. The highest level of homology (29%) was found with β_2-microglobulin and the C_H3 domain of IgGs. Lower degrees of homology were found with V_H domains (18% V_λIII, 21% V_H) and non-Ig proteins from the Dayhoff file (19% TMV coat protein, 18% chymotrypsinogen A). Moreover, the homologies with V domains and non-Ig proteins contained fewer identities at the rare amino acids (trp, cys and his) than the homologies with C_H3 and β_2-microglobulin. This level of homology between HLA-B7 and C_H domains is identical to that found in comparing different C_H domains of Ig. Therefore, it is concluded that HLA-A and -B heavy chains, like the β_2-microglobulin chain, contain at least one Ig-like domain, thus confirming an evolutionary link between HLA-A and -B and Ig. Other data show extensive identities between the various specificities of HLA-A and -B and H-2D and -K sequences such that it is clear that these four proteins are homologous.

613 CROSSREACTIONS OF MOUSE ANTI-Ia ALLOANTISERA WITH HUMAN Ia-LIKE ANTIGENS, Joan K. Lunney, Dean L. Mann, and David H. Sachs, NIH, National Cancer Institute, Bethesda, MD 20014.

Crossreactions between human and mouse Ia antigens have been detected by analyzing mouse anti-Ia alloantisera for reactivity with human cells. After absorption with pooled human platelets positive mouse alloantisera killed predominantly human B cells and not T cells. When a panel of mouse alloantisera was surveyed, only anti-Ia sera produced against the a, k, and d haplotypes resulted in the lysis of human B cells. Positive reactions for some of these sera were correlated in family studies with the inheritance of a particular parental HLA chromosome. The molecular specificity of these crossreactions was examined using sodium dodecyl sulfate polyacrylamide gel electrophoretic (SDS-PAGE) analyses. Lentil lectin purified detergent extracts of ^{3}H-leucine labelled human peripheral blood lymphocytes were complexed with mouse alloantisera. Only the sera that exhibited positive cytotoxic reactions with human B cells resulted in glycoproteins of 34,000 and 29,000 dalton molecular weight being detected by the SDS-PAGE analysis. Thus, by cellular distribution, HLA linkage, and immunochemical criteria, these mouse alloantisera detected human DR-like antigens. Sera prepared against limited portions of the mouse I region were next analyzed to delineate the specificity of the cross-reacting mouse antibodies. Anti-I-J,E,C sera caused precipitation of DR-like proteins while anti-I-A,B sera led to no significant precipitation of ^{3}H-labelled glycoproteins. These results support the hypothesis raised by recent N-terminal sequence data that human DR antigens are homologous to mouse I-E/C products.

614 EVIDENCE FOR MORE THAN ONE LOCUS CONTROLLING THE EXPRESSION OF HUMAN B CELL ALLOANTIGENS (DR), D. L. Mann, NIH, National Cancer Institute, Bethesda, MD 20014.

Interpretation of structural studies of human Ia-like antigens (DR) is dependent in part on the knowledge of the number of gene products studied. Heretofore, serologic analyses have related DR antigens to D antigens, e.g., those antigens responsible for stimulation in MLR which are considered the products of one locus with multiple alleles. However, family studies suggest that more than one allele controls DR antigens. Immunoprecipitation analysis of B cell alloantigens was performed to determine the number of gene products. Cell membranes of the tissue culture cell line, JY (HLA-A2, B7, DRW4, and DRW6) were labeled with ^{3}H leucine and solubilized with NP-40. B cell antigens isolated by lectin affinity chromatography were sequentially reacted with antisera detecting DRW4 or DRW6 antigens as well as an antisera (Ia 172) reacting with other DR antigens. Antigen-antibody complexes were isolated with Staph A, solubilized with SDS, and electrophoresed on 10% polyacrylamide gels. Each antisera precipitated 29-34,000 mw components. When first precipitating with an antisera detecting one antigen, subsequent treatment with the two other antisera isolated additional 29-34,000 mw components. Combinations of 2 antisera were used in pre-precipitation studies. Subsequent reactions with individual antisera substantiated the presence of 3 B-cell, Ia-like antigens. The results indicate that multiple genes control the expression of B cell alloantigens in man.

615 STRUCTURAL STUDIES OF MUTANT AND NON-MUTANT H-2D^{d} ANTIGENS, Roderick Nairn, John E. Coligan*, Thomas J. Kindt* and Stanley G. Nathenson, Depts. of Microbiology and Immunology and Cell Biology, Albert Einstein College of Medicine, Bronx, New York 10461 and *Laboratory of Immunogenetics, NIAID, National Institutes of Health, Bethesda, Maryland 20014

B10.D2 (M504) (H-2D^{dml}) is a mutant in the D gene of the mouse major histocompatibility complex. This strain is characterized by serologically detectable alterations as well as histogenic reactivity (mutual stimulation in MLR, cytotoxicity in CML, skin graft rejection, and GVHR reactivity) when compared to the parental strain B10.D2 (H-2D^{d}).

The H-2D glycoproteins from the parental non-mutant (H-2D^{d}) and mutant (H-2D^{dml}) strains when digested with cyanogen bromide yield fragments which can be purified and analyzed by comparative tryptic peptide mapping. These experiments demonstrate that the H-2D^{dml} antigen may have undergone a relatively complex change. Primary structural data for the cyanogen bromide fragments purified from the H-2D^{d} antigen has been obtained by the use of radioactive micro-sequencing methods. Comparison with results previously obtained in this laboratory for the H-2K^{b} antigen has allowed the H-2D^{d} fragments to be aligned and has demonstrated a significant sequence homology between H-2D^{d} and H-2K^{b}.

616 BIOCHEMICAL DETECTION OF I-E/I-C GENE PRODUCTS IN HAPLOTYPES H-2^b, H-2^f, H-2^q AND H-2^s WITH XENOANTISERA, William Lafuse, Brian Neely, David McKean and Chella David, Dept. of Immunology, Mayo Clinic, Rochester, Minnesota 55901

Products coded for by I-E and I-C subregions of the mouse major histocompatibility complex have been identified in haplotypes H-2^k and H-2^d. I-E subregion products have also been identified in haplotypes H-2^p and H-2^r on the basis of crossreactions with specific anti-I-E^k sera. I-E and I-C subregion products have not been defined in haplotypes H-2^b, H-2^f, H-2^q and H-2^s, in spite of extensive studies. This might suggest that I-E/I-C gene products are not highly polymorphic and might constitute ancestral gene products. The I-E and I-C gene products of the H-2^d, H-2^k, H-2^p and H-2^r haplotypes might constitute more recent mutational events. We have used xenoimmunizations to identify the I-E and I-C gene products in the 'silent' haplotypes. Rabbits were immunized with NP-40 solubilized extracts of lymphoid cells from mice expressing I^b, I^f, I^q and I^s. The rabbit antisera precipitated H-2 as well as Ia molecules. Upon removal of H-2K and I-A molecules with specific mouse alloantisera, the rabbit antisera precipitated an Ia molecule. Since Ia molecules have been mapped only to the I-A and I-E/C subregions it is likely that the Ia molecules detected by the rabbit antisera after removal of I-A subregion gene products are the I-E/C gene products. Additional sequential immunoprecipitations and absorption studies which further characterizes the Ia molecules immunoprecipitated by the rabbit antisera will be presented.

617 STRUCTURAL STUDIES ON A MODIFIED H-2K ANTIGEN ISOLATED FROM A MURINE THYMOMA. Fiona A. Hunter, John E. Mole, Jeff W. Paslay, and J. Claude Bennett, University of Alabama in Birmingham, Alabama 35294.

A glycoprotein with an apparent molecular weight of 70,000 has been immunoprecipitated by monoclonal anti-H2K^k antiserum from detergent solubilized, surface labelled membrane preparations of the AKR-derived thymoma, BW5147. Proteins precipitated by the same antiserum from AKR splenocytes and the ASL-1 lymphoma which express the H-2K^k antigen have a molecular weight of 45,000.

Several mechanisms for this modification have been postulated including viral directed modification of the H-2 antigen, inadequate cleavage of a precursor poypeptide of H-2 and transcription of normally silent DNA regions resulting in translation of regulator genes, inserted DNA regions or adjacent genes. Present research is directed towards structural studies of the modified antigen to determine if there is shared primary structure between the H-2K^k molecule, the modified antigen and the gp70 viral antigen. Additionally, antiserum against β-2 microglobulin is being used to determine if the modified antigen displays the same subunit structure as H-2 and HLA.

(Monoclonal antiserum was secreted by the 11-4.1 hybridoma obtained from the Salk Institute through the courtesy of L. Herzenberg.)

618 BIOCHEMICALLY, SYRIAN HAMSTERS HAVE AN MHC! J.T.Phillips, J.W.Streilein, and W.R. Duncan, U.Texas H.S.C. at Dallas, Texas 75235.

Syrian hamsters have been reported to display several anomalous immunologic responses: lack of MHC restriction in killing virus-infected targets; inefficient cellular collaboration in the IgM to IgG switch; and lack of suppressor cell generation during the course of vigorous allo-immune reactions. A genetic lesion at the hamster MHC equivalent, Hm-1, has been proposed to explain these aberrations. Utilizing newly developed hamster alloantisera, we have attempted to characterize biochemically the alloantigenic determinants of Hm-1. Glycoprotein fractions prepared from lentil lectin purified NP-40 extracts of radiolabelled lymphoid cells were immunoprecipitated with various experimental antisera. Upon SDS-PAGE, two types of determinants were discerned:(A)molecular species of 39,000 and 29,000 mol.wt. were immunoprecipitated from lymph node cells by alloantisera; (B)a single species of cell surface molecules with mol. wt. of 43,000 was co-precipitated with putative hamster B_2 microglobulin immunoprecipitated by a heterologous anti-human B_2m reagent. Type A and Type B molecules appear to be candidates for membrane glycoproteins comparable to those encoded by murine Class II(I) and Class I(K/D) H-2 genes, respectively. Preliminary linkage data suggest that the alloantisera identify antigens which segregate with determinants eliciting strong mixed lymphocyte reactivity. Our data provide biochemical support for the hypothesis that hamsters possess an MHC encoding for both Class I and Class II determinants. The data do not help resolve the dilemma regarding the putative immunologic aberrations ascribed to this species, but make the possibility less likely that the lesion resides at the level of the major histocompatibility complex.

619 TRYPTIC PEPTIDE ANALYSIS OF THE THIRD H-2 MOLECULE(H-2L) Stephen M. Rose, Ted H. Hansen, David H. Sachs, and Susan E. Cullen. Department of Microbiology and Immunology, Washington University School of Medicine, St. Louis,Mo. and Immunology Branch, NCI,National Institutes of Health, Bethesda, Md.

We have previously demonstrated the existence of a third classical transplantation molecule distinct from the known H-2K and H-2D molecules within the H-2 complex. This molecule has been designated H-2L and can be physically separated from the K and D molecules by sequential immunoprecipitation. The L molecule shares the same basic chemical properties as the K and D antigens in that it is a 45,000 dalton M.W. glycoprotein that is non-covalently associated with B_2-microglobulin. The H-2L molecules are determined by a locus which has not been separated from the H-2D by recombination. They can be specifically detected by a Balb/c-H-2^{db} anti-Balb/c antiserum in which the H-2^{db} is a mutant apparently lacking H-2L molecules. To further characterize the H-2L molecular structure and to examine the structural relationship of H-2L molecules to the previously studied H-2K and H-2D molecules, we performed comparative tryptic-peptide mapping analysis between L and D molecules of different haplotypes and analysis of L molecules of different alleles. The results unequivocally establish that the D and L molecules are structurally different and that the extent of difference is comparable to that found between the K and D molecules.

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620 Two-Gene Complementation for the Expression of Ia Antigens

Patricia P. Jones, Donal B. Murphy, and Hugh O. McDevitt, Dept. of Biological Sciences, Stanford Univ., Dept. of Medicine, Stanford Univ. School of Medicine, Stanford,CA 94305, and Dept. of Pathology, Yale Univ. School of Medicine, New Haven, CT 06510

We have published previously (J. Exp. Med. 148:925, 1978) that antibodies against the I-E^k subregion precipitate two polypeptide chains, one (E) coded for by I-E and the other (A_e) coded for by I-A. A_e is different from two other I-A subregion products (A_a and A_b) which normally are precipitated by anti-I-A antisera. A_e probably is brought down by anti-I-E antibodies because it is bound to E in the cell. We recently have extended and generalized these observations. In those haplotypes which express an I-E subregion product, e.g., k, d, p, and r, A_e is expressed on the cell surface. However, in cells of the b and s haplotypes, which do not synthesize E, A_e is found only in the cytoplasm. Surface expression of A_e of b and s haplotypes can be obtained if the I-A subregion is complemented with I-E from the k, d, p, or r haplotypes, either in F_1 heterozygotes or in recombinant strains. Thus it seems likely that the expression of A_e on the cell surface requires the presence of the E chain in the cell. Similar complementation studies as well as biochemical analyses indicate that the f and q haplotypes not only do not synthesize an I-E product, but also fail to synthesize detectable amounts of A_e. Different H-2 haplotypes therefore appear to vary in terms of which Ia polypeptide chains will be synthesized and expressed.

621 CHEMICAL CROSSLINKING OF Ia ANTIGENS, George F. Dancey, Susan E. Cullen, and Benjamin D. Schwartz, Howard Hughes Medical Institute Laboratory at Washington University and the Departments of Medicine and Microbiology and Immunology, Washington University School of Medicine, St. Louis, MO 63110.

Studies on the role of Ia antigens in the functioning of the immune system would be facilitated by a better understanding of their structure. For this reason, we have begun an examination of the polypeptide chain composition in membrane-bound and detergent solubilized murine Ia antigens via crosslinking with the cleavable reagent dimethyl 3,3'-dithiobisproprionimidate (DTBP). Products of the I-E subregion were solubilized from spleen cells with NP-40, partially purified by affinity chromatography on lentil lectin-agarose, exposed to DTBP, isolated by indirect immunoprecipitation, and analysed on SDS polyacrylamide gels. The I-E molecules could be almost completely crosslinked (75%) by DTBP as judged by the disappearance of the characteristic 35K (α) and 26K (β) m.w. polypeptide chains and the appearance of higher molecular weight material, the major species migrating as a protein of 67K m.w. When isolated and reduced with 2-mercaptoethanol, the 67K peak was found to be comprised of α and β chains. When I-E molecules were crosslinked on the cell surface, similar results were obtained with the exception that the degree of crosslinking was less (30%). These data suggest that the I-E α and β chains are associated in situ.

Preliminary results on DTBP crosslinking of antigens coded for in the I-A subregion suggest that these proteins can also be almost entirely crosslinked in detergent solution.

Supported in part by ACS grant IM-131 and NIH grants AI 13782 and CA 20500.

T and B Cell Markers and Differentiation

622 IMMUNOGLOBULIN SYNTHESIS BY LYMPHOID CELL LINES TRANSFORMED IN VITRO BY ABELSON MURINE LEUKEMIA VIRUS, Edward J. Siden, Naomi Rosenberg, Dan Clark and David Baltimore, Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, MA. 02139

The majority of cell lines derived by infection of murine bone marrow cells with Abelson murine leukemia virus synthesize a mu heavy chain but no dectable light chain. A minority of cell lines have been found which make no immunoglobulin, light chain alone, or transiently express a light chain as well as mu chain. Two lines have been studied in detail, one that makes mu chain and one that makes a kappa light chain. Synthesis of both polypeptides can be increased by modifying the culture conditions so as to decrease the growth rate of the cells. Although some kappa chain secretion was observed, neither secreted nor surface mu was dectected. We suggest that the mu-only phenotype may be and early, normal step in the pathway of B-lymphocyte maturation.

623 MECHANISM OF THE EXPRESSION OF MURINE THYMUS DIFFERENTIATION ANTIGENS. Ellen Rothenberg and Edward A. Boyse, Memorial Sloan-Kettering Cancer Center, New York, NY 10021.

Newly synthesized thymus-leukemia antigens (TL antigens) in murine tissues have been isolated by metabolic radiolabeling, detergent lysis, immune precipitation with specific antisera and fixed Staphylococcus aureus, and analysis of the precipitated materials by one- and two-dimensional gel electrophoresis. In normal TL^+ thymocytes and ASL.1 leukemia cells, which express TL.1,2,3 determinants, a 15-minute pulse of (^{35}S)methionine primarily labeled a 45000 dalton TL species which was not accessible to lactoperoxidase-catalyzed radioiodination. This TL species included an oligosaccharide moiety which was sensitive to endoglycosidase H. Over a period of hours, the pulse label could be chased into a form of TL with an apparent M_r of 46000 daltons, which was accessible to surface labeling and endoglycosidase H-resistant. Another surface form of TL, migrating diffusely with an apparent M_r of 48000 daltons, may also be derived from the 45000 dalton intracellular precursor. Labeled β_2-microglobulin was associated with 45000-dalton TL as well as with the surface forms. In contrast, in ERLD cells, a TL.1,2,4-positive leukemia from normally TL-negative mice, surface- and metabolically-labeled TL migrated with apparent M_r's of 48000-50000 daltons. The posttranslational processing steps involved in these conversions represent potential control points for the expression of these developmentally regulated antigens.

In further work, patterns of gene activity were studied in thymocytes cultured with and without mitogenic doses of Concanavalin A. In either case, TL synthesis was labile to culture while H-2 synthesis continued at the same rate. Synthesis of terminal deoxynucleotidyl transferase, however, was specifically shut off in the presence of Concanavalin A.

624 T CELL MARKERS, Donal B. Murphy, Yale University, New Haven, Conn. 06510.

Thymus derived lymphocytes (T cells) are a heterogeneous population of cells which mediate a number of immunological functions. They generate cytotoxic responses, exert helper and suppressive effects on humoral and cellular immunity, and are involved in delayed type hypersensitivity responses. The use of cell surface markers which are selectively expressed in different lymphocyte subpopulations has shown that at least some of these functions are mediated by distinct T cell subpopulations. For example, T lymphocytes bearing the Ly-1 determinant facilitate antibody production by B lymphocytes and enhance the generation of cytotoxic effector cells. In contrast, T lymphocytes bearing the Ly-2 determinant mediate cytotoxic responses and suppress both humoral and cellular immunity. During the past few years, studies with a number of other selectively expressed cell surface markers demonstrate further T cell heterogeneity. The Ia-4 determinant demarcates a subpopulation of Ly-2 bearing T cells which suppress immunity, while the Qa-1 determinant demarcates a subpopulation of Ly-1 bearing helper T cells which also have the property of inducing feedback suppression. Utilization of selectively expressed cell surface markers has not only allowed the identification of a number of functionally distinct T cell subpopulations, but has permitted further advances in elucidating the manner by which these cells interact with and regulate one another.

625 DETECTION OF ANTI-Ly SPECIFICITIES IN AKR ANTI-C3H ANTI-THY 1.2 SERUM. W.F. Davidson, I. Betel, S. O. Sharrow and B. J. Mathieson, National Institute of Allergy and Infectious Diseases, Bethesda, MD. 20014

Anti-Thy 1 sera have been used widely for both the detection and specific elimination of murine T cells. On the basis of strain distribution classical AKR anti-C3H anti-Thy 1.2 serum may contain antibodies with specificity for antigens other than Thy 1.2, namely, Ly 1.1 Ly 3.2, Ly 6.1, Ly 7.1, Ly 8.1 and Ala 1.1. By in vivo absorptions with selected inbred and congenic mice and analysis of antibody binding by flow microfluorometry we have detected three of these specificities; anti-Ly 1.1, anti-Ly 3.2 and anti-Ly 8.1. The fluorescence profiles obtained with anti Ly 1.1 and anti-Ly 3.2 sera were similar to those observed with alloantisera to these determinants. Thus, AKR anti-C3H anti-Thy 1.2 serum provides a convenient source of high titre anti Ly- t sera in addition to containing antibodies which react to antigens present on both T and B cells such as Ly 8. The implications of removing Ly 8^+ B cells remain to be determined.

626 SEROLOGICAL, BIOCHEMICAL AND FUNCTIONAL PROPERTIES OF Ia ANTIGEN EXPRESSION ON MURINE PEYER'S PATCH CELLS, Christopher J. Krco, Steven J. Challacombe, William Lafuse, Brian C. Neely, Chella S. David and Thomas B. Tomasi, Jr., Dept. of Immunology, Mayo Clinic and Medical School, Rochester, Minnesota 55901

The expression of antigens associated with the I-region (Ia antigens) of the mouse major histocompatibility complex (MHC) on Peyer's Patch cells (PPC) was studied. Approximately 45% of PPC from mouse strain CBA are killed by antiserum (A.TH anti-A.TL) specific for the I-region (anti-A,B,J,E,C) in complement dependent cytotoxicity assays. When PPC are tested with antisera specific for the I-A or the I-E subregions approximately 30% of the cells are killed. When the same three antisera were tested on spleen and lymph node cells essentially the same results were obtained. Antisera directed against the other I subregions did not lyse the PPC. On the other hand, antiserum specific for I-C gave a weak reaction. Immunoprecipitation studies with PPC gave substantial Ia peaks. Further studies on the serological and biochemical characterization of I region products on Peyer's Patch cells will be discussed. Studies using PPC in in vitro assays of immunocompetence with respect to mitogen and alloantigen responsiveness are in progress and will be reported.

627 INTERACTION BETWEEN Fc (IgG) RECEPTORS AND ALLOANTIGENS ON THE SURFACE OF BALB/c B LYMPHOMA LINES. Colette Kanellopoulos-Langevin, K. Jin Kim, David, H. Sachs and Richard Asofsky. NIAID, NCI, Bethesda, MD.

The availability of a series of spontaneous B cell lymphomas has permitted us to study the relationship between several B cell markers in homogeneous B cell lines as opposed to the heterogeneous splenic B cell populations previously studied. We have found that Fc (IgG) receptors, detected by erythrocyte-antibody (EA) rosetting technique could be inhibited by polyvalent anti Ia sera on Ia positive (Ia+) cell lines (A20, K46, X16C). Antisera directed against antigens coded by individual I-subregions were likewise inhibitory of EA rosettes. There was no such inhibition of an Ia negative (Ia-) cell line (M12). A 1 to 1 mixture of A20 (Ia+) and M12 (Ia-) cells was incubated with anti Ia serum and the resulting inhibition was 50%, suggesting that the alloantibodies reacted only with the Ia antigens on the same cells on which the Fc receptors were blocked. In addition, either Ia+ or Ia- cell lines could be inhibited with antisera directed against Mls-associated LyM antigens. Inhibition by anti H-2D sera was also tested and inhibition was obtained in all cases, although on some cell lines this inhibition was clearly distinct from that produced by anti Ia sera. The differences we have observed between the relationships of alloantigens and Fc receptors in these cell lines may reflect their origin from different distinct B cell sub-populations.

628 MOUSE LYMPHOCYTE ALLOANTIGENS CONTROLLED BY GENES ON THE X CHROMOSOME, Marc Zeicher, Edna Mozes, Yair Reisner, Peter Lonai, The Weizmann Institute of Science, Rehovot, Israel.

By immunization of F_1 male recipients with spleen cells of the identical F_1 female we could obtain antisera directed against alloantigens controlled by the X chromosome of the parental male strain (Ly-X alloantigens). Ly-X alloantigens were mainly detected on a thymus derived lymphocyte subpopulation present in thymus, spleen and lymphnodes (Lyt-X alloantigens). In the thymus, Lyt-X was only detected on subpopulations of mature, cortisone resistant Tla^- thymocytes namely on part of the Tla^- $Ly1^+2^+3^+$ cells and on part of the Tla^- $Ly2^+3^+$ cells but neither on the Tla^+ thymocytes nor on the Tla^- $Ly1^+$ thymocytes. In the spleen and the lymph nodes Lyt-X was only detected on about 40% of the $Ly1^+2^+3^+$ cells and on part of the $Ly2^+3^+$ cells but not on the $Ly1^+$ cells. In 4 out of 11 batches of pooled sera we could detect activity on both B and T cells. Absorption studies reveal that the Lyt-X alloantigens and the Lyb-X alloantigens are antigenically distinct. Lyb-X was detected on 50% of the B cells and may be an allelic form of Lyb-3. The strain distribution pattern of Lyt-X and Lyb-X was similar. Regulation of their expression was in agreement with Mary Lyon's theory of X-chromosome inactivation. Preliminary backcross experiments show that Lyb-X and Lyt-X are controlled by separate genes on the X-chromosome.

629 IMMUNECOMPLEX INDUCED MODULATION OF Fc-RECEPTOR EXPRESSION ON HUMAN T CELLS, Werner J. Pichler, Lawrence Lum, and Samuel Broder, Metabolism Branch,NCI,NIH, Bethesda, Maryland.

It has been proposed that receptors for Fc-IgG and Fc-IgM may be used as markers to identify distinct human T cell subsets. Reportedly, the T_μ cells, having receptors for IgM, represent helper T cells, while T_γ cells, having receptors for IgG, represent suppressor T cells after interaction with IgG-immunecomplexes (IC). We reinvestigated these T cell subclasses in healthy humans with a sensitive rosette method, namely bovine red blood cells coated with rabbit IgG (EA-IgG) or IgM (EA-IgM). Sixty-five to 91% of the T cells exhibited Fc-IgM receptors after culture and 5-35% expressed Fc-IgG receptors. These two populations can be seperated by interaction of T_γ cells with EA-IgG and Ficoll Hypaque gradient seperation. In the interphase one finds T-nonγ cells, which consist of over 90% T_μ cells and about 1% T_γ cells. In the pellet an enriched T_γ cell population is found (72-85%). After lysis of the bound EA-IgG, incubation of the cells at 37°C leads to capping, endocytosis and/or shedding of the Fc-IgG receptor. Culture of these cells leads to only partial reexpression of Fc-IgG receptors (5-20%), but expression of Fc-IgM receptors (67-83%) on these originally T_γ cells. The transition of T_γ to T_μ requires IC interaction of the T_γ cells and is blocked by NaN_3 and incubation at 4°C. Cytochalasin B, Cochicine and X-irradiation have no effect. Pronase treatment of these positively selected T_γ cells uncovers Fc-IgG receptors (which will not cap), and after 40hr in culture T cells bearing Fc-IgG and Fc-IgM receptors can be found. Therefore, Fc receptors do not appear to be markers for distinct T cell subsets, under some conditions. Rather, they may be the expression of a functional stage of T cells.

630 CELL SURFACE RECEPTORS OF A TRANSFORMED RABBIT SPLEEN CELL LINE, A.D. Strosberg[+°], V. Zurawski[x], A.B. Schreiber[+], B. Vray[•], C. Favre[§], P. Marche[§], E. Petit-Koskas[°] and G. Buttin[°]. [+]Free University of Brussels (V.U.B.), [x]Harvard Medical School, [•]Université Libre de Bruxelles (U.L.B.), [°§]Universités Paris VI and VII.

The rabbit spleen cell line TRSC-1, transformed by simian virus 40, was grown continuously in vitro for over two years (Strosberg et al., 1974 : Proc. Natl. Acad. Sci. 71, 263). The cells maintained a low production for anti-pneumococcal strain SIII antibodies during the first passages in vitro, but lost this capacity after about 30 transfers. Attempts to raise the initial low level of antibody by addition of antigen or by modifying growth conditions were unsuccessful. Various cell surface receptors were researched. Tests for cell surface immunoglobulins and Fc receptors were negative. Binding of lectins and anti-ß2-microglobulin antibodies on these cells will be studied. The effect of several types of mitogens and the capacity of these cells to perform phagocytosis will be analyzed.

Antibodies will be raised against the TRSC-1 cells and will be used to define cell subpopulations among normal rabbit B lymphocytes.

631 A STUDY OF HUMAN T LYMPHOCYTE SURFACE ANTIGENS, D. McMahon Pratt, S.F. Schlossman, and J.L. Strominger, Sidney Farber Cancer Institute, Boston, Massachusetts 02115

A high titered (1/600-1000) anti-human T lymphocyte serum (A99) was raised against the Brij 97 detergent solubilized crude membrane fraction isolated from the CEM T cell line. The antiserum when absorbed with B lymphoblastoid cell lines (including one derived from an HLA identical sibling of the CEM cell donor) was found to be specific for T lymphocytes. Analysis of indirect immunofluorescence by fluorescence activated cell sorter (FACS-I) demonstrated that the A99 antiserum reacted with normal peripheral blood T-lymphocytes and T lymphoblastoid cell lines but not with B lymphoblastoid cell lines or acute myelogenous leukemia cells, chronic lymphatic leukemia or chronic myelogenous leukemia cells. Immunoprecipitation data using ^{125}I radioiodinated antigenic material showed that at least four different molecular species are recognized by the A99 antiserum and are specific for the human T lymphocyte. The A99 antiserum precipitated 53,000, 96,000, 120,000 and 152,000 dalton molecular weight protein molecules. A 196,000 dalton protein molecule was found to be recognized by the A99 antiserum on CEM cells but was also detected in low but detectable quantities in immunoprecipitates from a B cell line. The antigens recognized by the A99 antiserum have been purified approximately 3000-fold through a 5 step biochemical procedure from the crude CEM membranes. Since, the elucidation of T lymphocyte functional mechanisms through the isolation and structural analysis of T lymphocyte specific plasma membrane components would provide information of great value to the future clinical treatment of autoimmune and immunodeficiency disorder and of organ transplant derived problems, this study should provide the basis for further biochemical and immunological studies of great interest.

632 CHARACTERIZATION OF A NON-H-2 LINKED GENE CLUSTER CODING FOR THE MURINE B CELL ALLOANTIGENS LYB 2, LYB 4, and LYB 6, Steven Kessler, Aftab Ahmed, and Irwin Scher, Department of Immunology, Naval Medical Research Institute, and Department of Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD 20014

We have defined as Lyb 6 the 45,000 d polypeptide, B lymphocyte surface membrane target of an antiserum raised in CBA/N mice against CBA/J spleen cells. Lyb 6 is identified by its mobility (reduced) in SDS-PAGE after immunoprecipitation from radioiodinated cells of appropriate mouse strains. The B cell localization of this antigen was verified by the inability of extensive absorptions with cells from nonlymphoid tissues to remove Lyb 6 reactivity from this serum, and by the ability to isolate this antigen from B cell populations but not from B cell-depleted populations. Because the strain distribution of Lyb 6 (further defined as the 6.1 allele) was strikingly similar to the published distributions of Lyb 2.1 and Lyb 4.1 (defined serologically), we performed linkage analyses for these and other markers in both BXD recombinant inbred strains and in (C57BL/6 x DBA/2)F_1 x C57BL/6 backcross mice. The results indicated conclusively that Lyb 2, 4, and 6 are products of distinct genes which are linked to the Mup-1 locus on chromosome 4. The pattern of recombinations observed establishes the gene order as Lyb 2-Lyb 4-Lyb 6-Mup-1, with a distance of approximately 4cM separating the Lyb 2 and Lyb 6 genes. At least one important B cell function, responsiveness to lipopolysaccharide in C3H/HeJ mice, has also been genetically mapped in this area by other workers. These findings suggest a role for this chromosome region in B cell development or in regulation of certain B cell functions.

633 INDUCTION OF SURFACE MARKERS IN THE B LYMPHOCYTE LINEAGE. By U. Hammerling, Sloan-Kettering Inst. New York, A. Ahmed, and I. Scher, Naval Med. Res. Inst., Bethesda.

Induction of phenotype conversion is a phenomenon occurring in B and T lymphocyte precursor cells that are committed to a new phenotype specified by a preordained differentiation pathway, but these cells depend on an appropriate extracellular inducing influence for expression of that phenotype. This inducing signal can be given in vitro by a variety of inducing agents, allowing the cells to advance in their differentiation pathway. The progression is assessed by measuring serologically the appearance of several new surface markers (e.g., IgM, Ia, CR, IgD, Pc.1). Because the inducible precursor B lymphocytes differ in their phenotypes with respect to several markers they can be formally separated into several subpopulations, representative of distinct differentiation compartments. Conversion of phenotype by induction in vitro is indicative of progression of cells from one compartment to the next, and this provides a means to determine the sequence of differentiation in the B cell lineage. New information indicates that the expression of Lyb2 precedes that of IgM; thus Lyb2 is one of the earliest markers of B cells. By contrast, Lyb5 is expressed late in B cell development coinciding with the expression of IgD and CR. On present evidence the ontogenetic order of expression of surface markers can be written as follows:

B Stem Cell ...	$Lyb2^+$	-	IgM^+	-	Ia^+	-	IgD^+	-- IgM^- $Lyb2^+$
							CR^+	Ia^- $Pc.1^+$
							$Lyb5^\pm$	CR^- $Ala.1^+$
	Pre B		Virgin B		Immunocompetent B			Plasma cell

634 AN ISOANTIBODY WITH SPECIFICITY FOR PROTHYMOCYTES, SOME THYMOCYTES AND A SUBSET OF PERIPHERAL T CELLS, R.S. Basch & J.N. Buxbaum, Departments of Pathology and Medicine, New York University Medical Center, New York, NY 10016

Inoculation of BALB/c mice with RLθ1-v4, a Thy-1 negative variant of the T-cell lymphoma RLθ1, obtained by immunoselection leads to the production of antibodies cytotoxic for hematopoietic thymocyte precursors and thymocytes. While 2/3 of cortical thymocytes are killed, medullary thymocytes are not affected by this reagent. Syngeneic, as well as allogeneic, cells are killed and no differences in susceptibility are found amongst mice differing at the TLa, Thy-1, Gv-1, Lyt-1, Lyt-2 or a variety of H-2 loci. Thymocyte precursors (measured by their ability to repopulate the thymus of irradiated mice) are highly susceptible to this antibody, but multipotential stem cells (CFU-s) and B cells are resistant. Although direct cytotoxicity for spleen or lymph node cells could not be demonstrated and neither the Con A nor PHA responses of peripheral T cells are affected, quantitative absorption studies indicate that small amounts of the antigen are present in spleen cell suspensions. Pretreatment of spleen cell suspensions with this antiserum reduces but does not abolish the capacity of these suspensions to respond to allogeneic stimuli in mixed leukocyte cultures. It appears likely that this serum is detecting a differentiation antigen characterizing T cell precursors and a subset of peripheral T cells.

635 MICE HAVE SERUM IgD, Fred D. Finkelman, Virgil L. Woods, Alice Berning, and Irwin Scher, The Uniformed Services University School of Medicine, The Naval Medical Research Institute, and The National Institutes of Health, Bethesda, MD 20014.

While serum IgD has been found in man, the monkey, and the rat, past attempts to identify it in mouse serum have been unsuccessful. We have looked for mouse serum IgD by analyzing the ability of sera to block FITC conjugated allospecific rabbit anti-mouse δ^a (Ra$M\delta^a$) staining of mouse spleen cells. The intensity of staining was quantitated with a fluorescence activated cell sorter. Staining of BALB/c spleen cells by Ra$M\delta^a$ was blocked by pre-incubating this antiserum with sera from BALB/c or DBA/2 mice, both of which have B lymphocyte surface (s) Ig of the a allotype. Five µl of BALB/c serum blocked staining to approximately the same extent as absorption with 10^6 adult BALB/c spleen cells. Staining was not blocked, however, by pre-incubating Ra$M\delta^a$ with serum from C57BL/6 mice which have sIgD of the b allotype, or serum from CB-20 mice, which are congenic to BALB/c mice but have the C57BL/6 C_H region genes. BALB/c serum which had been absorbed with Sepharose-bound rabbit anti-mouse Ig or Sepharose-bound hybridoma 10-4.22 anti-mouse δ^a no longer inhibited staining of BALB/c spleen cells by Ra$M\delta^a$. BALB/c IgMλ, IgGκ, and IgAκ plasmacytoma proteins also failed to inhibit staining. These considerations indicate that the serum inhibiting factor is IgD. While a conventional mouse anti-mouse δ^a could be substituted for Ra$M\delta^a$ in our assay, mouse serum does not specifically inhibit staining of B cells by FITC labeled hybridoma anti-mouse δ antibodies. Since we have shown indirectly that these monoclonal antibodies bind serum IgD, other considerations such as low avidity or the low number of determinants bound must be responsible for their lack of efficacy in our system.

636 RABBIT PERIPHERAL BLOOD LYMPHOCYTES WITH SHARED T AND B CELL PROPERTIES, Corey Raffel and Stewart Sell, Univ. of Calif., San Diego, La Jolla, CA 92093.

Rabbit lymphocyte surface immunoglobulin (sIg), phytohemaglutinin (PHA) receptors, and concanavalin A (Con A) receptors have been examined by electron microscopy. 70% of peripheral blood lymphocytes (PBL) are labeled by anti-immunoglobulin, whereas 100% of PBL are labeled by either PHA or Con A. When labeled cells are incubated at 37° for 30 minutes, 64% endocytose the sIg label, 45% endocytose the PHA label, and 55% endocytose the Con A label. These numbers correspond roughly to the percentages of cells transformed by each mitogen. When PBL are first incubated with either lectin labeling system, and then labeled for sIg, the following results are obtained:

FIRST LABEL	INCUBATION (37°, 30')	SECOND LABEL	LECT. ENDO. sIg^+	LECT. ENDO. sIg	NO LECT. ENDO. sIg^+	NO LECT. ENDO. sIg
PHA	Yes	sIg	31%	16%	34%	19%
Con A	Yes	sIg	21%	24%	41%	14%
PHA	No	sIg	---	---	70%	30%
Con A	No	sIg	---	---	70%	30%

Thus, between 20% and 30% of rabbit PBL endobytose T cell mitogens and bear sIg. Preliminary data indicate that less than 4% lectin endocytosing, sIg bearing cells can be found in spleen and lymph node cells.

637 THE REGULATION OF HUMAN LYMPHOCYTE DEVELOPMENT BY THE THYMUS, James F. Jones and Frank L. Meyskens, Jr., Depts. of Pediatrics and Medicine and the Cancer Center, University of Arizona, Tucson, AZ 85724

The development and maturation of T-lymphocytes is dependent on a normal hormonal and direct cellular interaction between thymus epithelial (TE) cells and prothymocytes. Analysis of these interactions in postnatal man is being performed. TE monolayers are established following enrichment using density gradient separation techniques. TE cell type and growth rate vary with age of the donor as determined by morphological criteria and measurement of macromolecular synthesis. Levels of terminal deoxynucleotidyl transferase (TdT), an enzyme found in prothymocytes and in the thymus, but not in peripheral T-lymphocytes, also vary with the age of the donor. TdT levels were present in the thymus of 3 patients with known T-cell dysfunction: severe combined immunodeficiency disease, DiGeorge's syndrome, and Down's syndrome. These observations suggest a functional relationship between TE cells and the expression of TdT in T-lymphocytes. Mixing experiments are in progress to test the effect of donor age and type of TE cell in culture on T-lymphocyte development by assessing changes in TdT levels and stable E-rosetting. We will also report on the effects of ATP and its metabolites on the *in vitro* regulation of TdT activity and the *in vivo* expression of TdT and the effect of these metabolites on T-cell development.

638 Ly 9, AN ALLOANTIGENIC MARKER FOR LYMPHOCYTE DIFFERENTIATION, Bonnie J. Mathieson, Susan O. Sharrow, Kim Bottomly, and Betty Jo Fowlkes. National Institute of Allergy and Infectious Diseases, Bethesda, MD. 20014.

A new lymhpocyte cell surface alloantigen, provisionally designated Ly 9, is detected as an extra specificity in sera from anti-Lyt immunizations. Ly 9.1, one of the apparently allelic specificities is detected in anti-Lyt 3.1 immunizations; (C3H/HeN X SJL/J)F_1 anti-C58 normal thymocytes. The alternative antigen expression, Ly 9.2, can be detected routinely in sera from 2 other Lyt immunizations; C57BL/6-H-2^k anti-CE/J normal thymocytes (anti-Lyt 2.1), and C58 anti-CE normal thymocytes (anti-Lyt 3.2).

This lymphocyte alloantigen has both a unique strain distribution and a unique cell/tissue distribution from previously reported cell surface antigens. Ly 9 is expressed on all thymocytes, lymphocytes and apparently on lymphocyte precursors in the bone marrow. This antigen is not expressed however on erythrocytes, epidermal cells, sperm, testis, brain, kidney, liver, or lung. Flow microfluorometry analysis and absorption typing reveals a quantitative difference between the level of antigen expression on cells from thymus versus spleen or lymph node. Cytotoxic elimination experiments confirm that the antigen is expressed on both T and B cells and on different T cell functional subsets.

639 EXPRESSION OF B CELL DIFFERENTIATION MARKERS OF BALB/c B CELL LYMPHOMA LINES AND THEIR HYBRIDOMAS. K. Jin Kim, Reuven Laskov, Colette Kanellopoulos-Langevin, Virgil L. Woods, Paul E. McKeever and Richard Asofsky. National Institute of Allergy and Infectious Diseases, Bethesda, MD 20014.

Balb/c B lymphomalines (K46, L10A, X16C, BALENTL 17, A20 and M12) have been reported to express various surface markers such as Ig, Fc receptors and Ia antigens. Biosynthesis and surface expression of monomeric IgM by K46, L10A and X16C has been confirmed by a biosynthetic labelling technique using ^{3}H- and ^{14}C-amino acid precursors and by a surface labelling technique using ^{125}I. Labeled molecules could be immunoprecipitated with class specific anti-IgM but not anti-IgA or anti-IgG. Some of these B cell lines appear to have IgD like molecules as judged by precipitation with anti-IgD antiserum obtained from several sources. Expression of the B cell differentiation antigens such as Ly-b5 and Ly-b7 are currently being studied.

Hybridomas were obtained from the fusion experiments between these B cell tumor cells with drug resistant cell lines derived from an IgG2b producing MPC-11 myeloma. Most hybridomas were able to secrete pentameric IgM but did not express significant levels of Ia antigens nor Fc receptors.

640 EXPRESSION OF 20α-SDH IN LYMPHOCYTE SUBPOPULATIONS, Laureen Pepersack, Cancer Biology Program, NCI Frederick Cancer Research Center, Frederick, MD 21701

Twenty-α-hydroxysteroid dehydrogenase (20α-SDH) mediates the conversion of progesterone to 20α-hydroxypregn-4-en-3-one and has been reported to be a possible enzymatic marker of T cells (Nature 266:632, 1977). To investigate the distribution of 20α-SDH expression in lymphoid populations of the thymus and spleen of the mouse, we used a variety of approaches. In the thymus, the lymphoid compartments include the cortisone-sensitive, major population, which expresses the enzyme terminal deoxynucleotidyl transferase (TdT), and the cortisone-resistant, TdT-negative, minor population (J. Immunol. 117:620, 1976). In contrast with TdT expression, cortisone treatment results in little loss of total 20α-SDH activity, suggesting that expression of 20α-SDH is associated with the minor, cortisone-resistant population. To further examine the reciprocal expression of these two enzymes, we examined a variety of lymphoma cell lines, all of which expressed TdT, and showed no detectable levels of 20α-SDH activity. In addition to the thymus, significant 20α-SDH activity is detectable in spleen extracts. Separation of splenocytes on nylon wool and anti-μ columns indicates that 20α-SDH activity is associated with the T-cell fraction. Consistent with this observation was our finding that cultures of macrophages and B-cell lines expressed no detectable levels of the enzyme. Depletion of splenocytes of T cells by anti-θ treatment resulted in >65% loss of 20α-SDH activity. These results strongly suggest that the expression of 20α-SDH may be an ideal enzymatic marker for mature, cortisone-resistant T cells. (Research sponsored by the National Cancer Institute under Contract No. NO1-CO-75380 with Litton Bionetics, Inc.)

Methods for Cloning T Cells

641 CELL SURFACE MARKERS AND FUNCTIONAL CHARACTERISTICS OF INDIVIDUAL AKR LYMPHOMAS, Laura J. Nell and Ellen R. Richie, M.D. Anderson Hospital, Houston, Texas 77030.

T and B cell surface markers and in vitro functional assays have been used to determine the stage of differentiation of individual AKR thymic lymphomas. Previously, we found that AKR lymphomas with similar cell surface phenotypes showed varying patterns of mitogen reactivity. Three additional spontaneous lymphomas have been characterized and maintained by in vivo passage. Cell surface markers examined include receptors for Fc of IgG (FcR) and C3 (C3R), surface immunoglobulin (SIg), Thy 1.1 alloantigen and the viral coat protein, gp 70. Two lymphomas (920 and 720) lack FcR and C3R while a third (105) displays both FcR and C3R. All 3 lymphomas bear Thy 1.1 and gp 70 antigens and lack SIg. The 720 and 105 spontaneous lymphomas responded to PHA and ConA, a characteristic of T cells at a relatively mature stage in their differentiation, whereas the 920 lymphoma was unresponsive. None of the lymphomas responded to LPS or dextran sulfate or to alloantigens. This data indicates that lymphomas with similar cell surface markers may differ in state of functional differentiation, whereas lymphomas which behave similarly in functional assays may display differing cell surface markers. To determine if the markers and functional characteristics of each lymphoma were stable, lymphoma cells were transplanted into syngeneic young recipients. Upon transfer, the 920 lymphoma cells failed to infiltrate recipient thymuses although peripheral lymphoid organs were completely infiltrated. In contrast, the functionally differentiated 720 and 105 lymphomas infiltrated recipient thymuses as well as peripheral lymphoid organs. The transplanted lymphomas maintained the initial surface marker and functional profiles of the spontaneous lymphomas. Cells from the 720 lymphoma infiltrated thymuses were further characterized by fractionation according to sedimentation velocity (centrifugal elutriation) utilizing gp 70 as a marker for leukemia virus. Cells which sedimented between 3.5 and 5.7 mm/hr/g responded to the mitogens and expressed gp 70 on their surfaces after mitogen stimulation.

642 GLUCOCORTICOID-HORMONE IMMUNE REGULATION:CONTROL OF T-CELL GROWTH FACTOR PRODUCTION, Steven Gillis, Gerald Crabtree and Kendall A. Smith, Dartmouth Med. Sch., Hanover, NH

Although several studies have shown that glucocorticoid hormones mediate profound metabolic effects on both resting and activated lymphocytes, the mechanism of corticosteroid-induced immunosuppression remains ill-defined. We recently described methodology allowing for the long-term culture of both human and murine antigen-specific cytolytic T-cells (CTLL) dependent upon a T-cell growth factor (TCGF) present in tissue culture medium conditioned by T-cell mitogen-activated lymphocytes. Using a microassay for TCGF activity (based on the ^{3}H-Tdr incorporation of CTLL in response to TCGF) we have examined the effects of glucocorticoids on both the replication of antigen-sensitized CTLL and on the production of TCGF. We found that after 48 hrs. of culture in the presence of 10^{-6}M dexamethasone (DEX), CTLL proliferation was only slightly decreased (10-20%) and there was no dimunution of CTLL cytotoxic activity. However, concentrations as low as 10^{-8}M DEX induced 100% inhibition of TCGF production by either Con A-activated rat spleen cells or PHA-stimulated human peripheral blood lymphocytes. Inhibition of TCGF production was found to be a glucocorticoid-specific phenomena. The amount of TCGF produced in the presence of DEX was directly correlative to the proliferative response observed in mitogen-activated lymphocyte preparations. These experiments suggest that glucocorticoid-mediated suppression does not act at the level of functionally differentiated effector lymphocytes (e.g. CTLL cells) yet controls the degree of expansion of antigen-activated populations by regulating the production of their proliferative signal, TCGF.

643 T-CELL MITOGEN AND ALLOANTIGEN SENSITIZATION OF NUDE MOUSE PRE-T-CELLS, Steven Gillis, Paul E. Baker, and Kendall A. Smith, Dartmouth Med. School, Hanover, NH.

Recently, we reported that T-cell growth factor (TCGF) derived from T-cell mitogen/antigen stimulated mononuclear cells allowed for the continuous proliferative culture of mitogen or antigen-activated T-cells. Further study of TCGF revealed that murine thymocytes produced minimal amounts of TCGF and exhibited poor proliferative responses to T-cell mitogens. However, proliferation and continuous growth of thymocytes was observed if TCGF was supplied exogenously along with a T-cell mitogen. These observations prompted an investigation of the effects of TCGF on thymocyte precursors present in athymic (nu/nu) mice. Using a microassay for TCGF (J. Immunol. 120:2027, 1978) we found that nu/nu spleen, lymph node or bone marrow cells did not produce TCGF and did not proliferate in response to T-cell mitogens. However, normal proliferative responses were observed in response to T-cell mitogens if TCGF was added exogenously. Additionally, alloantigen senstization (C57Bl/6) of BALB/c nu/nu spleen cells in the presence of TCGF, resulted in the generation of antigen specific cytolytic effector cells. Treatment of the effector cells with anti-Thy-1 serum and complement completely abrogated cytolytic activity. These cells have remained in TCGF-dependent proliferative culture for over six weeks and continue to mediate antigen-specific cytolysis (30% lysis at an effector:target ratio of 250:1). These results suggest that a primary function of the thymus is to effect the maturation of cells capable of producing TCGF and that prothymocytes present in nu/nu mice are capable of normal T-cell immune responses if TCGF is supplied exogenously.

644 PURIFICATION OF A T-CELL MITOGEN FROM LYMPHOCYTE CONDITIONED MEDIA (CM) J.W. Mier*[1], V.S. Kalynaraman*[2], M.G. Sarngadharan*[2], and R.C. Gallo[1], [1]National Cancer Institute and Litton Bionetics Laboratories, Bethesda, Maryland 20014.

Several years ago a report from this laboratory showed that CM from PHA stimulated human blood cells contained mitogenic factors for human hematopoietic cells.[1] Subsequently, pure populations of T-cells derived from normal human blood or marrow were grown in long-term liquid suspension culture for the first time by using this CM.[2,3] Here we report the purification of the active factor. CM was prepared by incubating human leukocytes in RPMI media containing 1% BSA and PHA for 3 days. The CM contained various biologically active substances, including the T-lymphocyte mitogen (TLM) and, as noted earlier, colony-stimulating factor (CSF).[1] After $(NH_4)_2SO_4$ precipitation, TLM was further purified by chromatography on a G-100 Sephadex column, from which the active fraction emerged with a molecular size of 20,000. Further purification of the protein involved DEAE-sepharose chromatography which removed several contaminating proteins. Isoelectric focusing was needed to separate the TLM from the more acidic CSF present in the CM, which previously copurified with TLM. The MW of the purified TLM was 12,000 on SDS PAGE. The failure to stain with PAS or to bind to any of 5 different lectin sepharoses showed that glycosyl moieties are not needed for biological activity and indicated that the protein was never glycosylated or that glycosyl moieties were lost in purification. Studies designed to correlate mitogenic activity with selective binding to cell membranes and efforts to define the physiologic role of the factor, especially in relation to immune deficiency states, are in progress. References: (1) Prival, J., Paran, M., Gallo, R., Wu, A: J. of the Nat. Cancer Inst., 53:1583-1588, 1974. (2) Morgan, D., Ruscetti, F., Gallo, R: Science, 193:1007-1008. (3) Ruscetti, R., Morgan, D., Gallo, R: J. of Immun., 119:131-138, 1977.

645 BIOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF FACTORS ENHANCING HUMORAL AND CELL MEDIATED IMMUNE RESPONSES, John J. Farrar, Philip L. Simon, William L. Farrar, and Janet Fuller-Bonar, The National Institutes of Health, Bethesda, Maryland 20014.

Culture supernatants from mouse spleen cells stimulated with either concanavalin A or allogeneic cells contain activities which reconstitute the anti-SRC PFC response of nude mouse spleen cells (T cell replacing factor; TRF) and enhance the generation of alloantigen-specific cytotoxic T cells (killer cell helper factor; KHF). Chromatographic analysis of these supernatants reveals at least three antigen-nonspecific factors with TRF and/or KHF activity. Factor 1 exhibits both TRF and KHF activity, has a molecular weight of 30,000-35,000 as estimated by gel filtration, and is apparently identical to T cell-derived mitogenic factor. Factor 2 exhibits KHF activity but no TRF activity, has a molecular weight similar to that of Factor 1, but is clearly separable from Factor 1 by either hydroxylapatite or blue Sepharose chromatography. Furthermore, Factor 2 is inseparable from Type II immune interferon. Factor 3 demonstrates: 1) TRF-like activity at high concentrations, especially in the presence of T cells, and 2) KHF-like activity under defined experimental conditions. Factor 3 has a number of the biochemical and biological characteristics of macrophage-derived lymphocyte activating factor (e.g. mitogenicity for thymocytes, 15,000 M.W., charge heterogeneity on DEAE-Sepahcel and PAGE). These data suggest that the control of both humoral and cell-mediated immune responses can be mediated in part by the same monkine and/or lymphokine. Furthermore, these immune responses are apparently modulated by a series of interdependent and sequential mediator-lymphocyte interactions which initiate and amplify immunological responsiveness.

646 T-CELL GROWTH FACTOR:THE SECOND SIGNAL IN THE T-CELL RESPONSE, Kendall A. Smith, Steven Gillis, Paul Baker, Douglas McKenzie, Dartmouth Med. School, Hanover, NH

T-cell growth factor (TCGF), derived from T-cell mitogen/antigen-stimulated mononuclear cells, selects for and supports the continuous growth of human and murine cytotoxic T-lymphocyte lines (CTLL). Furthermore, the in vitro generation of cytolytic T-cells was found to be dependent on TCGF. To investigate the parameters of TCGF production and action we used a microassay for TCGF (J. Immunol. 120:2027, 1978). Thy-1 positive spleen cells were found to be required for TCGF production, however, nylon-column purified splenic T-cells produced reduced amounts of TCGF (10% of unfractionated cells). The addition of 1-5% adherent spleen cells to purified T-cells restored TCGF production. Thymocytes produced 1% TCGF compared to spleen cells and the addition of adherent cells did not increase activity. Proliferating T-cells (mitogen-activated and CTLL) depleted TCGF from media, resulting in the cessation of proliferation and cell death. Furthermore, only activated (PHA, Con A, MLC, CTLL) T-cells absorbed TCGF activity. Absorption occurred rapidly (within 2 hours at 37°C) and was cell concentration dependent. Mitogen-free, partially purified TCGF supported the continuous proliferation of activated (PHA, Con A, MLC, MTLC) T-cells but did not initiate or sustain proliferation of unactivated cells. These data indicate that TCGF is the proliferative signal in the T-cell immune response and that: 1)both mature T-cells and adherent cells are required for TCGF production, 2)T-cell proliferation requires mitogen/antigen activation but proliferation is mediated solely by TCGF, and 3)TCGF probably interacts with activated T-cells via a TCGF-specific receptor. Therefore, further studies of the factors controlling TCGF production and utilization, may provide important information regarding the regulation of the T-cell immune response.

647 ACTIVITIES OF A PERMANENT T KILLER CELL LINE, Gunther Dennert, The Salk Institute, Post Office Box 1809, San Diego, California 92112

Permanent cell lines of T killer cells have been established by weekly restimulation of mouse mixed lymphocyte cultures with irradiated stimulator cells. One line (BALB/c lymphocytes sensitized to C_3H targets) has been kept in culture continuously for more than three years. This line can be frozen in liquid nitrogen and proliferates with a doubling time of about 30 hr if stimulated with spleen cells carrying antigens coded by the IA^k subregion of the H-2 gene complex. The line does not proliferate in response to the T cell mitogens Con A and PHA, nor can it be propogated in conditioned media prepared by culturing splenocytes in the presence of Con A or PHA. The cytolytic activity of this cell line is specific for targets carrying IA^k subregion coded antigens. Cytotoxicity by this line reaches plateau values at attacker to target ratios lower than 10:1. If mixed with bone marrow derived lymphocytes carrying IA^k subregion coded antigens, this line is able to stimulate a humoral response to sheep erythrocytes in a positive allogeneic effect.

648 COLONY-STIMULATING FACTOR FROM T LYMPHOCYTES. Marianne Frölich, David W. Golde, and Martin J. Cline. UCLA, Los Angeles, CA 90024

A factor (a CSF), secreted from a human T lymphocytic cell line, is capable of stimulating in vitro formation of colonies of macrophages and granulocytes from human bone marrow cells. The molecular properties of this CSF suggest a possible mechanism of regulation of the CSFs. We found the CSF to easily dissociate into a low and a high molecular weight factor. The low molecular weight factor, FI, initiates colony formation of a subset of the marrow cells. FII, the high molecular weight factor, is inactive by itself. Reconstitution of FI with FII causes a dramatic increase in the number of target cells that form colonies. The number of target cells responding to FI reconstituted with FII is frequently larger than the number of cells responding to untreated CSF. Lipid-free BSA is found to substitute for FII in activating FI. Lipid-free BSA is competitive with FII. We believe that dissociation and reassociation of this CSF (and of other CSFs) may be physiologically important in determining which cell population is activated in hematopoiesis.

Molecular and Cellular Characterization of Antigen Binding Receptors

649 PURIFICATION OF HAPTEN BINDING MOLECULES FROM ENRICHED POPULATIONS OF HAPTEN SPECIFIC T-CELLS, George K. Lewis, Peter Hornbeck, and Joel W. Goodman, Department of Microbiology and Immunology, University of California, San Francisco, California. 94143

Strain A/J mice immunized with azobenzenearsonate conjugates of autologous immunoglobulin(ABA-Ig) or tyrosine (RAT) produced ABA-specific suppressor cells and ABA-specific T-cell proliferation, respectively. Suppressors were enriched on ABA-coated surfaces (Lewis and Goodman, JEM:148, in press), while RAT-immunized T-cells were isolated as T-blast cells using in vitro cell culture and discontinuous density gradient centrifugation. Enriched ABA-specific T-cells were labelled for 4 hours with 35S-methionine, after which NP-40 lysates were prepared. ABA-specific molecules were purified from ABA affinity columns by sequential elution with hapten and urea, and the eluted molecules were analyzed by SDS-PAGE. Two groups of molecules were recovered from ABA-Ig immune T-cells with approximate molecular weights of 90-95K daltons and 35K daltons. Both of these molecules were also present in RAT-blast lysates, in addition to a molecule having a molecular weight of 140K daltons. These results suggest that different subpopulations of T-cells may have distinct types of antigen receptors, and offer a direct approach to the analysis of T-cell receptor diversity.

650 BIOCHEMICAL STUDIES OF MURINE SURFACE IMMUNOGLOBULINS, Roberta R. Pollock and Matthew F. Mescher, Department of Pathology, Harvard Medical School, Boston, MA 02115

The physiological forms of murine B cell surface IgM and "IgD" have been studied by isolation of iodinated surface immunoglobulins on a solid phase immunoabsorbant and analysis on SDS polyacrylamide gradient gels under non-reducing conditions. IgM constitutes 20-25% of the total iodinated surface Ig and has a molecular weight of 200,000 d., consistent with a μ_2L_2 structure. The remaining surface Ig is IgD; two-thirds (by radioactivity) of this IgD is a light form, IgD_2 (δ_1L_1), of 95,000 d. and one-third is a heavier form, IgD_1, of 150,000 d. No changes in mobility or relative amounts of the two forms are seen when isolation is done in the presence of protease inhibitors or following treatment with alkylating reagents to prevent disulfide rearrangements. Similarly, prolonged incubation of cell lysates has no effect. The low molecular weight of IgD_1 is inconsistent with a δ_2L_2 structure. It appears unlikely that this low molecular weight is an artifact of the SDS gel system used, as these same gels give the expected molecular weights for IgM, human IgG and the δ_1L_1 form of IgD (IgD_2). One explanation is that IgD_1 is a δ_1L_1 form associated with an additional protein. The protein would be inaccessible to lactoperoxidase-catalyzed iodination, as it is not detected on autoradiograms of SDS gels of reduced samples. This possibility is being investigated. Neonatal B cells lack IgD, but possess an additional surface protein which could be involved in their activation. This protein, isolated together with IgM on rabbit anti-mouse Ig-Sepharose, is 160,000 d. on non-reducing SDS gels and is 80,000 d. on reducing gels. Studies of this protein will also be presented.

651 ANTIBODIES THAT RECOGNIZE T CELL IMMUNOGLOBULIN: THE PRINCIPLE OF PHYLOGENETIC DISTANCE, Gregory W. Warr, Cancer Biology Program, NCI Frederick Cancer Research Center, Frederick, MD 21701

Immunoglobulin (Ig) determinants can be recognized in situ on the membrane of T cells and thymocytes in both mammals and lower vertebrates, when antisera to Ig are raised across wide phylogenetic distances. Two examples of this are 1) fowl antisera to mouse IgG $(Fab')_2$ fragments and 2) rabbit antisera to the IgM of fish. In both cases these antibodies react with serum, B cell and T cell (or thymocyte) Ig. The fowl antiserum recognizes both heavy chain (Fd) and light chain determinants. These determinants are expressed most strongly on intact Ig molecules, and may represent "interaction" determinants. Fowl antisera to the $(Fab')_2$ fragment of mouse IgG has the ability to react with idiotypic determinants found on circulating and lymphocyte membrane-bound Igs. Our rabbit antisera to teleost fish IgM recognize primarily peptide, not carbohydrate antigenic determinants on the thymocyte Ig. T cell or thymocyte Ig in the mouse, goldfish and trout consists of heavy chains (μ-like but antigenically distinct in the mouse) and light chains (κ-like but antigenically distinct in the mouse) by biochemical (^{125}I surface labeling, SDS polyacrylamide gels) and antigenic criteria. In addition, in some species the thymocyte or T cell Ig possesses physicochemical properties distinct from those of B cell or serum Igs, e.g. detergent solubility, and apparent mass of the heavy chain. (Research sponsored by the National Cancer Institute under contract no. NO1-CO-75380.)

652 CHARACTERIZATION OF ALLOANTIGEN SPECIFIC T CELL RECEPTORS, Bent Rubin, B.Hertel-Wulff and A.K.Kimura, Centre d' Immunologie, Marseille; Statens Seruminstitut, Copenhagen, and Dept. of Immunology, Uppsala.

Recent studies have shown that T and B lymphocytes express similar idiotypes (Id) on their specific antigen recognition molecules. We have produced rabbit antisera against the IgG fraction of mouse anti-H2 antibodies (Scand.J.Immunol. 7 523, 1998). Such rabbit antibodies were shown to be anti-Id antibodies and in the case of an antiserum against B6 anti-CBA antibodies we have shown that both anti-CBA T cells and antibodies from mice carrying the $Ig\text{-}1^b$ or $Ig\text{-}1^e$ allotypes expressed similar idiotypes.
T cell receptor mater ial from either MLC supernatants or from NP-40 solubilized anti--CBA T blasts were isolated on anti-Id antibody Sepharose columns. Specific antigen material from both sources were of about 72,000 molecular weight with no reactivity against anti-Ig reagents, anti-H2/Ia reagents, or Lens Culinaris- and Con A Sepharose beads. Preliminary studies have shown that the H2 type of $Ig\text{-}1^b$ allotype carrying mice may determine the quantitative expression of both Id positive T cell receptors and anti--bodies.
These results will be discussed in relation to current concepts on the structure of and the genetic regulation of expression of T cell receptors

653 V_H GENE PRODUCTS ARE PRESENT ON SUPPRESSOR T CELL DERIVED REGULATORY MOLECULES, Mark I. Greene, Bruce Allen Bach, Alfred Nisonoff and Baruj Benacerraf, Harvard Medical School, Boston, MA 02115

Antigen specific suppressor thymus-derived (T) cells (STC) are generated in A/J mice by the intravenous administration of azobenzenearsonate modified A/J spleen cells (ABA-spl). Conversely antigen specific effector T cells capable of transfer and manifestation of DTH, *in vivo*, or CMC and proliferation *in vitro* are generated by the subcutaneous administration of ABA-spl. Discrete subcellular proteins have been obtained from ABA-specific suppressor T cells which can limit the *in vivo* development of ABA specific DTH in A/J. Immunochemical analysis of such molecules has established that STC suppressor factor (SF) bear determinants recognized by anti-idiotypic antibody. Rabbit antibodies directed against the cross-reactive idiotypic structures found on anti-ABA antibodies of A/J mice can specifically bind to ABA specific STC SF. Furthermore all strains of mice tested produce ABA specific STC after i.v. immunization with syngeneic ABA-spl. However only C.AL-20 ($H\text{-}2^d$), which possesses a heavy chain allotype linkage group closely related to that of A/J, produces the CRI and the idiotype bearing SF. B10.A ($H\text{-}2^a$) which can make SF active in B10.A, produces SF which do not bear cross-reactive idiotypic determinants. Thus there is a correspondence between the presence of certain heavy chain allotype linkage group genes and the ability of STC to elaborate idiotype bearing SF. The role of H-2 genes in coding for components of regulatory molecules has also been established and will be presented.

654 CHARACTERIZATION OF T CELL-DERIVED MOLECULES INVOLVED IN ANTIGEN RECOGNITION, R.E. Cone, R.W. Rosenstein, K. Kondo, J. Murray, V. Ptak and R.K. Gershon. Yale University School of Medicine, New Haven, Ct. and the University of Krakow, Poland.

Detailed analysis of the structural and functional properties of immunoregulatory antigen specific T cell products should provide insight into the molecular basis of T cell recognition of antigens. We have utilized hapten affinity chromatography to purify T cell derived molecules (TSF) released by DNP or TNP-tolerant spleen cells in vitro which specifically abrogates the adoptive transfer of DH reactions by DNP or TNP-sensitized T cells. Analysis of purified TSF by SDS-PAGE, gel chromatography and isoelectric focusing demonstrates that TSF is a single 68Kd polypeptide chain which does not bear known Ig constant region determinants, exhibits heterogeneity characteristic of antibody molecules and is cytophilic for macrophages and Fc-receptor bearing S. aureus. TSF is markedly sensitive to proteolytic degradation and fragments to 50Kd, 34Kd and 26Kd polypeptides, all of which can bind antigen. The relationship of soluble TSF to cell membrane receptors for antigen was explored with rabbit antisera to TSF which bind T cell surface membrane components. Cell surface molecules bound by R-anti-TSF share some of the structural and functional properties of soluble TSF and are also bound by rabbit antisera against murine myeloma proteins which after appropriate absorbtion contain antibody activity to Ig kappa chains and variable region determinants. The antibody activity in the anti-Ig serum responsible for binding appears to be directed towards Ig variable region framework determinants. These results suggest that TSF may be related to a T cell membrane receptor for antigen.

655 EXPRESSION OF IDIOTYPIC DETERMINANTS BY T CELLS IN THE ABSENCE OF B CELL IDIOTYPE PRODUCTION, Ruth Benca Kaplan and José Quintáns, La Rabida-University of Chicago Institute and the Departments of Pathology and Pediatrics, University of Chicago, Chicago, IL 60649

Since mice with the CBA/N X-linked immune defect cannot produce antibody in response to phosphorylcholine (PC) antigens, (CBA/N ♀ x Balb/c ♂)F_1 (NBF_1) mice were used to characterize the PC-specific T cell receptor. An adoptive transfer system was used to test PC-specific helper activity, and it was found that the X-linked defect does not affect the ability of F_1 offspring to produce PC-specific helper T cells. Furthermore, inhibition of helper activity with anti-idiotypic antiserum demonstrates that T cell receptors expressing HOPC-8 idiotypic determinants are present in NBF_1 mice, including F_1 males with the B cell defect. These results indicate that adequate T cell priming does not require the presence of circulating antibody, and that the T cell receptor must be of endogenous origin. (R.K. supported by graduate training grant 1-T32 HD-07009; J.Q. supported by grant AI-14530, R.C.D.A. AI-00268 and a Basil O'Connor Starter Research Award from the National Foundation March of Dimes.)

656 PREPARATION OF AN ANTIGEN BINDING FRAGMENT FROM IMMUNE MURINE T-LYMPHOCYTES, Hugh J. Callahan, and Paul H. Maurer, Thomas Jefferson University, Philadelphia, PA 19107

BALB/c mice were immunized with the random sequence polypeptide $(Glu^{60}Ala^{40})_n$ (GA). Spleen and lymph nodes were used to prepare T-lymphocyte suspensions via nylon wool columns. These cells were radiolabeled with ^{125}I by the lactoperoxidase procedure and then ruptured by nitrogen cavitation. Membrane fragments were isolated by sucrose density centrifugation and subsequently solubilized in Triton X-100. Non-specific material was removed by passing the solubilized membranes through an immunoadsorbent composed of D-GA (i.e. $[D\text{-}Glu^{60}\text{-}D\text{-}Ala^{40}]_n$) covalently bound to Sepharose 4B. The non-adherent fraction was subsequently passed through L-GA Sepharose and the adherent material eluted with 3M NaCNS. After removal of salt and relabeling (chloramine-T), the eluate was readsorbed onto and eluted from a fresh L-GA column. This material bound almost completely to L-GA immunoadsorbents (30-85%) but at control levels (8-10%) to immunoadsorbents composed of D-GA, D-GAT (i.e. $[D\text{-}Glu^{60}\text{-}D\text{-}Ala^{30}\text{-}D\text{-}Tyr^{10}]_n$), or L-GAT. Polyacrylamide gel electrophoresis revealed only one component with a molecular weight of $\sim$66,000.

Membranes were also prepared from normal, non-immune BALB/c mice and fractionated over immunoadsorbent columns in the manner described. On the basis of incorporated radiolabel, the total amount of antigen binding membrane recovered was 15-20% of that obtained in immune animals. It demonstrated no specificity in binding to either L-GA or D-GA immunoadsorbents (20% in each case).

Supported by NIH Grant A107825

657 STRUCTURAL DIFFERENCES BETWEEN u CHAINS FROM CELL SURFACE AND SECRETED IgM. D. Yuan, J.W. Uhr, M. Knapp, S. Slavin, S. Strober and E.S. Vitetta Department of Microbiology, University of Texas Southwestern Medical School, Dallas; Division of Immunology, Department of Medicine, Stanford University School of Medicine, Stanford; Department of Medicine, A. Hadassah-HebrewUniversity School of Medicine, Jerusalem.

The murine tumor, BCL_1 is analogous in many respects to chronic lymphocytic leukemia. These cells bear 8s IgM on their surface and can be induced to secrete 19s IgM after stimulation with LPS in vitro. In our experiments, BCL_1 cells were labeled before or after LPS stimulation and the cell surface and secreted IgM prepared by immunoprecipitation. The u chains were eluted from SDS gels and digested with chymotrypsin and trypsin. Peptides were resolved by cation exchange chromatography. One peptide present on the u chains of secreted IgM was not present in u chains of cell surface IgM; while a second peptide present on the u chains of cell surface IgM was not present in the u chains of secreted IgM. The same peptide differences have been demonstrated using u chains from IgM synthesized by normal BALB/c splenocytes. Further characterization of these peptide differences may define the manner of attachment of cell surface IgM to the plasma membrane of the B cell.

658 ANALYSIS OF RABBIT T CELLS FOR V_H DETERMINANTS, Jens Chr. Jensenius, Lawrence E. Mole and Alan Johnstone, Institute of Medical Microbiology, Odense University, DK-5000 Denmark.

T lymphocytes have been analyzed for the presence of H chain like molecules without associated L chains according to the predictions of the Binz and Wigzell model for T cell antigen receptors. Rabbit Ig carry an allotypic marker in the V_H region (Aa), i.e. a marker which would be expected to be present on H chains produced by T cells. Conventional anti-a1 alloantisera were found to contain large proportions of antibody reacting with determinants present on a1/b4 Fab$_\gamma$ but not on a1/b4 Fab'α or a1 V_H, whereas a goat anti-rabbit IgG antiserum after absorption with a2a2/b4b4 serum showed equal reactivity with these fragments as well as Fd and untreated Ig. A radio-immunoassay was constructed using this hetero-antiserum and ^{125}I-labelled purified a1 V_H fragment. Detergent extracts and culture supernatants of rabbit T lymphocytes, purified by negative selection, were analyzed by this assay as well as by a L chain specific assay. The amount of material estimated by the two assays were very similar (about 10^3 7S molecules per cell, probably originating from contaminating B cells) indicating the absence of production of V_H carrying molecules by rabbit T cells.

659 DETERGENT BINDING OF B-CELL IG, Yong Sung Choi and John Lifter, Sloan-Kettering Institute for Cancer Research, Rye, New York 10580

We have studied the binding of detergent by B-cell and plasma cell Ig by charge-shift electrophoresis and isopycnic centrifugation. Plasma cell Ig (intracellular or secreted) did not bind detergent. In contrast, B-cell Ig bound detergent (20~23 molecules Triton X-100 per Ig molecule). With respect to detergent, B-cell Ig is similar to other hydrophobic integral membrane proteins. Since detergent binding was not affected by the extent of glycosylation of Ig, the hydrophobicity of B-cell Ig was most likely due to the chemical nature of the peptide chain. We also show that detergent bound only to the Fc portion of mouse B-cell IgM and IgD but not to the Fab.

These studies suggest that (1) the Fab part of B-cell receptors is the hydrophilic area of the protein molecule, and (2) the hydrophobic C-terminal may be unique to B-cell Ig for its association with plasma membrane. Ig synthesized and secreted by plasma cells may not have the hydrophobic peptide.

660 DO T AND B CELLS SHARE V_H FRAMEWORK DETERMINANTS? Ronald L. Wilder, Irwin Scher, and Rose G. Mage, NIAID, NIH and National Naval Medical Center, Bethesda, Maryland.

A large body of published data indicates that T and B cell antigen receptors share idiotypic determinants. The extent to which V_H framework determinants are represented on T cell receptors is unresolved and controversial. We have studied this question in the rabbit using anti-a(V_H framework) allotype sera and a goat anti-rabbit V_H serum, prepared against isolated V_H derived from pooled IgG from 50 rabbits (a1+a2+a3 allotypes). Flow microfluorometric analysis of activated spleen cells from hyperimmune animals (Freund's adjuvant plus picryl chloride) demonstrated virtually identical frequencies of cells stained with the anti-V_H framework antisera compared to anti-light chain antisera. Additional studies were performed with radiolabeled plasma membranes from such "activated" spleen cells. After solubilization in 0.05% Triton X-100 (4/1 Triton/protein weight ratio), the membranes were analyzed by standard immunoprecipitation techniques. Absorption with anti-Ig constant region immunoabsorbents removed all radioactivity precipitable by the specific anti-V_H framework antisera compared to controls, whereas absorption with non-specific immunoabsorbents left V_H-bearing molecules with heavy chain molecular weights of sIgM (and ? sIgD). Thus, a population of cells or a class of Ig bearing V_H framework specificities in the absence of light chain was not demonstrated. Although these studies do not support the concept of similar V_H framework determinants on T and B cell receptors, they do not exclude a much more limited sharing of determinants or a very minor T cell subpopulation which expresses V_H framework that would not be detectable by the methods used in these studies.

661 IgD ON MURINE B LYMPHOCYTES RESPONDING TO A THYMUS-INDEPENDENT ANTIGEN, Linda Brown-Buck and Ellen S. Vitetta, University of Texas Southwestern Medical School, Dallas, TX

It has been demonstrated by blocking studies that the presence of membrane IgD is not essential for the stimulation of B lymphocytes by certain thymus-independent antigens such as TNP-Brucella (TNP-BA) but is required for stimulation by thymus-dependent antigens such as TNP-SRBC It has not been ascertained, however, whether cells responding to TNP-BA express IgD or whether they comprise a subpopulation of B lymphocytes which bear only IgM. To examine this question, adult murine spleen cells were treated with anti-μ, anti-δ, or anti-Ig in the presence of complement. The anti-δ serum used in these studies was the monoclonal product of a hybridoma cell line. It was shown to bear activity for δ-chains but not for μ- or L-chains by SDS-polyacrylamide gel electrophoresis. Treated cells were cultured with TNP-BA or TNP-SRBC and direct plaque forming cells to TNP were assessed after four days. Our results indicated that both IgM and IgD are present on a large proportion of B lymphocytes capable of responding *in vitro* to both TNP-BA and TNP-SRBC. Similar results were obtained when neonatal cells were used. These results suggest that while IgD is present on the majority of cells responding to both types of antigens, it may not be an obligatory receptor for the antigen dependent triggering of a TNP-BA precursor.

662 T CELL ACTIVATION AND THE SYNTHESIS AND SECRETION OF MIXED LEUKOCYTE REACTION SUPPRESSOR FACTOR. John W. Belmont, Robert R. Rich, Susan Solliday Rich. The Howard Hughes Medical Institute Laboratory and the Department of Microbiology and Immunology, Baylor College of Medicine, Houston, Texas, 77030.

Little is known of the mechanisms by which T cells are activated to synthesize and secrete I region-controlled immunoregulatory molecules. Thus, we characterized one such product, mixed leukocyte reaction suppressor factor(MLR-TsF), with respect to kinetics of secretion and sensitivity to a variety of metabolic inhibitors. Spleen cells from alloantigen-immunized mice released active MLR-TsF after freeze-thaw lysis; upon restimulation with the priming alloantigen, primed cells secreted factor into culture supernatants at a linear rate. Colchicine-and-vinblastine-sensitive structures were not required for activation and secretion of MLR-TsF, but these processes were cytochalasin B sensitive. DNA synthesis played no role in MLR-TsF secretion (as determined by resistance to mitomycin C treatment and irradiation). However,new protein synthesis was required as indicated by the potent inhibitory effects of cycloheximide. Timed addition and removal of cycloheximide defined a broad period of requirement for protein synthesis, starting at culture initiation and lasting 12-16 hours. Cell-associated MLR-TsF activity was also inhibited when cycloheximide was added to cultures and cell lysates assayed at later intervals. These experiments demonstrated that (1) MLR-TsF is present in an active cell-associated, as well as secreted, form, (2) it is synthetized de novo in culture (3) the activation and secretion processes do not require DNA synthesis or colchicine-and vinblastine-sensitive structures (4) the processes do require cytochalasin B-sensitive structures and protein synthesis. (Supported in part by USPHS Grant AI 13810).

Synthetic Membranes as Models for Immunological Recognition and Function

663 INDUCTION OF VIRUS-SPECIFIC, H-2 RESTRICTED MURINE CTL BY LIPOSOMES, Matthew F. Mescher, Robert Finberg and Steven Burakoff, Dept. of Pathology, Harvard Medical School, Boston, MA 02115

Liposomes containing Sendai virus membrane proteins and partially purified H-2^d antigens are able to stimulate a virus specific, H-2 restricted secondary CTL response. Liposomes were prepared by mixing deoxycholate-solubilized virus membrane proteins, lectin-purified H-2^d antigens and phospholipid, followed by removal of the detergent by dialysis in the presence of calcium. When placed into culture with spleen cells from B6D2F_1 (H-2^b/H-2^d) mice previously immunized with Sendai virus, these liposomes stimulated the generation of a secondary CTL population specific for Sendai virus-coated P815 (H-2^d) tumor cell targets. No lysis was found using Sendai-coated EL4 (H-2^b) targets, thus demonstrating that the viral proteins are recognized in the context of the H-2 antigens present in the liposomes. Reciprocal results were obtained using liposomes containing viral proteins and H-2^b antigens. Mixing experiments demonstrated that T cell recognition requires that both the viral proteins and the H-2 antigens be present in the same membrane. Thus, no CTL response is obtained if liposomes containing only viral proteins are added to cultures along with liposomes containing only H-2 antigens. The Sendai virus membrane proteins have now been purified by DEAE chromatography in the presence of Triton X-100 and experiments are in progress to determine which of these proteins is required for CTL induction.

664 IN VITRO STIMULATION OF MOUSE SPLEEN CELLS BY HAPTENATED LIPOSOMES TO GIVE A PRIMARY PLAQUE-FORMING CELL RESPONSE, Gillian M. K. Humphries and Harden M. McConnell, Department of Chemistry, Stanford University, Stanford, CA 94305

Liposomes, prepared from phosphatidylcholine, highly purified cholesterol and N-dinitrophenyl-aminocaproylphosphatidylethanolamine, co-cultured for four days with mouse spleen cells in Marbrook flasks, stimulate production of antibody-secreting cells. These cells generate direct plaques in dinitrophenylated sheep red blood cell layers, using the Cunningham-Zvenberg modification of the Jerne plaque assay. The response is dependent upon the hapten density in the plane of the liposomal membrane.

In common with several other afferent in vitro immune responses, this liposome-mediated stimulation is suppressed by a low concentration of oxidised cholesterol.

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665 CELL MEDIATED LYSIS OF ARTIFICIAL LIPID VESICLES CONTAINING CELL MEMBRANE PROTEINS, Nurit Hollander, Qasim S. Mehdi, Todd Lewis, Irving L. Weissman, Harden M. McConnell, and Joseph P. Kriss. Stanford University, Stanford, CA 94305.

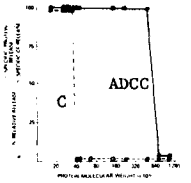
The successful use of artificial lipid bilayer membranes as targets for cytotoxic lymphocytes is described. Artificial lipid vesicles were made under strictly specified conditions from a mixture of dimerystoyl lecithin, dipalmitoyl lecithin and cholesterol, had both human eye muscle membrane proteins and membrane proteins of LSTRA or EL4 tumors inserted into the bilayer wall, and contained ^{51}Cr marker. Incubation of vesicles with lymphocytes sensitized in mixed lymphocyte cultures against allogeneic cells resulted in specific ^{51}Cr release from vesicles. Thus, vesicles containing LSTRA (H-2^d) membrane proteins were exclusively damaged by lymphocytes sensitized against BALB/c (H-2^d) spleen cells, whereas vesicles inserted with EL4 (H-2^b) membrane proteins were exclusively damaged by anti-C57Bl/6 (H-2^b) effector cells. Permeability changes were not induced either by lymphocytes sensitized to an inappropriate H-2 haplotype, or by unsensitized spleen cells. ^{51}Cr release could be demonstrated within 3 hours incubation at low effector cell to target vesicle ratios. We therefore propose this system as a model to study mechanisms of lymphocyte-mediated cytolysis.

666 STIMULATION OF SECONDARY MOUSE CYTOTOXIC T CELLS WITH HLA-A AND -B ANTIGENS IN PHOSPHOLIPID VESICLES, Victor H. Engelhard, Steven Burakoff, and Jack L. Strominger, Harvard University, Cambridge, MA 02138

Detergent soluble human HLA-A and -B antigens, purified from the lymphoblastoid cell line JY were incorporated into phospholipid vesicles in an antigenically active form by a detergent dialysis procedure. Protease treatment and detergent solubilization indicated that the association of the protein with the vesicles was similar to its association with the native cell membrane. These preparations stimulated secondary cytotoxic T lymphocyte (CTL) activity in C57BL/6 mice previously primed with intact JY cells. The amount of killing stimulated depended both on the total amount of HLA added per culture and upon its density on the liposome surface. Maximal stimulation was achieved using vesicles with an HLA density similar to that on intact JY cell surfaces. The CTLs exhibited specificity since they preferentially lyzed JY targets rather than targets of the HLA-unrelated lymphoblastoid line, Raji. Preliminary evidence suggests that the specificity of HLA-vesicle stimulated CTL is greater than that of cell or membrane-stimulated CTL. The results show that purified human histocompatibility antigens alone are sufficient to induce a secondary CTL response in mice, and that the CTL obtained can distinguish among different human allospecificities.

667 INTERACTION OF HAPTEN-BEARING FLUORESCENT LIPOSOMES WITH MURINE MYELOMA TUMORS AND OTHER CELLS, Lee D. Leserman, John N. Weinstein, Robert Blumenthal, Susan Sharrow, John J. Moore, and William D. Terry, NIH, National Cancer Institute, Bethesda, MD 20014. Cell-liposome interactions were studied with sonicated liposomes containing the water-soluble fluorophore carboxyfluorescein and the antimetabolite methotrexate. Liposome membrane phosphatidylethanolamine was substituted with the dinitrophenyl or phosphorylcholine hapten. When these liposomes were incubated with myeloma cells only those liposomes corresponding to the binding specificity of the surface immunoglobulin of the tumor became associated with the cells, as confirmed by fluorescence microscopy and flow microfluorometry. This binding was inhibitable by the appropriate soluble hapten. Measurements of the incorporation of ^{3}H-deoxyuridine showed that myeloma cells were sensitive to free methotrexate but that encapsulation of the drug in liposomes markedly reduced the drug effect whether or not cells bore the appropriate surface immunoglobulin. Specifically bound liposomes did not deliver methotrexate to the cytoplasm because they were not internalized by myeloma cells. Methotrexate encapsulated in hapten-bearing liposomes was more effective than free drug only when the liposomes were opsonized by exogenous anti-hapten antibody and presented to (non-myeloma) cell lines capable of endocytosing the liposomes. These studies illustrate the use of liposomes as stable markers of specific lymphoid cell surface receptors.

668 MEMBRANE PORES INDUCED BY CYTOTOXIC LYMPHOCYTES AND COMPLEMENT, Charles B. Simone and Pierre Henkart, NIH, National Cancer Institute, Bethesda, MD 20014. Previous studies on the nature of the lytic event induced in target cells by cytotoxic lymphocytes suggest that an increase in target membrane permeability is responsible for cell death. To probe such membrane damage in the absence of colloid osmotic lysis, we have studied the action of complement (C) and antibody dependent cell mediated cytotoxicity (ADCC) on resealed human TNP-erythocyte ghost targets. The release of fluorescent markers of known molecular weight was monitored using both fluorometry and fluorescence microscopy with identical results. In each experiment the resealed ghost contained the small membrane impermeable dye carboxyfluorescein (CF) and a rhodamine-labeled protein. CF is released from these ghosts only under conditions of C and ADCC lysis in which ^{51}Cr is released from intact red cells. As shown in the figure, in an extensive series of double label experiments, sharp size cut-offs were observed for the release of the proteins from the resealed ghosts by C and ADCC. Specific CF release of 75% or greater was observed in all cases. Human lymphocyte and neutrophil ADCC effectors released proteins from 17.5-480x10^{3}d but not larger proteins; monocyte effectors did not release the 480,000 d protein but released all smaller proteins. C lysis released 17.5-34.6x10^{3}d proteins, but not larger ones. We conclude that discrete pores are formed in the target membrane by the action of cytotoxic lymphocytes and that such pores are larger for ADCC than for C. Functional pore diameters estimated from the largest proteins released are ~50 Å for complement and 120-165 Å for ADCC.



669 H-2 RESTRICTED ADHERENCE OF CYTOLYTIC T LYMPHOCYTES TO SCHISTOSOMULA OF SCHISTOSOMA MANSONI, M.A. Vadas, A.E. Butterworth, S.J. Burakoff, E. Martz and A. Sher, Department of Medicine and Pathology, Harvard Medical School, Boston, MA 02115
We have demonstrated recently that, after passage through mice, schistosomula acquire host histocompatibility antigens in a form detectable by immunofluorescence (Sher, Hall and Vadas, J. Exp. Med. 148:46, 1978). We have now shown that these acquired antigens are also recognizable by T lymphocytes. Mouse peritoneal exudate cells rich in alloreactive cytolytic T lymphocytes (CTL) adhere specifically to schistosomula passaged through the appropriate strains. T cells purified on nylon wool columns demonstrate the same pattern of adherence as the unpurified peritoneal exudate cells, and Thy-1 antigen is demonstrable on the surface of the majority of adherent cells. The presence of CTL in the adherent cell population was confirmed by demonstrating that T cells, after binding to the parasite, can lyse tumor targets. In spite of the attachment of functional CTL, no damage to the parasite was detectable. These organisms could, however, be damaged by anti-H-2 serum and complement. This model has now allowed us to demonstrate that the adherence of anti-TNP or anti-minor histocompatibility antigen (H-m) CTL to schistosomula bearing these antigens is H-2 restricted. The CTL were incubated with schistosomula bearing a) only the appropriate H-2 antigens, b) only the modifier antigens (TNP or H-m) or c) both these antigens. Adherence was only demonstrable to parasites bearing H-2 and the appropriate modifier. In addition, we have shown that the two antigens, H-m and H-2, necessary for the adherence of anti-H-m CTL can be acquired by the parasite independently of one another by sequential passage through the appropriate strains.

670 BINDING OF ALLOANTIBODY AND ALLOIMMUNE CYTOTOXIC T LYMPHOCYTES TO LIPOSOMES CONTAINING H-2. Carol C. Whisnant, Richard B. Zelman, and D. Bernard Amos. Duke University Medical Center, Durham, NC 27710.

Detergent solubilized, partially purified mouse histocompatibility (H-2) antigens have been reconstituted in phosphatidylcholine (PC) and phosphatidylcholine/cholesterol (PC/CHOL) lipid vesicles. Preliminary studies indicate that these are large unilamellar vesicles (d_{av} = 0.5 micron) with lipid:protein mole ratios of 80:1 (PC) and 200:1 (PC/CHOL). The H-2 antigens in these lipid vesicles are recognized both by alloantibody and by cytotoxic T lymphocytes (CTL) as demonstrated by specific inhibition of lysis of target cells by alloantibody and complement and by inhibition of specific conjugate formation between target cells and IPEL (immune peritoneal exudate lymphocytes), a population rich in CTL. Inhibition of % specific conjugate formation per μg protein by detergent soluble H-2^b and PC/CHOL/H-2^b is shown below.

		DOC-SOL H-2^b(1 μg)	PC/CHOL H-2^b(6 μg)
IPEL anti-EL4(H-2^b)	19.9%	4.1%	6.9%
IPEL anti-RL1(H-2^d)	13.9%	14.8%	16.8%

Currently we are studying the effects of lipid composition and lipid:protein ratio on binding of alloantibody and CTL to lipid vesicles. Lipid vesicles containing H-2 will be used as artificial "target cells" for lysis by alloantibody and complement, antibody-dependent cell-mediated cytotoxicity, and by alloimmune CTL.

671 LATERAL DIFFUSION OF MEMBRANE ANTIGENS AND A LIPID PROBE IN LYMPHOCYTES, Paul R. Dragsten, Joseph Schlessinger, Pierre Henkart, John N. Weinstein, Robert Blumenthal, NCI, NIH, Bethesda, Md. 20014.

Fluorescence photobleaching recovery measurements were used to determine the rate of lateral diffusion of surface immunoglobulin (sIg--detected with rhodamine-IgG and -Fab fragments of goat anti-mouse IgG), Thy 1 antigen (detected with rhodamine-RAMB), and a lipid probe (3,3'-dioctadecylindocarbocyanine iodide) in the plasma membrane of mouse spleen lymphocytes. Sixty to eighty percent of the detectable sIg and Thy 1 antigen are free to move in the plane of the membrane, with diffusion constants of about 3×10^{-10} cm^2/sec. sIg diffusion could be restricted by crosslinking with appropriate concentrations of bivalent (IgG) anti-Ig (in the presence of azide to inhibit capping). The extent of immobilization of sIg was dependent on the concentration of anti-Ig used, and correlated well with the frequency of cap formation in the absence of azide. Immobilization of Thy 1 was not readily observed with the IgG RAMB, and the antigen did not cap. Addition of a second layer of crosslinking antibody (goat anti-rabbit IgG) did produce both capping and immobilization. It appears from these results that, under crosslinking conditions sufficient to induce cap formation, sIg and Thy 1 antigen are essentially immobile with respect to diffusion over distances of a micron or greater. The fluorescent lipid probe gave $D=(1.5 \pm .3) \times 10^{-8}$ cm^2/sec, with essentially 100% of the probe free to diffuse in the plane of the membrane. We also measured the lipid probe's diffusion coefficient on individually identified T and B lymphocytes by double staining experiments using fluorescein-conjugated anti-mouse Ig or RAMB. No differences in D were observed between these two cell populations.

672 CELL SURFACE MODULATION OF CYCLIC AMP PHOSPHODIESTERASE, Dolores Feld Takemoto*, M. Michael Appleman', and Sol Kaplan*, Dept. Pediatrics UCLA, Los Angeles, Ca. 90024*, Cellular and Molecular Biology, Univ. Southern Calif., Los Angeles, Ca. 90007'

The cyclic AMP phosphodiesterase (PDE) from leukemic human peripheral blood lymphocytes is found to differ in several properties. When compared to normal lymphocytes, the leukemic lymphocyte PDE has a loss of inhibition by cyclic GMP and a 10-fold higher specific activity. In an effort to determine the mechanism by which the PDE becomes altered, we have developed a system to induce this change in normal human lymphocytes. Following membrane perturbation with con A, the PDE from the normal cells becomes gradually altered until it fully resembles the enzyme found in leukemic lymphocytes. The change occurs both in specific activity and in enzyme inhibition by cyclic GMP. Addition of a crude guanylate cyclase inhibitor prevents this change and can be reversed by the exogenous addition of 8-bromo cyclic GMP. These results suggest that membrane perturbations can affect the intracellular levels of cyclic GMP and, thus, induce the synthesis or expression of new enzymes. The relevance of this change in PDE to cell growth control is discussed.

Early Events in Lymphocyte Activation

673 PHYLOGENY OF T AND B LYMPHOCYTES, Edwin L. Cooper, Department of Anatomy, School of Medicine, University of California, Los Angeles, Ca.

My chief concern in immunobiologic research, along with a number of colleagues, has been the origin of T and B lymphocytes. I have focused on phylogeny of T lymphocytes, their evolutionary development, by using the earthworm as an invertebrate model. This phylum is the first to show a full array of leukocyte types, which are now known to be related structurally in phylogeny to vertebrate lymphocytes, macrophages and granulocytes. According to emerging information on membrane characteristics, at least one of the worms' leukocyte types forms spontaneous rosettes with SRBC and preliminary evidence suggests the presence of receptors for the lectin isolated from Helix. Both are acceptable criteria for delineating human T lymphocytes. Worm lymphocytes also possess receptors for Con A and they can be stimulated with PHA. These characteristics suggest that T-cell prototypes evolved among advanced invertebrate species. Our second model has focused primarily on the anuran amphibian, the first terrestrial vertebrate to possess bone marrow, presumably from which stem cells are derived. Adult frogs reconstituted with bone marrow after irradiation are able to mount cell-mediated immune responses suggesting the presence of T lymphocytes or their precursors within marrow. On the basis of our own studies and those from other laboratories, stressing relevant membrane data, we conclude that T and B cells diverged early in evolution. My research interests have been supported recently by USPHS Grant HD 09333.

674 THE ROLE OF PROTEIN PHOSPHORYLATION IN THE ACTIVATION OF LYMPHOCYTES BY LPS, Kathleen Kelly and James Watson, University of California, Irvine, California, 92717

LPS stimulates B lymphocytes to cell division and antibody synthesis. To analyze the biochemical events involved in the mitogenic activation of lymphocytes we have examined the genetical and biochemical basis of a mutation found in C3H/HeJ mice. A defect at a single locus, Lps, found on chromosome 4, prevents the activation of lymphocytes from C3H/HeJ mice by LPS. The expression of the Lps locus appears to be required for an early event in mitogen activation. We are examining the involvement of protein phosphorylation in the delivery of an activation signal to lymphocytes. A variety of extracellular mediators, for example hormones, stimulate the phosphorylation of specific intracellular substrates. In many cases, the phosphorylation of a protein substrate results in an alteration in the activity of that substrate. Both cyclic nucleotide-dependent and independent phosphorylation mechanisms have been described. We are exaining the phosphorylation of protein substrates soon after the delivery of a mitogenic signal to the cell. This is done by labeling the endogenous ATP pools with $^{32}PO_4$ and analyzing on two-dimensional polyacrylamide gels those proteins in the cell which are phosphorylated following mitogenic activation. Phosphorylated proteins are analyzed for subcellular location and time of appearance after mitogenic stimulation. By comparing phosphorylated protein patterns from normal mouse strains to the C3H/HeJ strain, we may determine whether C3H/HeJ lymphocytes receive a transmembrane signal after LPS stimulation.

675 INDUCTIVE EVENTS IN B LYMPHOCYTE TUMOR SUBSETS, Peter Ralph, Christopher J. Paige and Paul W. Kincade, Sloan-Kettering Institute for Cancer Research, Rye, NY 10580

The response of B tumor lines to lymphocyte mitogens in culture was studied. Immature B cell lines 70Z/2 and 70Z/3 (J. Immunol. 121:641,1978), lacking surface immunoglobulin (sIg) could be induced for sIg by 1-10 μg/ml LPS which ultimately was toxic. In agreement with the marker studies of A.W.Harris for early and late B tumors, these lines were very sensitive to the toxic effects of corticosteroids (10^{-7}M) and thymidine (10^{-4}M). Mature B cell tumors WEHI-231, 38C-13 and BCL, with easily detectable sIg were resistant to toxic effects of LPS. Surface μ induction by LPS occurred in the presence of toxic concentrations of actinomycin D, puromycin, cycloheximide, thymidine and dexamethasone, suggesting rearrangement or translocation of preformed heavy chains. Induction of surface κ expression was apparently more sensitive to inhibitors of protein synthesis suggesting a requirement for newly-formed light chains. The ulex plant lectin also induced sIg in a clone of 70Z/3, which allows study of surface receptors for an inductive agent in a model B cell system with a known hapten inhibitor, L-fucose.

676 CHARACTERIZATION OF LYMPHOID CELL SURFACE PROTEASES, R.Jerrold Fulton and David A. Hart, The University of Texas Health Science Center, Dallas, Tx 75235

Proteases can act as B cell mitogens and substutute for T cell help in a primary antibody response in vitro. Furthermore, protease inhibitors can block activation of lymphocytes by B and T cell mitogens and antigens. We have characterized the endogenous proteases found in cultures of thymocytes and lymph node cells. Cultures of viable cells were found to exhibit neutral endopeptidase activity toward radiolabeled protein substrates added to the culture medium. Proteases were not secreted into the medium by either cell type in serum free culture but appeared to be bound to the surface of viable cells. Cell surface proteases were not inhibited or released from the cells by incubation in 1 mM EGTA; however, incubation in 1mM EDTA released 24±5% of lymph node cell surface protease activity into the culture medium. The cell surface proteases of both populations were partially inhibited by the serine protease inhibitors DFP, PMSF and TPCK, but not by TLCK, EACA, SBTI or iodoacetamide. The bacterial protease inhibitor antipain was also inhibitory whereas leupeptin and elastinal were not. Cell surface proteases were partially inhibited by $ZnCl_2$ (50 uM), but not by several other divalent cations. Simultaneous addition of DFP and $ZnCl_2$ or antipain and $ZnCl_2$ resulted in additive inhibition of the cell surface proteases. These results indicate that viable thymocytes and lymph node cells bear at least two distinct surface proteases which could be involved in growth control mechanisms.

677 LOW DENSITY LIPOPROTEINS INHIBIT EARLY EVENTS IN LECTIN-INDUCED LYMPHOCYTE PROLIFERATION. David Y. Hui and Judith A. K. Harmony. Chemistry Dept., Indiana University, Bloomington, IN 47405.

Interaction of phytohemagglutinin (PHA) with human peripheral lymphocytes initiates a sequence of events leading to cell proliferation. Biochemical alterations that occur shortly after mitogenic stimulation include enhanced calcium uptake, accelerated turnover of phosphatidylinositol (PI) and a transient increase of cellular cyclic 3',5'-guanosine monophosphate (cGMP). Low density lipoproteins (LDL) inhibit these early events, and inhibition correlates with the immunosuppressive ability of LDL.

Within the first hour after PHA (3 µg/ml) addition in the absence of LDL, $^{45}Ca^{2+}$ uptake increases 9-fold, intracellular cGMP increases 2-fold, and PI turnover as measured by ^{32}P incorporation into PI increases more than 5-fold. However, when the cells are preincubated with LDL (150 µg/ml protein) for 2 h prior to the addition of PHA, no increase in cell-associated $^{45}Ca^{2+}$ or cGMP occurs. Enhanced incorporation of ^{32}P into PI decreases by approximately 50%. LDL-induced inhibition is not due to a change in the amount of PHA required for maximum cellular response: if a suboptimal concentration of LDL (15 µg/ml) is added, the optimal PHA concentration which elicits $^{45}Ca^{2+}$ and cGMP accumulation remains at 3 µg/ml. LDL have no effect on ^{125}I-PHA binding to lymphocytes, although LDL inhibition is directly due to the interaction of the lipoproteins with the lymphocyte membrane. Addition of heparin, which removes all LDL bound to the cell membrane, effectively reverses the inhibitory effect of LDL on PHA-stimulated lymphocyte activation.

678 ELECTROCHEMICAL EVENTS IN LYMPHOCYTE COMMUNICATION, Janis E. Lochner, Philip Blume, Arthur Malley, and G.V.F. Seaman, Good Samaritan Medical Center, Oregon Regional Primate Center, and University of Oregon Health Sciences Center, Portland, OR 97201.

Although, it is generally believed that the decisive event involved in triggering the lymphocyte subsequent to an encounter with antigen occurs at the cell surface, little insight into the nature of this event exists. We have applied analytical particle electrophoresis, a biophysical technique sensitive to perturbations in the peripheral components of the membrane, to study early events associated with lymphocyte stimulation. Initially, it was shown that incubation (5 min. 37°C) with Concanavalin A (Con A) at concentrations as low as 10^{-17} g/ml would induce a 30% increase in the electrophoretic mobility of murine T cells. The electrophoretic response could not be attributed to the binding of lectin molecules to the surface of each lymphocyte in the population in view of the short incubation period and the fact that at the threshold concentration of Con A required to initiate the response only about 200 molecules of Con A were present. Consequently, a cellular communication mechanism was postulated to mediate the electrokinetic effect. A factor released by a few cells that bound Con A was envisioned to effect the change in electrokinetic properties of the population of cells. Such a factor has been experimentally identified. Purification of the factor by gel filtration on Bio-Gel P-2 in PBS showed the factor to have a molecular weight between 2000 to 3000. Further purification of the factor by high pressure liquid chromatography (HPLC) analysis is in progress. Cultured human cells, CCRF-CEM which have been characterized as T cells show an electrokinetic response similar to the murine T cells upon exposure to Con A.

679 ACTIVATION OF CYCLIC AMP-DEPENDENT PROTEIN KINASE DURING LYMPHOCYTE MITOGENESIS, David O. Lucas, University of Arizona, Tucson, AZ 85724

Human peripheral blood lymphocytes have been cultured with several mitogenic or non-mitogenic lectins. Responses were assayed in terms of activation of cyclic AMP-dependent protein kinase, induction of ornithine decarboxylase (ODC), and increases in RNA and DNA synthesis.

Mitogenic stimulation with Con A, PHA, or PWM resulted in activation of cAMP-dependent protein kinase at about 4 hours, followed closely by induction of ODC, then RNA synthesis beginning at about 12 hours. Non-mitogenic lectins (wheat germ agglutinin and Agaricus bisporus lectin) do not cause increases in any of the parameters tested. These data suggest that activation of cAMP-dependent protein kinase may be an "early" event in mitogenesis.

However, supramitogenic doses of PHA and Con A are poorly mitogenic but strongly activate cAMP dependent protein kinase, as does dibutyryl cAMP which is not mitogenic, but rather inhibits mitogenesis. Therefore activation of this kinase is not a simple or sufficient signal.

Experiments with the addition of alpha-methyl mannoside to Con A stimulated cultures demonstrate that cells become committed to progress to DNA synthesis at about 8-12 hours and that subsequent cAMP dependent protein kinase activation is unimportant.

It is proposed that discrete activation of compartmentalized cAMP dependent protein kinase at 4-12 hours is an important component of early mitogenic events.

680 ANTIBODY AFFINITY IN CBA/N MICE. K.E. Stein, J.J. Mond, C. Brennan, O. MÄkelÄ and W.E. Paul, NIAID, NIH, Bethesda, MD 20014 and Univ. of Helsinki, Helsinki, Finland.

The CBA/N mouse carries an X-linked trait which results in a profound inability to respond to thymus independent antigens of the TI-2 class. It has been suggested that its B cell defect might also lead to an inability to produce high affinity antibody in response to immunization with thymus-dependent antigens. We have addressed this question by studying the anti-hapten response to hapten-protein conjugates at various times after immunization. Defective (CBA/N x DBA/2N)F_1 male and control (DBA/2N x CBA/N)F_1 male mice were immunized with DNP_{10}-KLH in CFA and bled at 2,4 and 6 weeks after immunization. The animals were boosted at 6 weeks or 6 months after primary immunization. The affinity of anti-DNP antibodies was measured in an ammonium sulfate precipitation assay using ^{3}H-DNP-Lysine. We found no significant difference in the affinity of anti-DNP antibodies in defective and control mice at any of the times tested. For example, at 6 weeks following primary immunization the mean affinity of anti-DNP antibodies was 5.7×10^6 M^{-1} in defective mice and 5.4×10^6 M^{-1} in control mice while for animals boosted 6 months after priming and bled 1 week after boosting it was 59×10^6 M^{-1} in defective mice and 53×10^6 M^{-1} in control mice. Similarly, defective F_1 male and control F_1 female mice immunized with NBrP-CGG or ABA-HOP-CGG developed anti-hapten antibodies of similar relative affinity as measured by a haptenated-phage inactivation assay. We conclude from these studies that CBA/N mice are capable of producing high affinity antibodies to haptens on protein carriers and that within the period studied, the appearance of high affinity antibody occurs with the same time course in defective and control animals.

681 SUPPRESSION OF IMMUNOGLOBULIN-PRODUCTION IN NORMAL HUMAN B LYMPHOCYTES BY TWO T CELL SUBSETS DISTINGUISHED FOLLOWING IN-VITRO TREATMENT WITH ADENOSINE. Chaya Moroz and Ronald H. Stevens, UCLA, Los Angeles, CA 90024

Adenosine inhibits the ability of a distinct T cell subset to form E-rosettes. The adenosine sensitive E-rosette forming cells (ES) were separated from T cells which formed E-rosettes in the presence of adenosine (ER). The effect of the two T cell subsets was investigated using an in-vitro system of powkweed mitogen-stimulated immunoglobulin (Ig) production. It was found that the maximum production of Ig by 0.4×10^6 B cells could be reproducibly suppressed 40-90% when adenosine treated ES cells were present. The addition of ER cells suppressed Ig production only when present in high T to B cell ratios. Further studies revealed that adenosine activated the suppressor activity of ES cells, which were otherwise non-effective. In contrast, the suppressor activity of ER cells was demonstrated prior to any treatment. Moreover, the activity of ES cells was irradiation resistant whereas that of ER cells was irradiation sensitive. Thus, two types of suppressor T cells were identified; one in the ES and the other in the ER subpopulation. Both cell types suppressed the production of all major Ig classes. Similar to the adenosine effect, a non-rosetting activated suppressor T-cell subset was obtained following in vitro treatment with theophylline. Adenosine did not have an additional effect on the rosetting capacity and the suppression of Ig synthesis exhibited by the theophylline treated T cells, suggesting that both drugs affect the same cell population in a similar manner.

Role of Antigen-Binding Cells in the Immune Response

682 ENHANCED IMMUNOGENICITY AND TOLEROGENICITY OF ANTIGEN BOUND TO AUTOLOGOUS CELL MEMBRANES, Frank L. Adler, Masahiro Nakao, Louise T. Adler and Marvin Fishman, St. Jude Children's Research Hospital, Memphis, TN 38101

Antibody production against T2 phage antigens is induced *in vitro* in response to the addition of 10^{-3} to 10^{-5} µg of solubilized phage protein nitrogen to 2 x 10^7 nucleated rabbit spleen cells. Antigen-specific suppressor cell activity is induced with 10^{-1} to 10^{-2} µg amounts of this antigen. Lymphnode cells incubated with tolerogenic amounts of antigen and then washed and sonicated transfer sufficient antigen to autologous spleen cells to evoke either tolerance when used in amounts equivalent to 10^6 tolerized cells, or antibody formation, when used in amounts equivalent to 10^2 to 10^5 tolerized cells. Both activities appear to be associated with antigen sedimented by centrifugation at 25000 x g/1 hr. These findings suggest that in this system the association of an extraneous antigen with autologous membrane components results in the amplification of signals common to tolerance and immunity responses. Whether binding in itself or modification of the antigen is responsible is under further study.

683 SUBPOPULATIONS OF B CELLS DEFINED BY ANTIGEN BINDING, Julia Greenstein, James Leary, John Kappler and Philippa Marrack, University of Rochester, Rochester, NY 14642

Antigen binding is being used as a probe to separate and define functional subpopulations of unprimed murine B cells. Fluoresceinated TNP-BSA binds from 2% to 10% of the cells in an anti-Thy 1 and C' treated spleen, when stained at a saturating concentration of antigen. These antigen binding cells are then sorted using a multi-parameter cell sorter. Cells which bind the antigen (fl+) are separated from cells which do not demonstrate binding (fl-). These cells are sorted sterilely and then assayed for function in a limiting dilution plaque forming assay. The fl+ cells have been shown to be enriched for a response to TNP-LPS and TNP-SRBC with a concurrent decrease in the fl- population.

We are separating the fl+ population, stained with a saturating concentration of antigen into subpopulations of antigen binding cells, with different densities of antigen binding receptors, using the parameters of fluorescence and light scatter-time of flight. These fl+ subpopulations will differ in the surface density of antigen receptors. The cells will be assayed for the plaque forming responses to various TNP antigens and for the isotype of immunoglobulin which they secrete. By combining the separation of these subpopulations with the responses to various antigens and mitogen reactivity, functional subpopulations of B cells will be defined.

684 PHOSPHORYLCHOLINE BINDING CELLS IN MICE UNABLE TO PRODUCE ANTIBODY TO THE SAME HAPTEN, Ruth Benca Kaplan and José Quintáns, La Rabida-University of Chicago Institute and the Departments of Pathology and Pediatrics, University of Chicago, Chicago, IL 60649

To determine whether the inability to synthesize antibodies against phosphorylcholine (PC) antigens is caused by a lack of committed cells in neonatal mice and mice with the CBA/N immune defect, we counted PC-binding cells by autoradiography, using PC-coupled fowl gamma globulin (FGG) as a probe. Studies comparing adult and neonatal Balb/c mice showed that the frequency of PC-binding spleen cells is roughly equal in both groups, although neonates cannot mount anti-PC humoral responses until 1 week of age. Mice with the CBA/N X-linked defect are also unable to produce plaque-forming cell responses to PC, but can provide normal PC-specific helper T-cell activity. Thus lack of responsiveness cannot be explained by a deletion of V-region genes, but may instead represent an absence or defect of certain B-cell subpopulations. Antigen binding studies of spleen cells from CBA/N and affected F_1 mice demonstrated that X-linked defective mice have PC-binding cells in frequencies of the same order of magnitude as control mice. CBA/N mice possess cells committed to the PC specificity but may fail to undergo a differentiation step necessary to reach immune reactivity. In both neonatal and CBA/N mice, non-responsiveness to PC antigens cannot be attributed to a lack of committed cells as determined by the ability to bind antigen. (R.K. supported by graduate training grant 1-T32 HD-07009; J.Q. supported by grant AI-14530, R.C.D.A. AI-00268, and a Basil O'Connor Starter Research Award from the National Foundation March of Dimes.)

685 RECEPTOR-LIGAND INTERACTIONS IN THE INDUCTION OF B CELL TOLERANCE.
Catherine Desaymard. U 23 INSERM. Hôpital St-Antoine. Paris 75012. France.

The cellular events involved for B cell inactivation by thymus independent (TI) antigens were investigated in vitro. Induction of tolerance is dependent on the time and the temperature of cell exposure to tolerogen, however it is independent on the presence of extracellular calium. These results suggest that tolerance is actively acquired by cells and involved a restricted number of cellular processes.Cell treatment with drug known to affect the microfilament and the microtubule systems, before the cell exposure to tolerogen, decreased their susceptibility to tolerance. Inhibition of tolerization was obtained in similar conditions as ligand induced receptor capping inhibition. The nature of the receptor which is mobilized by tolerogen was defined by the use of specific antisera. There was an additif tolerogenic effect in cells exposed to tolerogen and anti-μ serum. B cell tolerance was induced by non or subtolerogenic doses of TI antigen when cells were subsequently treated by anti-μ but not anti-δ sera. Taken together, these data and the discriminative role of epitope density in immunity and tolerance provided evidence on the mechanism of B cell tolerance triggering. B cell inactivation would result from the capping and endocytosis or shedding of a large number of IgM receptors by highly substituted TI antigens.

686 CHANGES IN THE SURFACE Ig CLASS OF ANTIGEN-BINDING CELLS AFTER IMMUNIZATION IN VIVO,
S. Kanowith-Klein, E. S. Vitetta, E. Korn and R. F. Ashman

The binding of sheep erythrocytes (SRC) to specific SRC binding cells (SRC-ABC) from mouse spleen was blocked by preincubation with antisera specific for μ, δ or γ chains, revealing the surface Ig phenotype. SRC-ABC populations from non-immune animals were $\mu^+\delta^+\gamma^-$or $\mu^-\delta^-\gamma^-$, the latter group including the T-ABC. The significant ABC populations present 5 days after in vivo immunization were $\mu^+\delta^-\gamma^-$ (27%), $\mu^+\delta^-\gamma^+$ (22%), $\mu^-\delta^-\gamma^+$ (16%), $\mu^+\delta^+\gamma^-$ (16%), $\mu^+\delta^+\gamma^+$ (15%) and $\mu^-\delta^-\gamma^-$ (8%). Immunization caused a decline from about 50% to about 25% in the proportion of δ^+ ABC, while γ^+ ABC increased from 0% to 45%. There was no significant change in the % of μ^+ ABC. Most sIgG and virtually all sIgD occurred on cells bearing sIgM, with the "transitional" $\mu^+\delta^+\gamma^+$ cells indicating that some of the sIgG was expressed on cells which formerly bore sIgD. About 30% of non-immune ABC were inhibited by extremely low concentrations of anti-μ (1/5000), but these "sensitive ABC" were undetectable 5 days after immunization, perhaps because of affinity maturation.

687 SPECIFIC BINDING OF RADIOLABELLED MEMBRANE VESICLES BY T CELLS ACTIVATED IN THE MIXED LYMPHOCYTE REACTION, Bruce E. Elliott, Zoltan Nagy, and Bela Takacs, Cancer Research Division, Department of Pathology, Queen's University, Kingston, Ontario, Canada.

An assay was developed to quantitate binding of radiolabelled membrane vesicles by T cells activated in the primary mixed lymphocyte reaction. T blasts generated in unidirectional reciprocal MLR combinations were found to bind much more effectively membrane vesicles prepared by nitrogen cavitation from allogeneic stimulator than from syngeneic spleen and lymph node cells which had been previously labelled biosynthetically with ^{3}H-leucine. Specific inhibition of binding with cold vesicles prepared from stimulator cells but not from responder cells was observed. The number of specific antigen binding cells is proportional to the concentration of membrane vesicles used. The proportion of labelled cells increases between 15 and 60 minutes of incubation with membrane vesicles, but thereafter remains constant. The binding of stimulator material is H-2 specific: cells bind membrane vesicles from congenic mouse strains only if they share the H-2 antigen of the original stimulator strain. Responder blasts bind labelled stimulator membrane fragments after as early as 2 days of primary MLR culture; no binding of membrane vesicles by small lymphocytes was detected between 2 and 5 days of culture. However, between 8 and 12 days of culture a significant proportion of small lymphocytes bind specifically stimulator membrane vesicles. The recognition function is sensitive to trypsin treatment but is regenerated within 5 to 6 hours. By this approach, binding of radiolabelled vesicles is quantitated independently of alloantibodies, and binding of syngeneic material can be assessed.

688 HIGH FREQUENCY OF SPECIFIC ANTIGEN-BINDING CELLS IN PROTEIN ANTIGEN STIMULATED LYMPH NODES. J. A. Clarke, L. Adorini*, A. Miller, and E. Sercarz. University of California, Los Angeles, California 90024.

An antigen-specific increase in rosette-forming cells (RFC) occurs in the draining lymph nodes of mice from 5-10 days after primary immunization with 10 μg hen eggwhite lysozyme (HEL) in complete Freund's adjuvant (CFA). From a background level of 0.2% the frequency of RFC in the lymph nodes draining the base of the tail in the B10.A responder mouse reaches 6-8%. These RFC are completely inhibitable by soluble HEL but not by ribonuclease, and vice-versa with RNAse stimulated RFCs. A majority of these RFCs are sensitive to rabbit anti-mouse thymocyte sera + C, and binding is inhibited completely by chicken anti-mouse Fab sera. Antigen-specific rosettes can be induced in responder mice with all lysozymes investigated. However, in the nonresponder B10, the RFC response to the non-immunogenic HEL is complex. The tail-draining nodes do not form rosettes after priming with HEL whereas RFC are found in the parathymic lymph nodes (PT-LN) after i.p. injection of HEL-CFA. These PT-LN RFC, early in the response, are HEL-specific and do not bind the closely related immunogenic lysozyme of the ring-necked pheasant. The relationship between this interesting specificity class of RFC and the induction of suppressor T cells in the non-responder is being investigated. *Postdoctoral Fellow of the Cancer Research Institute.

689 ANTIGEN BINDING AND SURFACE IMMUNOGLOBULIN EXPRESSION BY T AND B LYMPHOCYTES, D. DeLuca, Cancer Biology Program, NCI Frederick Cancer Research Center, Frederick, MD 21701.

Lymphocytes from adult bone marrow, adult spleen, adult thymus, and maturing neonatal spleen have been studied to determine the clonal specificity of antigen binding (AB) and the possible immunoglobulin (Ig) nature of the AB receptor. The lymphoid nature of B-AB cells has been established by use of pure populations derived from B-lymphocyte colony-forming cells grown in agar. Using fluorescent-labeled protein antigens, anti-Ig reagents made in rabbits or chickens, anti-H-2 sera and anti-Thy 1.2 sera in double labeling experiments, we found that B-AB cells had localized antigen patches with Ig detected with either rabbit or chicken anti-Ig reagents. T-AB cells did not show detectable Ig with rabbit reagents, but did show copatching of antigen with chicken anti-$(Fab')_2$. T-AB cells did not show patch correspondence with anti-H-2 serum or anti-Thy 1.2 serum. AB studies of whole colonies of bone marrow B lymphocytes indicate that a lot of colonies (15%) specifically bind each of the large protein antigens used, a direct test of the clonal nature of AB capacity. Few clones (3%) bound two antigens concurrently. These results parallel those found when the frequency of AB Ig-bearing cells from whole bone marrow or neonatal spleen at birth is determined. Adult spleen lymphocytes have a much lower percentage of B cells binding antigen (∿ 2%) and a correspondingly lower frequency of double AB cells (∿ 0.2%). Since this shift in double AB frequency occurs during the first week after birth, and since velocity sedimentation studies show multiple AB cells to be associated with pre-B cells, it may be that B-AB cells are clonally derived populations which become more restricted in their AB capacity as they mature. (Sponsored by NCI contract no. NO1-CO-75380 with Litton Bionetics, Inc.)

690 ANTIGEN-DRIVEN CHANGES IN ANTIGEN-BINDING CELLS RESPONDING IN VITRO, R. F. Ashman and J. E. Merrill

Spleen cells from non-immune mice cultured with sheep erythrocytes (SRC) in vitro exhibit 5-10 fold expansion of the specific SRC-binding cells (ABC). The inhibition of ABC generation by hydroxyurea and the observation of ABC incorporating ^{3}H-thymidine by radioautography demonstrated an important contribution of cell division to this ABC increase. Both T and B-ABC were maximal at day 4, but the early increase in ABC during the first day was restricted to T-ABC and was not cell-division-dependent. Inhibition of antigen binding by heavy-chain specific antisera showed that the non-immune B-ABC were mainly $\mu^+\delta^+\gamma^-$. Abruptly on the third day of the response, surface IgG appeared, mostly on cells already bearing IgM and IgD. But from day 4 on, most B-ABC were $\mu^+\delta^-\gamma^+$. Cytophilic IgG did not provide the new surface IgG, as no IgG PFC were detectable. The expression of sIgG was totally antigen-dependent, though the loss of IgD was not. Yet both processes required new gene expression in that they were sensitive to 5 μg/ μl BUdR.

691 RAPID, QUANTITATIVE MEASUREMENT OF CELL SURFACE RECEPTOR SITE DENSITIES, James F. Leary, Julia Greenstein, Noel L. Warner, James H. Jett, Anita Stevenson, University of Rochester (JFL, JG), Rochester, N.Y., University of New Mexico (NLW), Albuquerque, N.M., Los Alamos Scientific Laboratory (JHJ, AS), Los Alamos, N.M.

Quantitative measurements presently made on fluorescent-antigen binding cells and on immunofluorescently stained cells by laser flow cytometers do not distinguish between small cells of high receptor site density and large cells of low receptor site density. Since receptor site density may play an important role in immunologic function we have devised two methods of measuring this quantity in addition to the usual total fluorescence/cell measurement. Cell Coulter volume (on the Los Alamos cell sorter) or light scatter time-of-flight (TOF) (on Ortho, Becton Dickinson, or Coulter flow cytometers) is measured simultaneously with fluorescence, and the fluorescence/(Coulter volume)$^{2/3}$ or fluorescence/(TOF)^{2} is measured on a cell-by-cell basis. These ratios are proportional to the number of receptors/cell surface area if the cell is nearly spherical in shape. Densities of antigen binding sites on B-cells and densities of allo-antigens and other markers of differentiation on B and T lymphoma cells are being measured by these methods.

692 B CELL MATURATION IN CBA/N MICE, Cheryl A. Whitlock and James D. Watson, University of California, Irvine, CA 92717.

CBA/N mice have an X-linked defect which results in an apparent block in the maturation of B cells. The aim of these studies is to examine how this defect affects surface Ig on B cells and the responsiveness of these cells to various antigens and mitogens. Lymphocytes from CBA/N and (CBA/N x DBA/2) F_1 male mice are unable to respond to certain T-independent antigens (TNP-Ficoll), and show lowered responses in culture to the T-dependent antigen, SRBC. Limiting dilution analysis of 9-week old F_1 hybrid mice reveal the frequency of SRBC-specific precursor B cells to be 3 to 4-fold lower in males than in female littermates.

The X-linked defect also affects the development of B cell responsiveness to LPS. B cells from young (5 week) F_1 males do not support significant mitogenic or polyclonal responses to LPS; whereas, B cells from older (12 week) males support responses comparable to those of F_1 females. Experiments are in progress to examine age-related changes in B cell responsiveness to LPS and SRBC, and to correlate these changes to the expression of cell surface Ig.

693 REGULATION OF THE IMMUNE RESPONSE BY TESTICULAR CELLS

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In preparation for investigating embryonic antigens of different haplotypes of the T/t locus of the mouse we defined conditions for lymphocyte stimulation in vitro against autologous and allogeneic testicular cells. Two subpopulations of testicular cells are obtained by differential protease treatment: collagenase releases a fraction enriched in Leydig cells (fraction I); trypsinization produces Sertoli cells and germ cells (fraction II). Autologous or allogeneic fraction I testicular cells induce lymphocyte proliferation, but no stimulation is caused by fraction II testicular cells. In cocultivation experiments with other stimulators, autologous fraction II cells suppress the response against allogeneic spleen cells. This effect is dose dependent on fraction II testicular cells and specific with respect to the responder strain. The results may suggest a special role of certain testicular cells that relates to the immunologically privileged status of the testis.

Discrimination Between Self and Non-Self

694 SUPPRESSOR CELLS DO NOT MAINTAIN HGG TOLERANCE INDUCED IN UTERO, C.A.Waters and Erwin Diener, University of Alberta, Edmonton, Alta.,Canada T6G 2H7

Most models of self tolerance are based on extrapolations of data gained from autoimmune phenomena or from experiments with adult or neonatal animals with antigens which were deliberately selected for their tolerogenic properties. Furthermore the current thinking on mechanism(s) of self tolerance largely disregards the potential importance of molecular structure of self antigens or their mode of presentation to the relatively undifferentiated immune system during ontogeny. In view of these considerations we felt it appropriate to utilize a self tolerance model which permits access of various test antigens to the fetus well before immunocompetent cells arise. The tolerogenic potency of human γ-globulin (HGG), bovine serum albumin (BSA) and a synthetic haptenated peptide of defined geometry (TNP-18) administered in utero was assessed in Balb/cCr mice. Contrary to expectations, only deaggregated HGG but not BSA or TNP-18 possessed in utero tolerogenicity even though label studies indicated that all three antigens crossed the placenta. As for HGG, unresponsiveness in the offspring was specific and complete for both T and B cell populations. Foster nursing experiments demonstrated that a four day period of intrauterine HGG transfer was sufficient to induce tolerance in the offspring. Unresponsiveness began to wane in the absence of additional administration of HGG at about 11-12 weeks of age. It was during but not before this period of tolerance breakdown that HGG-specific suppressor cells could be found, in contrast to the observations made when HGG tolerance is induced in adult animals.

695 AGING AND DEVELOPMENT OF AUTOIMMUNITY, by M.A. Berman, L.H. Perrin, P.H. Lambert, P.A. Miescher. WHO Immunology Research and Training Centre, Dept. of Medicine, 1211 Geneva 4, Switzerland.

Stimulation of mouse spleen cells by LPS or Fc fragments of IgG results in an increase of IgM plaque forming cells (PFC) to sheep RBC, horse RBC, goat RBC and to certain haptens, but not to human RBC(HuRBC). HuRBC were therefore used to detect antibodies directed to mouse, bovine, rabbit, and human IgG coupled to their surface. Direct PFC were detected using spleen cells from normal one year old CBA or Balb/c mice, 5 month old NZB/W, and young or old mice stimulated in vivo with LPS or Fc fragments. Anti-μ development performed according to Dresser (Nature 274:480, 1978) gave a 2 to 5 fold increase in PFC to human IgG coated HuRBC: $120/10^6$ in old CBA, but $998/10^6$ after LPS stimulation, and $424/10^6$ after Fc fragments. $168/10^6$ were seen in 5 month old NZB/W and $840/10^6$ in 1 year old NZB/W. $< 1/10^6$ were seen in 7 week old CBA, but $64/10^6$ after LPS stimulation and $6/10^6$ after Fc fragments. Anti-μ serum developed plaques were very large and one third of these plaques were ring shaped. Surprisingly, spleen cells of old CBA mice showed as many PFC to Fab as to Fc coated HuRBC. 10 to 100 times less PFC were detected using HSA-coated HuRBC (with or without anti-μ serum) in old mice. The spontaneous IgM antibodies of old mice toward IgG or those induced by LPS or Fc fragments could be interpreted in several ways: a) lack of suppressor activity towards a major endogenous antigen, b) stimulation of IgM rheumatoid factor production by the large amount of immune complexes found in older mice, c) regulation of the immune response by IgM.

696 EVIDENCE FOR CENTRAL OR INTRINSIC UNRESPONSIVENESS IN B CELLS TO T DEPENDENT ANTIGENS, D. Elliot Parks and William O. Weigle, Scripps Clin & Rsch Fnd, La Jolla, CA 92037

Substantially fewer B lymphocytes specific for protein antigens respond to nonspecific B cell activation than to antigen-mediated stimulation. Some antigen-binding cells detected by polyclonal B cell activation may differ from the antigen-reactive B cells stimulated by antigen. Although polyclonal activation has been reported to stimulate small numbers of B cells to produce antibody to protein antigen in tolerant individuals, the significance of these nonspecifically stimulated B cells and their relationship to the mechanisms of induction and maintenance of the tolerant state remain unclear. Substantial numbers of antigen-specific plaque-forming cells cannot be detected by the injection of LPS or LPS and antigen in mice rendered unresponsive to HGG as adults when B cells are unresponsive as assayed by adoptive transfer and antigen challenge. Responsive B cells can only be demonstrated in vivo by challenge with LPS and antigen when they can also be detected by adoptive cell transfer (approximately 7 weeks after tolerization). When spleen cells are removed from tolerant mice at various times after tolerization, washed extensively, and transferred into lethally irradiated recipients to circumvent any possible blockade or masking of responsive B cells by tolerogen in the donors, the kinetics of reacquisition of responsive B cells in the recipients parallels that of the donors. This is true whether the mice reconstituted with tolerant spleen cells are challenged with LPS alone or with LPS and antigen. The inability to detect responsive B cells until 7 weeks after tolerization to the soluble protein antigen HGG indicates that an irreversible, central unresponsiveness resulting in an intrinsic defect in B lymphocytes can be established to this T dependent antigen. Supported by NIH Grant AI-07007 & ACS Grant IM-42H.

697 MATURATION LEVEL OF B CELL SUBSETS IN TNBS TOLERANCE, John M. Fidler, Department of Immunopathology, Scripps Clinic and Research Foundation, La Jolla, CA 92037.
The B cell subpopulations involved in TNP-specific immunological unresponsiveness induced by trinitrobenzenesulfonic acid (TNBS) were investigated using in vitro antigen-specific and polyclonal responses. While tolerance 4 days after TNBS injection was stable to TNP-LPS challenge and was previously shown to be due to functional deletion, receptor blockade was observed up to 12 hr after tolerogen injection. 90% tolerance was obtained within 1 hr of TNBS treatment and this unresponsiveness was partially reversible. These tolerant cells suppressed the response of co-cultured control splenocytes. Using LPS as a probe for immature B cells, 60% tolerance in high affinity TNP-specific B cells was induced within 12 hr of TNBS treatment and complete unresponsiveness by 24 hr. In contrast, no significant decrease in the response to the mature B cell activator, PPD, occurred until day 2. Furthermore, tolerance was also induced by lower TNBS doses in LPS than in PPD reactive cells. In comparison to the TNP-LPS response, 50% tolerance was observed 1 hr after TNBS injection upon antigen-specific stimulation by TNP-Ficoll (which, like PPD, activates mature B cells), with unresponsiveness increasing gradually to completion on day 2. Thus, immature B lymphocytes, which give polyclonal responses to LPS and antigen-specific responses to TNP-LPS, are rendered tolerant to TNBS more rapidly and at lower doses of tolerogen than mature B cells, which react polyclonally to PPD and specifically to TNP-Ficoll.

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698 ANALYSIS OF TOLERANCE SUSCEPTIBILITY OF TI_1, TI_2, and TD B-CELL SUBSETS WHICH RESPOND MONOCLONALLY TO PHOSPHORYLCHOLINE (PC). J.C. CAMBIER AND M.J. NEALE, DUKE UNIVERSITY MEDICAL CENTER, DURHAM, NC 27710. Susceptibility of Balb/c B-cells responsive to PC SRBC, PC Brucella abortus (Ba) and R36A to in vitro tolerance induction using $PC_{40}HgG$ was studied. Anti-Thy 1 and complement treated splenocytes from adult mice were exposed to varied concentrations of $PC_{40}HgG$ for 24 hours before being washed exhaustively and recultured in the presence of SRBC primed, irradiated T-cells and immunogen. Direct anti-PC and anti-SRBC PFC responses were assayed four days after initiation of the cultures. All anti-PC responses were found to be monoclonal as determined by blocking of PFC formation by anti-$TEPC_{15}$ idiotype antibody. Thymus independent responses to PC Ba were highly susceptible to tolerance induction while thymus dependent responses to PC-SRBC were not. The anti-PC response to R36A, a putative thymus independent antigen of the TI_2 subclass, was found to depend on SRBC primed irradiated cells being present in the culture. Furthermore, this response was sensitive to anti-Thy 1 and complement treatment. The R36A induced anti-PC response was resistant to tolerance to a degree similar to that of the PC-SRBC response. These results indicate that differences in receptor affinity for antigen between TI and TD B-cells do not determine the observed differences in tolerance susceptibility. Studies of the role of IgD in determining tolerance susceptibility of these B-cells populations are in progress and will be discussed.

699 AUTOREACTIVITY TO SYNGENEIC THYROGLOBULIN IN GOOD RESPONDER MICE, Yi-chi M. Kong, Mostafa ElRehewy, Noel R. Rose, and Alvaro A. Giraldo, Wayne State University School of Medicine, Detroit, MI 48201
Using three different adjuvants, previous studies on the T-cell-based difference in responsiveness to mouse thyroglobulin (MTg) between good and poor responder mice have provided indirect evidence for the presence of MTg-reactive T cells in good responder mice. To explore further the autoreactivity of such cells, MTg was given repeatedly (4x/wk for 4 wk) to good responder C_3H ($H\text{-}2^k$) and DBA/1 ($H\text{-}2^q$) and poor responder BALB/c ($H\text{-}2^d$) mice in the absence of adjuvant. MTg antibodies reached high levels only in good responder mice given high doses of MTg. To eliminate stimulation by alloantigenic determinants and reduce the chance of denaturation, thyroid extracts from syngeneic mice were prepared freshly each week and injected into good and poor responder strains. Again, significant antibody titers were observed only in good responder mice. The antibody was specific for MTg since 1) it was not inhibited by extracts of other organs and 2) it reacted strongly with the closely related rat thyroglobulin but very poorly, if at all, with thyroglobulin from other species. Histology revealed mononuclear cell infiltration of the thyroid of good responder, but not poor responder, mice, regardless of the strain used to provide the MTg.
The data demonstrate that MTg-reactive cells may be stimulated by repeated, high doses of this self-antigen, without the aid of adjuvant, to override normal regulatory influences. Since MTg-reactive T cells were found in good responder mice, the previously shown *Ir-Tg* gene(s) probably codes for T-cell autoreactivity to self-determinants. (Supported by NIH grants CA-18900 and AM/AI-20023.)

700 ANTIGEN-INDUCED PROLIFERATION TO FOREIGN AND SELF-ANTIGENS, Carole G. Rombal1 and William O. Weigle, Scripps Clinic and Research Foundation, La Jolla, CA 92037

In vitro antigen-induced proliferation has been used to further examine the immune status of lymphocytes to thyroglobulin antigens and their relationship to autoimmune thyroiditis. In preliminary experiments, spleen cells from rabbits primed with human gamma globulin (HGG) were shown to proliferate (as assayed by incorporation of ^{3}H thymidine) in vitro after addition of HGG to cultures. The responsiveness to HGG, as well as the responsiveness to the T cell mitogen concanavalin A (Con A), was shown to reside in the cell population eluted after nylon wool passage of whole spleen cells. In other experiments, both spleen and peripheral blood lymphocytes from rabbits primed to bovine thyroglobulin (Bov Tg) gave a proliferative response to Bov Tg. This response was also shown to reside in a population of nylon wool nonadherent cells. Rabbit thyroglobulin (Rab Tg), however, failed to induce a proliferative response in Bov Tg primed spleen cells. In addition, rabbits immunized with Rab Tg in complete Freund's adjuvant (CFA) failed to proliferate in vitro after stimulation with either Bov Tg or Rab Tg. In contrast to the inability of Rab Tg to induce a proliferative response in Tg-primed lymphocytes, rabbits immunized with either Bov Tg or Rab Tg in CFA produce antibody to Rab Tg and develop thyroid lesions. These results are consistent with a state of tolerance at the T cell level to autologous Tg, which can be circumvented by administering either heterologous or homologous Tg in CFA, resulting in stimulation of competent B cells to produce autoantibody and thyroid lesions.

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701 GENETIC CONTROL OF EXPERIMENTAL AUTOIMMUNE MYASTHENIA GRAVIS (EAMG) IN MICE, P. Christadoss, V.A. Lennon and C.S. David, Mayo Clinic, Rochester, Minnesota 55901

The EAMG model in rat has provided us with considerable information on several aspects of myasthenia gravis. Mouse and human studies have suggested a role for the major histocompatibility complex (MHC) in susceptibility to the disease. In order to determine definitively whether or not susceptibility to autoimmune myasthenia gravis is H-2-linked, we set up a model on congenic mice. Torpedo acetylcholine receptor (TAR) with complete Freund's adjuvant and B. pertussis was injected into B10 congenic mice expressing the different independent haplotypes. The animals were screened for (1) autoantibody to mouse muscle receptors (radioimmunoassay); (2) quantity of acetylcholine receptors (AChR) in muscle (moles of AChR/gm of muscle); (3) the clinical signs of EAMG; and (4) T cell proliferation to TAR. The results shown in the table suggest that one or more H-2-linked genes are involved in the susceptibility to EAMG. Studies with recombinant strains, and backcrosses are in progress to further define and map the gene(s) involved and will be reported.

Strain	H-2 Haplotype	Autoantibody to mouse muscle receptors	T cell proliferation to TAR (stim. index)	# Mice with bioch. changes	# Mice w/EAMG
B6,B10	b	high	>15	5/5	2/5
B10.K,B10.S	k,s	intermediate	4.00	0/5	0/5
B10.P	p	low	not tested	not tested	0/6
B10.G	q	moderate	6.62	2/5	0/5
B10.D2	d	moderate	not tested	not tested	1/6
B10.RIII	r	moderate	11.9	not tested	0/4

702 INHERENT AND ACQUIRED UNRESPONSIVENESS TO LYSOZYME. Robert J. Scibienski, Sch. of Med. Univ. of Calif., Davis, Calif. 95616.

Injection of newborn mice with 5 mg of chicken lysozyme (CL) renders the animals unresponsive to this antigen. When assessed at 6-8 weeks of age these mice fail to mount primary responses and fail to acquire memory following immunization with CL in FCA. In contrast, when such tolerant mice are challenged with CL coupled to LPS the primary anti-CL response is equivalent to that seen in non-tolerant controls. Nevertheless, this immunization fails to induce immunological memory. Furthermore, prior injection of tolerant mice with CL/FCA or CL-LPS depresses their responsiveness to a subsequent challenge with CL-LPS. These results imply that the neonatally tolerized animals contain a suppressor mechanism which is activated by primary challenge with antigen. This has been confirmed by cellular cotransfer experiments.

A second type of unresponsiveness to lysozyme is evident in C57Bl/10 mice, which are inherently non-responsive to CL under certain conditions. Thus, immunization of B10 mice with CL/FCA or CL-LPS fails to prime for a subsequent anamnestic response. On the other hand, the primary response of B10 mice to CL-LPS is unaffected. This profile is thus similar to that seen in mice with acquired tolerance. However, primary and secondary lymph node responses are unaffected in B10 mice whereas they are suppressed in mice with acquired tolerance. Furthermore, again in contrast to mice with acquired tolerance, prior injection of B10 mice with CL/FCA or CL-LPS does not interfere with their ability to subsequently respond to CL-LPS. These findings suggest that the mechanisms which underlie these two forms of non-responsiveness are different.

703 FAILURE OF NZB SPLEEN TO RESPOND TO PRE-THYMIC BONE MARROW SUPPRESSOR CELLS, Michael J. Dauphinee, Norman Talal, Veterans Administration Medical Center, San Francisco and Department of Medicine, University of California, San Francisco, California.

The spontaneous autoimmune disease that develops in NZB mice is associated with a disordered state of immunologic control in which the generation of regulatory factors or the response to them may be abnormal. We find a suppressor cell present in DBA/$_2$ and NZB bone marrow (BM) which is a precursor of T lymphocytes because it can be induced to express θ-antigen by thymosin. Primed spleen cells from NZB mice are resistant to suppression by either DBA/$_2$ or NZB BM suppressor cells. NZB BM, however, can suppress primed DBA/$_2$ spleen cell responses. Thus NZB spleen cells appear refractory to suppressor signals. The resistance of NZB spleen to BM suppression is associated with the B cell enriched fraction.

704 LOW DOSE TOLERANCE INDUCTION TO GAMMA GLOBULIN IS FC DEPENDENT. Suzan Friedman and Henry H. Wortis, Tufts University School of Medicine, Boston, Mass. 02111

Most strains of mice are easily tolerized by a single injection of as little as 10-100 μg of heterologous gamma globulin. The mechanism of tolerance induction to this multivalent protein antigen is unknown. Either each epitope of gamma globulin induces tolerance to itself, or there exists a unique sequence which induces tolerance to all the determinants.

To differentiate between these two possibilities, we compared the ability of rabbit gamma globulin (RGG) and its fragments to tolerize CBA mice. Mice were first given intraperitoneal injections of RGG or its fragments (≤ 100 μg) in saline. Subsequently, they were challenged with these antigens in adjuvant. Antibody production was measured by immune elimination and/or a solid phase radioimmunoassay. We found that (1) RGG tolerizes CBA mice to both Fab and Fc. (2) one or multiple injections of Fab'2 or Fab primes for a response to Fab and (3) Fc tolerizes for Fc. Therefore, the RGG molecule is not uniform: Rabbit Fab primes under conditions in which Fc tolerizes. There is a highly tolerogenic sequence in the Fc but not the Fab fragment.

Tolerance to heterologous Fc can be triggered either by a unique epitope in the Fc or a molecular-cellular interaction (e.g., Fc-Fc receptor) other than epitope binding. Data are presented in support of one of the two models.

705 THE SPECIFICITY OF NZB ANTI THYMOCYTE ANTIBODY (NTA), Jan Moynihan, John W. Kappler and Philippa Marrack, University of Rochester, Rochester, NY 14642.

New Zealand mice develop a naturally occuring thymocytotoxic antibody (NTA) with age. This antibody has been shown by others to interfere with T cell functions *in vivo*. In particular it causes a loss in suppressor T cell activity when injected into mice. We have been studying the cytotoxic properties of this antiserum *in vitro*. In the presence of complement NTA kills precursor and effector suppressor T cells, induced by concanavalin A, which act by inactivating a nonspecific, T cell-derived helper factor. Under the same conditions NTA has no effect on T cells which make the helper factor. In preliminary studies NTA appears to have specificity for long-lived, anti-thymocyte serum sensitive, memory T cells, rather than short-lived, anti-thymocyte serum resistant, virgin T cells. This is true even though concanavalin A incubation induces suppressor cells from both these populations of T cells. We conclude that NTA has specificity for memory suppressor T cells, and that this specificity may contribute to the development of disease in New Zealand Black mice.

706 B CELL TRIGGERING AND TOLERANCE TO FLUORESCEIN-CONJUGATED ANTIGENS. David W. Scott
Division of Immunology, Duke University Medical Center, Durham, N. C. 27710.
Recent evidence suggests the existence of B cell subsets responsive to TI- or TD- forms of the same antigen. In the present study, we determined whether subsets can be described which differ in their expression of surface IgD, their sensitivity to in vitro tolerance induction, and their ability to be stimulated by different forms of the same hapten. We found that the primary in vitro IgM response to several "TI" antigens could be partially inhibited with an anti-IgD reagent. These responses were generally resistant to in vitro tolerogenesis. Moreover, anti-IgD pretreatment could facilitate tolerance induction in certain subpopulations.

Using a two-stage culture system, we found that the responses to all FL-antigens could be greatly increased by preculture with optimal amounts of several of these antigens (e.g., FL-ficoll), and that this "cross-priming" was reversed by BUdR and light treatment of the stimulated cells. "Cross-priming" was independent of T cells, antigen-IgD receptor interaction and PBA receptors. These studies indicate that B cell subsets vary in their sensitivity to anti-IgD and tolerogenesis, and that optimal antigen-IgM interaction with any of these subsets can provoke an initial triggering event without subsequent differentiation to PFC.
(Supported by AI-10716).

707 H-2 DETERMINISM IN ESTABLISHMENT OF TOLERANCE OF CLASS I ANTIGENS. J.W.Streilein and J.Klein, UTHSCD,Dallas,Tx 75235 and Max Planck Inst.,Tubingen,Fed.Rep.Germany.
Loci within H-2 promote and restrict the expression of neonatally-induced tolerance to MHC alloantigens with a specificity rivaling that which presides over the development and expression of T effector cell function. Neonatal mice were inoculated with hematopoietic cells from H-2 D disparate donors(some differed from neonates at flanking H-2 regions to the left(I-JEC) and right(Qa,Tla)). At maturity, putative tolerant mice were challenged with skin grafts from 3 *different groups of donors*: I-genotypically identical to the tolerizing inoculum; II-expressing the D tolerogen plus flanking determinants of a third haplotype;III-expressing the D tolerogen plus flanking determinants of host genotype. I region loci influenced the acceptance rate of Group I grafts;host alleles within IA conferred high or low resistance to tolerance induction, while IJ disparity strongly promoted tolerance. Loci flanking H-2 D to the left restricted tolerance expression:Group II & III grafts were rejected within 20 days. The relationship between D tolerogen and IJ phenotype on test grafts was critical: grafts bearing D tolerogen with its original IJ were accepted, even if disparity at flanking loci was present; alternatively, grafts bearing D tolerogen with a different IJ were destroyed, even if no new adjacent specificities were present. We conclude that tolerance is promoted by Class II genes acting in both donor and host, and is restricted by genes within or near IJ. These findings imply that maintenance of self/non-self discrimination may be governed by the IJ region of H-2: Class I determinants become self-markers in a specific IJ context and can be regarded subsequently as self only when expressed in that same context.

708 ACQUIRED SELF TOLERANCE TO THE INTRACELLULAR LIVER PROTEIN F, Guil Winchester, Tumour Immunology Unit, Zoology Department, University College London, Gower Street, London WC1 6BT, England.
F is an intracellular differentiation antigen, M.W.40,000 daltons, found in the liver of all mammalian species but not functionally identified. Mice are polymorphic for F; two allelic types, I and II, can be identified on the basis of immunogenicity. Liver from type II mice will immunize responder mice of type I and vice versa. Anti-F antisera cannot serologically distinguish between the two F-types, implying that although mice are T-cell tolerant of self F they lack B cell self tolerance. F is expressed co-dominantly and F_1 hybrid between type I and type II is tolerant of both F-types. However in a(I x II) $\rightarrow$ II^{1}BM chimaera the BM cells mature in the absence of F of type II and are able to respond to immunization by type II. Responsiveness can be inhibited by injections of F in soluble form during the recovery period. This demonstrates that self tolerance to the differentiation antigen F is acquired. These chimaeras offer a way of directly testing the prediction that self tolerance in T but not B cells reflects the levels of self antigen seen by the immune system. Injections of type I F during the recovery period tolerize T cells to the allovariable T_H determinant: injections of type II F - present in the host - will only result in unresponsiveness if the B cells have been tolerized. Results show that at doses of 10^{-8}M F 3 x week T cells are completely tolerized and B cells not at all; and at 10^{-11}M T cells are still partially tolerized, while B cells appear to be primed. Preliminary results suggest F is present in low amounts in serum. These are based on the inhibition by serum of ^{125}I-F binding to anti-F in a RIA.

Mixed Lymphocyte Reactions

709 MLR SUPPRESSOR T CELLS INDUCED BY MULTIPARITY: G. Chaquat, U 23 INSERM, 75571 Paris, France.

Mitomicyn treated spleen cells of allogeneically pregnant mice added on a MLR of maternal strain responder cells against paternal strain stimulators can, in contrast with control cells from normal or isogeneically pregnant animals, cause a marked suppression (MLRs are performed in heat inactivated mouse serum, and regulatory cells harvested at day I4 of at least a 2nd or 3rd pregnancy).
The suppressory cell is a T cell, of Ly 2, Ia+ phenotype. The suppresion can also be abrogated by replacing normal mouse serum by antiidiotypic antiserum in the same conditions in the culture medium.
Cross recognition requirement at the target level show that the suppression, although non-specific at first insight, is in fact probably highly specific of Ia determinants.
The system is genetically restricted, to the I-C, S, G subregions, although a certain amount of suboptimal suppression can be detected in certain combinations where responders and cells from allopregnant mice share only S-G. Further analysis point also towards a background control of this MHC restriction.
The suppressor cell acts via a soluble factor, as shown by double chamber experiments. This factor is now under analysis.
Preliminary data suggest that this regulation could also play a role in controlling CTL generation. Studies conducted with placental extracts suggest suppressor cells generation to be induced by placental substances under study.

710 HYBRID I REGION ANTIGENS AND I REGION RESTRICTION OF RECOGNITION IN MLR. C. G. Fathman, Department of Immunology, Mayo Clinic and Medical School, Rochester, Minnesota 55901

Recent studies in our laboratory have demonstrated the existence of a unique hybrid MLR stimulating determinant on F_1 stimulator cells of a hybrid between strains A/J and C57Bl/6. These determinants have been demonstrated to be encoded by gene products localized within the I region of the MHC and the recognition of these products has likewise been demonstrated to be controlled by products of the I region of the H-2 complex. Using appropriate recombinant mice it has been possible to further localize the site of interaction which leads to the production and recognition of this hybrid MLR stimulating determinant. Prerequisites for expression of the hybrid MLR stimulating determinants between strains A and B6 include an I-E/C region of the k haplotype and an I-A of both b and k haplotype. F_1 mice derived from recombinant strains which are deficient in any one of these three subregions fail to express the F_1 MLR stimulating determinant. Thus, we have postulated that the product recognized by strain A present on (A x B)F_1 hybrids is an altered form of $I\text{-}A^b$ resulting from genes residing within the I-E/C region of haplotype k and the recognition of this altered form of $I\text{-}A^b$ is restricted by $I\text{-}A^k$ region products.

711 T CELL RESPONSES TO M LOCUS DETERMINANTS: EVIDENCE THAT THE Mls^a ALLELE IS IDENTICAL TO THE Mls^d ALLELE, Katherine L. Molnar and Jonathan Sprent. Univ. of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104.

Two gene loci, the major histocompatibility complex (MHC) and the M (Mls) locus, code for determinants which stimulate strongly in mixed lymphocyte cultures (MLC). The Mls locus exhibits at least four alleles. The Mls^a and Mls^d alleles stimulate as strongly as incompatibilities at the MHC (10-40 fold above background), whereas the b and c alleles stimulate only weakly (2-4 fold above background). Recent experiments suggest that the strong Mls^a allele is identical to or very crossreactive with the Mls^d allele. The following evidence supports this hypothesis: (i) C3H/HeJ($\underline{H\text{-}2^k}$, $\underline{Mls^c}$($\underline{k}$,$\underline{c}$))lymphocytes negatively selected to Mls^a determinants (ie. depleted of alloreactivity by acute blood to lymph recirculation through (BALB/c($\underline{d}$,$\underline{b}$) x AKR/J($\underline{k}$,$\underline{a}$))F_1,fail to respond to CBA/J($\underline{k}$,$\underline{d}$); (ii) C3H/HeJ($\underline{k}$,$\underline{c}$) lymphocytes negatively selected to Mls^d by filtration through (BALB/c($\underline{d}$,$\underline{b}$) x CBA/J($\underline{k}$,$\underline{d}$))F_1 fail to react against AKR/Cum($\underline{k}$,$\underline{a}$), Dl.C($\underline{d}$,$\underline{a}$), and DBA/2($\underline{d}$,$\underline{a}$); (iii) C3H/HeJ lymphocytes which have been positively selected (ie. stimulated in MLC) to Mls^a (AKR/Cum($\underline{k}$,$\underline{a}$)) either $\underline{in\ vivo}$ or $\underline{in\ vitro}$ respond equally well to CBA/J($\underline{k}$,$\underline{d}$) as to the priming strain; (iv) C3H/HeJ ($\underline{k}$,$\underline{c}$) lymphocytes postively selected to Mls^d (CBA/J($\underline{k}$,$\underline{d}$)), either $\underline{in\ vivo}$ or $\underline{in\ vitro}$ respond well to AKR/Cum ($\underline{k}$,$\underline{a}$) AKR/J($\underline{k}$,$\underline{a}$), and to all strains which exhibit the Mls^a allele; and (v) AKR/Cum($\underline{k}$,$\underline{a}$) and CBA/J($\underline{k}$,$\underline{d}$) fail to respond against one another in MLC. These data imply that only one Mls encoded allele ($Mls^a \equiv Mls^d$) stimulates strongly in MLC.

712 DIFFERENTIATION OF MLC-INDUCED SUPPRESSOR T CELLS AND KILLER CELLS BY IN VITRO DRUG TREATMENT, Anthony Schwartz, Yale University, New Haven, CT 06510.

In an in vitro allogeneic mixed leukocyte culture (MLC) specific (but not "promiscuous") cytotoxic T lymphocytes (CTL) and two populations of suppressor T cells, one "promiscuous" (PS) and one specific (SS) are generated. SS suppress CTL induction only in an MLC stimulated by the homologous antigen. It has been difficult to determine whether CTL inhibition by SS is due to suppression of responders or to premature killing of stimulators in the second culture. Pyrilamine, a histamine$_1$ antagonist which also has attributes of a local anesthetic was found to profoundly inhibit CTL and PS generation while it does not inhibit induction of SS. The suppressive effect of SS on CTL induction could not be overcome by increasing stimulators in the second culture. Our results confirm that PS and SS belong to different T cell sets. Both suppressor cell sets also differ from CTL, since suppression could be demonstrated when CTL were not detectable at the time of transfer. Effects similar to those brought about by pyrilamine also could be induced by local anesthetics. Since pyrilamine can be added as late as 72 hours after establishing MLC's, members of this membrane-active drug class might interfere with late cell-cell interactions. Histamine also can inhibit CTL generation and has effects on suppression. However, it is unlikely that histamine agonist or antagonist effects mediate the influence of pyrilamine on these T cell functions, since histamine only affects the MLC when added less than 2 hours after culture and thus probably acts on an early T cell triggering event(s). Supported by U.S.P.H.S. Grant AI-10497

713 SEROLOGICAL DISTINCTION BETWEEN B CELL 'DR' ANTIGENS AND THOSE RESPONSIBLE FOR STIMULATION IN MIXED LYMPHOCYTE CULTURE. C.M. Steel, V. Van Heynigen, B.B. Cohen & D.L. Deane. MRC Clincial & Population Cytogenetics Unit, Edinburgh, Scotland.

Isolated plasma membranes from cultured human B lymphoid cell lines have been use to raise antisera in rabbits. After absorption with T cell lines these antisera were shown to react in complement-dependent microcytotoxicity tests with all B lymphoid lines tested and with peripheral blood lymphocytes. Similar antisera have been shown by other workers to be directed against B cell 'DR' antigens. These antisera are potent inhibitors of mixed lymphocyte reactions but they also imapir lymphocyte responses to soluble mitogens. One cell line (EB_1), derived from a Burkitt's Lymphoma biopsy, was found to be a consistently weak stimulator of allogeneic blood lymphocytes in MLC despite having all the other characteristics of a B lymphoid line, including 'DR' antigens. When EB_1 cells were used to absorb rabbit anti-B cell sera, the titre of the antisera, in microcytotoxicity tests and in quantitative assays of cell surface binding, fell dramatically. EB_1-absorbed anti-B cell sera have little effect on lymphocyte responses to soluble mitogens but, even at high dilution, retain the ability to inhibit mixed lymphocyte reactions, whether the stimulating cells are from B lymphoid lines or peripheral blood B cells. Immunofluoresence and immunoferritin studies suggest that the antigens detected by EB_1-absorbed anti-B cell sera are present in small amounts but uniformly distributed over the surface of B lymphoid cell line cells. The same antigens appear to be expressed on peripheral blood B cells. These findings indicate that the B cell 'DR' antigens detected serologically in microcytotoxicity assays are distinct from those responsible for stimulation of allogeneic T cells in MLC.

714 H-2K SPECIFIC TUMOR REACTIVE CYTOTOXIC T LYMPHOCYTES GENERATED IN MIXED LYMPHOCYTE REACTION, by Masahiro Imamura, Robert Justice, Barbara Pope and W. John Martin, Bureau of Biologics, FDA, Bethesda, Md. 20014

Transplantation studies using transplacentally induced lung tumors of C3Hf mice provide strong evidence that C3Hf mice have deviated from C3H mice in their expression of an H-2K region coded alloantigen. Thus several of these tumors will grow preferentially in C3H mice and specific radioresistant immunity can be induced in C3Hf mice by pre-immunization with normal tissues of mice known to express the H-2K haplotype. In the present study we have further characterized the H-2K region difference between C3H and C3Hf mice using the mixed lymphocyte reaction (MLR). Reciprocal stimulation is regularly observed in the primary but not in a secondary MLR. Cytotoxic T cells generated in MLR between viable C3Hf derived responding spleen cells and C3H derived X-irradiated stimulating spleen cells were cytotoxic for C3H cells and B10.A (4R) cells (MHC haplotype kkbbbb) but not for B10 (bbbbbb) or C3Hf cells. These cells also killed the C3Hf derived lung tumor 85. In vitro generated tumor reactive cytotoxic T lymphocytes were effective in suppressing tumor growth when tested in C3Hf recipients using the Winn assay. Cytotoxic cells derived from C3H anti-C3Hf MLR were cytotoxic for C3Hf but not for any other allogeneic strain tested. These cells did not lyse the lung tumor 85. In spite of strong reciprocal MLR no evidence of serological reactivity against the altered alloantigen was obtained.

715 TROPHOBLAST CONTROL OF ALLOGENEIC RECOGNITION IN VITRO. J.A.McIntyre and W.P.Faulk, Med. Univ.of SC, Charleston,SC and Blond McIndoe Transplant. Centre East Grinstead, England.
Genetical differences between allogeneic cells are expressed by antigens of the major histocompatibility complex (MHC) and matching of these antigens is currently the most effective means of selecting organs or tissues for clinical transplantation. The most successful biological graft however, is the trophoblast during pregnancy. Trophoblast membranes lack all known MHC antigens indicating the presence of an as yet undefined mechanism responsible for its success. We have investigated the ability of trophoblast membranes to modulate certain lymphocyte functions by studying the effects of heterologous anti-trophoblast serum and trophoblast membrane fractions on lymphocyte responses in vitro. Reagents used in this study were either the first peak (TA_1) obtained by chromatography of detergent solubilized trophoblast membranes or rabbit antisera raised to this peak (anti-TA_1). MLC reactions are significantly suppressed in the presence of anti-TA_1 and totally abolished when exposed to microgram concentrations of TA_1. Both reagents were most effective when added early to culture and ineffective if added after 24 hours. Dose-response studies showed similar inhibition curves although TA_1 was the more potent. The unique ability of these reagents to affect only allogeneic recognition reactions prompts us to suggest that a mechanism exists whereby allogeneic stimulation produces specific reaction products which can either be recognized and suppressed by anti-TA_1 or competitively inhibited by TA_1. We have also identified antigenically cross-reactive TA_1 proteins on certain lines of human transformed cells, suggesting that the host-parasite relationship in human cancer may invoke an analogous process to that employed by trophoblasts in the host-parasite condition of human pregnancy.

716 THE MURINE MIXED LYMPHOCYTE RESPONSE REPRESENTS A T CELL RESPONSE TO NON-T, NON-B, SPLENIC ADHERENT CELLS IN THE MIXED LYMPHOCYTE REACTION, Gerald B. Ahmann, Paul Nadler, Alan Birnkrant, Karen S. Hathcock, and Richard J. Hodes, NIH, National Cancer Institute, Bethesda, Md. 20014
The proliferative response in the murine MLR provides a model for cell-cell recognition. In this response system it has been well established that the responding cell is a T cell, whereas the stimulating cell has not been conclusively characterized. It has been demonstrated that Ia antigens (encoded within the H-2 region) and Mls antigens (non-H-2 encoded) are strong MLR stimulating determinants and are expressed on B cells and macrophages. Experiments were therefore performed to establish which of these cells is the predominant stimulator. Stimulating spleen cell populations were depleted of adherent cells and T cells by passage through Sephadex G10 and subsequently treating with a rabbit antimouse brain sera (RAMB) and complement. This resulting population, which was enriched for B cells ($78\pm4\%Ig^+$) and depleted of latex ingesting cells ($<0.5\%$ $latex^+$), was unable to stimulate an MLR in unseparated responder spleen cells. In contrast, a glass adherent, radioresistant, RAMB negative spleen cell population ($67\pm13\%$ latex ingesting) stimulated a strong MLR when added in cell numbers that comprised only 5% of the unseparated stimulator spleen population. Treatment of these splenic adherent cells (SAC) with anti-Ia reagents and C abolished their ability to stimulate an MLR. Using subregion specific anti-Ia reagents, these SAC were found to express Ia determinants encoded by genes in I-A,B,J and/or I-E/C. In conclusion, MLR may represent a model in which T cells recognize and respond to Ia and Mls antigens only when they are expressed on splenic adherent cells.

717 QUANTITATION OF H-2 AND THY 1.2 EXPRESSION ON MURINE T CELL LYMPHOMAS AND NORMAL CELLS BY FLOW MICROFLUOROMETRY, Craig W. Spellman, and Noel L. Warner, The University of New Mexico School of Medicine, Albuquerque, NM 87131.
Studies on H-2 antigens with specific typing sera and flow microfluorometry (FACS system) indicate that the quantitative expression of these phenotypic markers varies greatly between mouse strains carrying the same H-2K or D region haplotype. This phenomenon has been observed for normal thymus and spleen cells and T cell lymphomas. Both genetic arguments and appropriate absorptions suggest that H-2 products are being detected. Other investigators have reported differential expression of non- H-2 gene products associated with particular haplotypes (for example, Tla), and differences in H-2 region expression between haplotypes. However, these data suggest that genetic controls exist which influence H-2 antigen expression within the same haplotype. In conjunction with this serological work, investigations are in progress using cold target inhibition assays to determine if antigens recognized by cytotoxic effector cells also exhibit quantitative expression. Further studies using the fluorescence activated cell sorter indicate that various murine T cell lymphomas, of a given haplotype, also express different amounts of Thy 1.2. Because the expression of certain antigens changes during normal cell maturation, the tumors are being studied from the perspective that quantitation of these markers may define descrete stages of T cell differentiation. As such, the tumors are then being used as models for functional capacities in relation to a specific differentiation state.

718 PHYLOGENY OF THE MAJOR HISTOCOMPATIBILITY COMPLEX: AN EXAMPLE OF CONVERGENT GENE EVOLUTION? N. Cohen, University of Rochester, Rochester, New York 14642.
The importance of the MHC in so many immunological phenomena has led to research into the evolutionary history of this gene complex. Immunological and recent immunogenetic studies with cold-blooded vertebrates suggest that MHC phylogeny may be a saga of convergent evolution of class II region genes (i.e., I region homologues). Functional markers associated with this region in mice, suggest its existence in advanced fishes and anuran amphibians and its absence in primitive fishes, primitive amphibians, and some, if not all, reptiles. Salamanders are the best studied group of vertebrates that appear to lack the complete homologue of the MHC. They reject skin allografts chronically but appear to have a predominant histocompatibility (H) locus which may be ancestral to class I region genes. Although a MLR locus has been provisionally identified in at least one species of salamanders, it is neither a H-locus nor is it functionally linked to the predominant H-locus. Moreover, this putative MLR locus is minimally polymorphic (i.e., only 2 codominant alleles) and is associated with very poor *in vitro* stimulation. *In vivo* immunization with skin grafts fails to alter the incidence, kinetics, or magnitude of the MLR. Finally, salamanders, which only have IgM immunoglobulins, fail to produce antibodies to antigens (serum proteins, ferritin) which, in mice, involve thymus dependent I region-associated cell interactions. Although salamanders do produce antibodies to SRBC and Salmonella H antigens, these responses are thymus-independent in these amphibians.

Role of Surface Immunoglobins and of Mitogen Receptors in B Cell Triggering and Tolerance

719 B CELL TOLERANCE IS NOT DUE TO COMPLETE CLONAL DELETION, Daniele Primi, George K. Lewis and Joel W. Goodman, Department of Microbiology and Immunology, University of California, San Francisco, CA 94143
B cell tolerance has been attributed to either receptor blockage or clonal deletion. To distinguish between these alternatives, BDF_1 mice were tolerized to fluorescein (Fl) by two injections of 1 mg of Fl-D-G,L. The D copolymer was used as a carrier because of its resistance to degradation. Tolerance was assessed by plaque-forming cell responses of spleen cells from injected and normal mice cultured in the presence of immunogenic quantities (0.1 μg/ml) of Fl-LPS, Fl-Dextran or Fl-Ficoll. Antibody responses were not made by cells from tolerized animals. However, when cells from these animals were cultured for 3 days with mitogenic doses of LPS (100 μg/ml) high and low affinity anti-fluorescein antibody responses were observed (576 ± 40 high affinity anti-Fl PFC per culture for tolerant mice versus 840 ± 84 for controls). In order to determine whether the antigen-unresponsive B cells could also be induced to proliferate, spleen cells from tolerized mice were cultured for 6 days at low density with a mitogenic dose of LPS, under which conditions virtually every lymphocyte capable of being activated grows continuously as a clone of cells. At the end of the culture period, numbers of anti-Fl PFC comparable to controls were detected, implying that the B cells unresponsive to antigen were capable of proliferation as well as differentiation when activated by a different mechanism. These results are compatible with receptor blockage but not with clonal deletion as the mechanism of antigen-induced B cell tolerance.

720 The Interaction between Surface Immunoglobulin Receptors and Mitogens in Lymphocyte Activation. Allen J. Rosenspire and Diane M. Jacobs, Dept. of Microbiology, SUNY at Buffalo, Buffalo, NY 14214 (Supported in part by USPHS NIH Grant CA20078).

A general model for the B lymphocyte triggering mechanism has been developed. The model is based upon the physical concept of an allosteric transmembrane mitogen receptor that is assumed to mediate signal transduction via a single second messenger. The mitogen receptor, however, is also assumed capable of interacting with surface immunoglobulin, so that immunoglobulin-antigen interactions may be capable of modulating receptor response to mitogen. The model can be made precise to the extent that a mathematical description of its behavior can be given. Subsequent analysis shows that this general model is capable of mimicing the behavior expected of the more standard one or two-signal models of lymphocyte activation under different circumstances. We have performed a series of in vitro experiments utilizing the T-independent antigen TNP-LPS with mouse spleen cells and show that, in this system at least, the experimental results can be interpreted in a systematic manner, in qualitative agreement with the predictions of the model. These same experiments cannot easily be reconciled with previous models of B cell activation.

721 Ia POSITIVE CELLS ARE REQUIRED FOR OXIDATIVE MITOGEN RESPONSES, M. Laurie Phillips, Sharon Hill, Richard L. O'Brien, and Jeffrey A. Frelinger, University of Southern California, Los Angeles, CA 90033. Addition of anti-Ia sera to cultures of mouse spleen cells stimulated with the oxidative mitogens, neuraminidase/galactose oxidase (NaGO) or sodium periodate, inhibits the subsequent proliferative response 30-70%. Anti-H-2K or D sera showed less than 20% specific inhibition. Subregion specific sera were less inhibitory than sera directed at the whole I region, but generally J,E, C region sera produced greater inhibition than A sera. Treatment with anti-Ia + C resulted in 90% inhibition of the NaGO response. Essentially complete inhibition was seen when the antiserum was directed against either the I-A or I-J, E and C regions, 65% with anti-I-J sera and 50% with I-E, C serum plus C. Allelic exclusion was not apparent since the proliferative response of F_1 hybrid mice was abolished by treatment with antiserum directed at either parental haplotype. The response was sensitive to rabbit anti-mouse brain serum , anti-Ly-1 or anti-Ly-2. The anti-Ia depleted response could be only partially restored in 2/10 attempts using thioglycolate stimulated, T cell depleted, peritoneal exudate cells. Further addition of 5 or 10% normal spleen cells to the Ia depleted spleen, did not restore NaGO stimulation. When large numbers (15%) of T cell depleted splenic adherant cells are used as a source of Ia^+ macrophages, 50-80% of the response can be restored. These results suggest that at least two cell types are required for NaGO stimulation: a T cell and a macrophage, at least one of which must be Ia^+.

722 IN VITRO STIMULATION OF MURINE LYMPHOCYTES BY RAUSHCER LEUKEMIA VIRUS, J. Eric Bubbers, John H. Elder and Frank J. Dixon, Scripps Clinic and Research Foundation, La Jolla, California 92037.
Murine lymphocytes cultured 4d. in the presence of Rauscher murine leukemia virus freeze-thaw, 100,000xg supernatants demonstrate increased ^{3}H-thymidine uptake. The stimulatory capacity has been shown to co-purify with the major envelope glycoprotein, gp70, and can be specifically removed from virus supernatants by absorption with anti-gp70 antibody-linked Sepharose. Cells from all strains tested, including C3H/HeJ and BALB/Wehi nu/nu, were found to respond. Peak response was after 3-4d. in culture and cells responded even after brief exposure to virus supernatants. When gp70 concentrations comparable to endogenous serum levels were used, both spleen and mesenteric lymph node cells responded routinely with stimulation indices of between 5 and 10; although bone marrow cells responded poorly and neither thymocytes nor cortisone-resistant thymocytes responded at all. Lymphocyte stimulation was independent of the presence of adherent cells and T and B-cell enriched populations responded equivalently.

723 DEXTRAN SULFATE CAN POTENTIATE THE MITOGENICITY OF LPS FOR MURINE SPLEEN CELLS Monte Wetzel and John R. Kettman. Southwestern Medical School, Dallas, Texas,75235
In vitro culture of murine spleen cells with the B cell mitogens Dextran Sulfate (DXS) and Lipopolysaccharide (LPS) yielded a synergistic growth response. The magnitudes of the proliferative responses seen in cultures with LPS alone or with both LPS and DXS are comparable. However, when both mitogens are present, peak responses are achieved 2 or more days earlier. In contrast to synergy in growth, LPS alone leads to a greater polyclonal antibody synthesis (PCA) response than when combined with DXS. DXS by itself stimulates weak proliferation and PCA responses. Small spleen cells sedimenting from 2.5 to 4.0 mm/hr and with diameters from 5u to 7u gave similar proliferation and PCA responses when compared to unfractionated cells. The lack of a T cell requirement for synergy is indicated since enhanced growth in the presence of both mitogens is seen in nude mouse spleen cells or normal murine spleen cells which have been treated with an anti-T cell reagent and C'. At high cell densities, the apparent synergy with both mitogens is not seen. This may explain discrepant results which have been reported in the literature. Sequential addition experiments showed that the order of addition of the mitogens is irrelevant, arguing that the major function of DXS is not maturational. By limiting dilution analysis, we have conclusively shown that 2 mitogens can act on the same cell.

724 INDUCTION OF T-CELL PROLIFERATION BY ANTISERA TO THE T-CELL SURFACE. Barry Jones, Hazel Dockrell and Charles A. Janeway. Yale University, Pathology Department and Department of Immunology, Middlesex Hospital Medical School, London, England.
Reaction of non-immune mouse spleen cells with rabbit anti-brain associated Thyl sera (anti-Bathyl) stimulated a proliferative response which correlated with the appearance of blast cells in the cultures between 29 and 45h. The response was dependent on T-cells (Thyl.2 positive cells); but highly purified T-cells prepared by passage over rabbit anti-MIg columns (<1% Ig positive) failed to respond. It appeared that an ancillary cell was required, since the response of the purified T-cells was reconstituted by the addition of Mitomycin C treated spleen cells. There was no requirement for identity at the H2 locus between the responding T-cells and the ancillary component:

Ancillary cell stain:	Proliferation of C3H x BALB/c F_1 T cells Δ dpm $\pm$ SD
C3H x BALB/c F_1	109,979 $\pm$ 2017
AKR	121,249 $\pm$ 5539
B6	106,973 $\pm$ 1850
None	1,045 $\pm$ 856

The complete absorption of mitogenic activity from anti-BaThyl by Thyl.2 positive cells indicated that antibody to Thyl was the important mitogenic agent. The possibility that antibodies against other T-cell antigens could be stimulatory was unlikely since high titre anti-thymocyte sera were poorly mitogenic, and could be completely absorbed by brain tissue without affecting their ability to react with the T-cell surface as judged by cytotoxic assays.

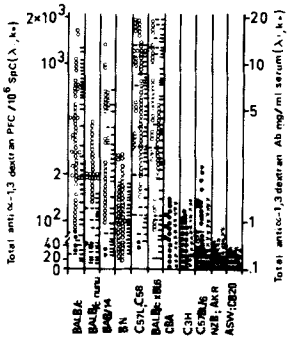
725 ANTIGEN SPECIFIC COMPONENT OF T CELL HELP IN PWM STIMULATED HUMAN PERIPHERAL BLOOD CELL CELL CULTURES, Eric M. Macy, Ronald H. Stevens, UCLA, Los Angeles, CA 90024
Booster immunization of normal individuals with Diptheria and Tetanus toxoid results in the enhanced ability of their lymphocytes to be stimulated in vitro by pokeweed mitogen (PWM) to produce immunogen specific IgG. T cells from these individuals appear to have antigen specific helper activity for in vitro IgG production. Limiting numbers of irradiated T cells were added to $2x10^5$ B cells shown to contain an excess of potential anti-tetanus toxoid (IgG-Tet) and anti-diptheria toxoid (IgG-Dip) IgG producing cells. The number of potential IgG-Tet and IgG-Dip producing B cells was determined by diluting out B cells in the presence of $5x10^5$ irradiated T cells. At two weeks post-boost about 1 in $1x10^4$ B cells were IgG-Tet producing and 1 in $3x10^4$ were IgG-Dip producing. E-rosette positive cells were used as T-cells and the E-rosette negative fraction was used as the B cell source eventhough it contained from 20 to 50 percent monocytes. The T cells were irradiated to minimize suppressive effects in the nine day PWM driven micro-culture system used. All cultures were assayed for IgG-Tet, IgG-Dip and total IgG using a quantitative radio-immune-assay. The helper activity of the T cells as measured by IgG produced diluted out according to a Poisson distribution assuming single reactive units were responsible for the antigen specific IgG produced by the B cells. The IgG-Tet and the IgG-Dip responses diluted out independently and cultures at the limiting dilution of T-help were observed to produce IgG-Tet or IgG-Dip but not both in the majority of cases even though sufficient potential IgG-Tet and IgG-Dip producing B cells were present. The frequency of helper cells for IgG-Tet varied from 1 in $2.0x10^4$ to 1 in $3.4x10^5$. The mean precursor frequency for the IgG-Dip helper T cells varied from 1 in $1.4x10^4$ to 1 in $9.1x10^4$.

726 GENETICS OF THE IMMUNE RESPONSE TO HAPTEN-LPS CONJUGATES, R. Skelly, A. Ahmed and D.C. Morrison, Scripps Clin. & Res. Fdn., LaJolla, CA, and NMRI, Bethesda, MD
Immunization of mice with hapten-lipopolysaccharide (LPS) conjugates results in both hapten-specific and polyclonal antibody (Ab) production. Since little was known about the Ab response to the polysaccharide moiety of hapten-LPS conjugates, mice were injected with either hapten-LPS or unsubstituted LPS and the splenic Ab response specific for the polysaccharide was measured. Some mice (BALB/c, A/J) produce equivalent amounts of anti-LPS Ab when injected with either hapten-LPS conjugates or LPS. However, other mouse strains (C3H/St, C57BL/6J, DBA/1J, DBA/2J, Swiss) make less anti-LPS Ab in response to hapten-LPS, compared with their response to an equivalent dose of LPS. The differential Ab response is independent of T cells, antigen dose, kinetics of Ab synthesis, hapten, or LPS serotype. The difference in anti-LPS Ab responses is also independent of the ability of these mice to respond to the lipid A moiety of the LPS as measured by in vivo polyclonal Ab synthesis. The differential anti-LPS Ab response occurs in both sexes, although quantitative differences in the amount of anti-LPS Ab produced by ♂ and ♀ mice to LPS do occur. However, in hybrid mice from poor responder x responder parents (B6AF1), the responder phenotype is sex linked; ♂ mice are differential anti-LPS Ab responders, while ♀ mice make equivalent amounts of Ab whether injected with hapten-LPS conjugates or LPS. Preliminary experiments using B10 recombinant strains of mice suggest that the d haplotype at the D end of the H-2 region also regulates the Ab response to hapten-LPS conjugates. These data indicate that the specfiic anti-LPS Ab response to hapten-LPS conjugates differs from the response generated by unsubstituted LPS and that the Ab response is influenced by H-2 and sex-linked genes.

727 MITOGEN RESPONSIVENESS OF A MURINE B CELL LINE, Christopher, J. Paige and John Lifter, Sloan-Kettering Institute, Rye, NY, 10580.

We have previously described a murine cell line, 70Z/3, which has many properties of a pre-B cell (J. Immunol. 121:641,1978). Under normal culture conditions the majority of cells synthesize μ heavy chains detectable by immunofluorescence in the cytoplasm but not on the surface. Other classes of Ig (γ, α, δ) including light chains are absent as are Ia antigens. In addition, most of the cells possess neither FcR or CR although more than half express Lyb2. A most fascinating property of 70Z/3 cells is their responsiveness to the B cell mitogens, LPS and DxS. Exposure to LPS leads to the surface expression of both μ and κ chains. In contrast, incubation with DxS leads to the expression of μ only. As these results are based on immunofluorescence studies which may have failed to detect a low level of κ, additional experiments using more sensitive serological analysis of radiolabeled cell lysates are in progress. LPS and DxS also differ in their effect on proliferation of 70Z/3 cells. Even low levels of LPS inhibit cell growth and eventually lead to cell death whereas 70Z/3 cells can be continuously cultured in the presence of DxS. Characterization of the Ig synthesized by 70Z/3 cells reveals free heavy and light chains and 7S IgM whose heavy and light chains are noncovalently associated. The Ig is hydrophobic as demonstrated by SDS binding properties evident in charge-shift electrophoresis. We have been unable to detect secretion of Ig by 70Z/3 cells either before or after LPS induction. Thus, this cell line provides a model not only for the characterization of pre-B cells but also for assessing the differential effects of B cell mitogens and for studying the synthesis and assembly of Ig in early B cells.
Supported by NIH Grant AI-12741 and NSF Grant PCM 75-19734.

728 MONOCLONAL VS POLYCLONAL STIMULATION BY B-1355 POLYGLUCAN, Clara Bell, The Memorial Sloan-Kettering Cancer Ctr, New York,NY 10021.



Polyglucan B-1355, containing α-1,3(35%) & α-1,6(57%) glucosyl linkages, possesses both antigenic and mitogenic moieties. Differences in the serum & PFC antibody (Ab) responses to the α-1,3 epitope have been noted in genetically distinct strains of mice (Fig. 1). Strains of which BALB/c,Ig-1^{a1}, is a prototype showed a rapid, high magnitude response of the λ class & those of which C57Bl/6,Ig-1^{b}, is a prototype, a slow, low magnitude response in the κ class. The λ Ab response was shown to be controlled by a C_H-Ig-1^{a1}-linked autosomal dominant V_H-gene(DEX^+) & by an unlinked $V_{\lambda 1}$-gene, the κ Ab response, being under a separate control. Both the λ & the κ Ab responses matured relatively late in ontogenic development. In contrast, no differences in the DNA proliferative responses have been noted in the two genetically distinct anti-α-1,3 dextran Ab prototype responders and no drastic maturational differences were observed. This suggest that subsets reactive with B-1355 are not all amenable to stimulation to monoclonal Ab production , reactivity and stimulatability being under apparent separate controls. (Recipient of USPHS Career Develop.Award AI 70235. Sup. by SKI Funds for cancer study)

729 REGULATION OF IDIOTYPIC (Id) EXPRESSION OF ANTI-α-1,3 DEXTRAN (dex) ANTIBODIES (Ab), Clara Bell, The Memorial Sloan Kettering Cancer Ctr, New York, NY 10021.

Inbred, Ig-1^{a1} strains of mice, of which BALB/c is a prototype respond to polyglucan B-1355 containing relative high percentages of the α-1,3 glucosyl linkage (37%) with a rapid, high magnitude response in the λ class. The response appears to be controlled by a C_H-Ig-1^{a1}-linked autosomal dominant V_H-gene (DEX^+) and by an unlinked $V_{\lambda 1}$-gene and to be Id related to 3 α-1,3 dex-binding BALB/c myelomas (MP). The cross-reactive, IdX,-104 E-J-558 - determinants shared by the 3 MP are dominant in the primary and secondary anti-α-1,3 dex response of BALB/c prototypes. The individual, IdI,-104 E & J-558- determinants shared by these MP are minimally expressed in the secondary response. The majority of the adult primary splenic B-precursors responsive to this epitope are of the IdX clonotypes; a low frequency of the IdX,IdI clonotype. Day 1-3 BALB/c neonates possess neither IdX nor IdX,IdI precursors. They contain progenitors which can however be abrogated from expressing the IdX-linked IdI clonotype during ontogenic development, by neonatal suppression with anti-IdI Ab. In this study pretreatment with anti-IdX Ab abrogated the appearance of the IdX- & IdX,IdI- associated with the λ Ab to α-1,3 dex elicited by subsequent B-1355 hyperimmunization, enabling the emergence of the IdX^-, IdI^-clone.

Since the IdX clone is also dominant in BALB/c nu/nu mice it was not clear whether abrogation of the IdX clone is governed by Id-related T suppresors reactive with complementary B surface structures, B-IdX- reactive clones or regulatory elements governing differentiation of individual Id B-clones. An adoptive transfer of the IdX-suppressed state was obtained by reconstituting adult irradiated syngeneic recipients with the anti-IdX suppressed BALB/c B-1355 hyperimmunized splenocytes B+T. (Rec. USPHS Career Develop. Award AI 70235 & SKI Cancer Funds.

730 EVOLUTION AND MITOGEN INDUCED LYMPHOCYTE ACTIVATION, Richard K. Wright and Edwin L. Cooper, University of California, Los Angeles, CA 90024

Lymphocyte activation has been analyzed using the plant lectin Concanavalin A (Con A). As a mitogen, Con A induces mitosis in lymphocytes after recognition and interaction between the lectin and cell surface oligosaccharides composed of α-gluco- and α-mannopyranosyl residues. Mitogenesis is inhibited after adding these sugars during the first 18-20 hrs of culture; once activated, lymphocyte responses cannot be inhibited. Activation of small and medium lymphocytes causes their morphological transformation into lymphoblasts with uropods, euchromatic nuclei, a distinct nucleolus, a well developed Golgi apparatus and various membrane bound bodies. Ribosomes of lymphoblasts are no longer free but aggregated, forming polysomes. Lymphocyte mitogenesis is also sensitive to temperature changes. Mitogen responses can be enhanced if lymphocytes are pre-incubated at low temperatures and then shifted to normal temperatures. At low temperatures, activation does not occur. In contrast, lymphocyte activation at normal temperatures followed by shifts to low temperatures inhibits mitosis. All of these results, using leopard frog splenic lymphocytes, confirm homology with those of mice, rats and humans. Thus, many biological characteristics of vertebrate lymphocytes developed early and have been conserved phylogenetically since the first terrestrial tetrapods, the amphibians, evolved 350 million years ago during the Devonian period. Supported by NIH Grant HD 09333.

731 THE USE OF ANTIGEN AND POLYCLONAL ACTIVATORS TO GENERATE ANTI-ARS PRODUCING HYBRIDS Stella M. Robertson, J. Donald Capra, and John R. Kettman. Southwestern Medical School, Dallas, TX 75235

Hybrid cells producing anti-ARS (ā-ARS) antibodies of four isotypes were generated by the fusion of A/J spleen cells with MPC-11 myeloma cells. Spleen cells from mice hyperimmunized to *p*-azophenylarsonate-KLH (ARS-KLH) were cultured for 4 and 7 days in the presence of dextran sulfate and LPS with or without ARS-KLH. Both cultured and uncultured cells were hybridized with MPC-11 cells using PEG. After 3 to 6 weeks the wells were scored for anti-ARS binding activity in a solid phase radioimmunoassay. Hybrids derived from uncultured A/J cells produced ā-ARS Ab in 11% of the wells plated. No ā-ARS hybrids were detected from fusions of non-mitogen treated cultured spleen cells. The frequency of ā-ARS hybrid wells produced from fusions of mitogen stimulated cells was 3-5% and the frequency of fusions from mitogen + antigen stimulated cells was between 10-33%. Of the ā-ARS hybrid wells, the ten which grew successfully were all derived from mitogen or mitogen + antigen stimulated spleen cells. All hybrids displayed the parent isotype (γ2b) plus an additional isotype, with a preponderance for μ. Upon subsequent transfer ā-ARS hybrids were identified which produce only one type of heavy chain (by gel diffusion, μ, γ2b, γ3, γ1). Other hybrids are possibly double or triple producers. The two hybrids which appear to be secreting molecules which bear the ā-ARS Ab cross-reacting idiotype are under study. Data from analysis of the A/J spleen cell populations used for hybridization indicate that the cells which were successfully hybridized and grown may have originated from a population of blast cells which were antigen sensitive but were not ā-ARS antibody secreting cells.

732 T CELL SUPPRESSION OF LPS-DRIVEN IgM SYNTHESIS, Deborah B. Cleveland and Paul K. Pattengale, University of Southern California, Los Angeles, CA 90033

An *in vitro* culture and assay system was designed to measure lipopolysaccharide (LPS)-driven IgM synthesis using a modified Jerne hemolytic plaque assay with Protein A-conjugated sheep red blood cells (SRBCs). This technique, as originally described by Gronowicz et al (Eur J Immunol 6:588,1976), allows for the enumeration of class-specific antibody-secreting B cells. It was found that 10^6 cultured Balb/c spleen cells stimulated with a mitogenically optimal dose of 50μg of LPS produced an average of 15,460 IgM-secreting B cells after 72 hours of incubation (average of 10 experiments). This contrasted to non-mitogen-treated controls which produced a background level of 1030 IgM-secreting B cells per 10^6 cultured spleen cells. When syngeneic Balb/c spleen cells preincubated for 24 hours with 2μg/ml of Concanavalin A were co-cultured with equal numbers of LPS-stimulated indicator spleen cells for 72 hours, significant suppression in the numbers of IgM-secreting B cells was seen. For 10 co-culture experiments an average of 80% suppression was observed (ie, approximately 3100 IgM plaque-forming cells per 10^6 spleen cells). Percent suppression was determined by comparing the plaque-forming cell responses of LPS-stimulated co-cultures after addition of Con A-treated spleen cells (ie, 2μg/ml Con A) to those LPS-stimulated co-cultures after addition of Con A-untreated spleen cells (ie, 0 Con A). Further studies showed that Con A-generated suppression was found to be sensitive to treatment with anti-T cell sera and complement. Preliminary experiments show that Con A-induced suppressor T cells are dependent upon the presence of cooperating T cells in the LPS-stimulated B cell indicator system for the mediation of suppression. In contrast, the LPS-driven IgM response is not dependent on the presence of cooperating T cells.

733 VARIOUS MECHANISMS OF NON-SPECIFIC B CELL ACTIVATION. Yvonne J. Rosenberg and Jacques M. Chiller, National Institute of Allergy and Infectious Diseases, Bethesda, MD. 20014

Using a reverse plaque-forming cell (PFC) assay which detects total Ig secreting cells or those secreting Ig of one particular class, regardless of specificity, in vivo experiments have been done to study different mechanisms which may result in non-specific activation of B cells. It is shown that the isotype of the induced PFC clearly reflects the cellular requirements for stimulation of B cells in the different cases. For example, it has been long known that mitogens e.g. LPS, which require only minimal signals from accesory cells results in early increases in PFC restricted to the IgM class. It is now shown that polyclonal B cell activation can occur via two different T-cell dependent mechanisms. First, during malaria infections, a marked B cell activation occurs. Analysis of this response reveals that all PFC classes increase in a parallel fashion suggesting a role of T cells derived lymphokines which act non-specifically on all B cell precursors. Reinfection of mice preciously given malaria however results in preferential activation of IgG classes. The second form of non-specific B cell activation occurs following either immunization with classical T-dependent antigens e.g. sheep erythrocytes, agg. human gamma globulin, or heterologous serum treatment and requires the participation of T helper cells specific for inducing antigen. In this case, the non-specific increases are class restricted, responses occuring predominantly in the IgG (particularly IgG1) class and, in one case, also IgA. The latter increases are compatible with the existence of a second Ig-specific helper mechanism.

734 ENHANCED THYMIDINE INCORPORATION BY MURINE SPLEEN CELLS CULTIVATED *IN VITRO* IN PRESENCE OF A SYNTHETIC IMMUNOADJUVANT (N-ACETYL-MURAMYL-L-ALANYL-D-ISOGLUTAMINE) AND VARIOUS ANALOGS. C.Damais, M.Parant and L.Chedid. Immunothérapie Expérimentale, Institut Pasteur, 75015 Paris, France.

A part of the bacterial peptidoglycan monomer which was obtained by synthesis (N-acetyl-muramyl L-alanyl-D-isoglutamine) referred to as muramyl dipeptide or MDP, has been shown to be the minimal structure required for duplicating the activity of Mycobacteria in Freund's Complete Adjuvant. Several synthetic analogs of this molecule are equally adjuvant-active even when administered with the antigen in an aqueous solution *in vivo* or *in vitro*. MDP incubated with mouse spleen cells stimulates the incorporation of ^{3}H-thymidine and increases the number of blast cells. This non-specific *in vitro* activity is well demonstrated with cells from high-responder mouse strains (DBA/2, Balb/c) whereas lymphocytes from C57Bl/6 or C3H responded weakly. Amongst the analogs of MDP only these which are adjuvant-active *in vivo* (increase of humoral antibodies) are capable of enhancing the thymidine incorporation by spleen cells *in vitro*; on the other hand, after polymerization of an inactive analog ,the paraaminophenyl MDP, the two properties can be separated : the oligomer is not adjuvant-active but is able to stimulate thymidine uptake even by spleen cells from an MDP low-responder strain.
MDP stimulates B-lymphocytes since enhanced incorporation is observed with splenocytes from nude mice or with B-enriched population obtained with various techniques whereas no enhancement is shown with thymocytes and T-cells. Macrophages which seem to be the target cells of MDP in other test systems do not play a role in thymidine uptake.

Cell-Cell Recognition and Regulation

735 CONTINUOUSLY PROLIFERATING ALLOSPECIFIC T CELLS. I. DEMONSTRATION AND SPECIFICITY OF HELPER FUNCTION IN THE POSITIVE ALLOGENEIC EFFECT TO SHEEP RED BLOOD CELLS.
J. Douglas Waterfield, Gunther Dennert*, Susan L. Swain, and Richard W. Dutton. Dept. of Biology, Univ. of California, San Diego, La Jolla, CA 92093 and *Salk Institute, La Jolla, CA 92037

Allospecific mouse T cells, positively selected in one-way mixed lymphocyte culture were maintained for three years in tissue culture by sequential restimulation. Such proliferating T cells were tested for their ability to induce a positive allogeneic effect: activating an *in vitro* primary humoral response to sheep erythrocytes. It was found that such T lymphocytes could function as helper cells. Helper activity was shown to be specific in that the B cells activated had to share MHC antigens with the strain used for selection of the cell line. Intra-H-2 mapping showed that genes in the K, IA, and possibly IB subregion(s) played an important role in coding for the determinants recognized in induction of the positive allogeneic effect. Supernatant factors from this cell line could substitute for the T cells in activation of the *in vitro* humoral response. However, such supernatants exhibited no strain specificity. Therefore, the genetic restrictions governing the positive allogeneic effect are a consequence of the alloantigenic recognition receptors intrinsic to the helper T cells and not to any biologically restricting properties of the allogeneic effect factor itself.

736 IMMUNE SUPPRESSION *IN VIVO* WITH FOWL GAMMA GLOBULIN-MODIFIED SYNGENEIC CELLS, David H. Sherr, Baruj Benacerraf and Martin E. Dorf, Harvard Medical School, Boston, MA 02115

Intravenous administration of syngeneic spleen cells coupled with the palmitoyl derivative of fowl gamma globulin (p-FγG) results in a profound state of FγG-specific tolerance in C57BL/6 mice. Administration of p-FγG coupled syngeneic cells specifically reduces both the primary and secondary hapten and carrier specific PFC response to TNP-FγG. Since the haptenic response is affected, the tolerance functions at the level of the FγG specific helper T cell. As few as 10^3 p-FγG spleen cells carrying only 1 ng of p-FγG can induce tolerance. At least a 2 day induction period is required. This nonresponsiveness is long lived, lasting over 120 days. Tolerance can be induced with X-irradiated cells coupled with p-FγG or with the membrane fraction of sonicated p-FγG coupled spleen cells. Spleen cells from tolerized mice can transfer suppression to normal syngeneic recipients. Treatment of tolerant spleens with anti-Thy 1.2 antiserum + C eliminates the suppressor cell activity. In addition, thymocytes and purified splenic T cells from tolerized mice can transfer suppression to normal recipients. Thus, at least a component of this nonresponsiveness is mediated by suppressor T cells. The data suggest that this method of inducing T-cell nonresponsiveness is generally applicable.

737 HELPER T CELLS WHICH RECOGNIZE H-2 DETERMINANTS ON ACCESSORY CELLS DO NOT RECOGNIZE H-2 DETERMINANTS ON B CELLS, Alfred Singer, Karen Hathcock, and Richard Hodes, NIH, National Cancer Institute, Bethesda, MD 20014.

$(AxB)F_1 \rightarrow P_A$ chimera helper T cells for PFC responses to TNP-KLH are able to recognize and cooperate with accessory cells for P_A but not P_B. In vitro and in vivo experiments were performed to assess the existence of a similar requirement for helper T cell recognition of P_A H-2 determinants on B cells. In vitro experiments were performed in which unprimed spleen cells were separated into functionally distinct populations of T cells, B cells, and accessory cells. In such experiments it was observed that $(AxB)F_1 \rightarrow P_A$ chimera helper T cells only cooperated with P_A accessory cells, but that in the presence of P_A accessory cells, cooperated with B cells from either P_A or P_B. In vivo adoptive transfer experiments also demonstrated that $(AxB)F_1 \rightarrow P_A$ T cells, which were unable to cooperate with (B+accessory) cells from P_B, did cooperate with P_B B cells upon the addition of P_A accessory cells. It is concluded that (1) although a strict requirement exists for helper T cell recognition of H-2 determinants expressed on accessory cells, no such requirement exists for T cell recognition of H-2 determinants on B cells and (2) there is no requirement for helper T cell recognition of the identical determinants on both accessory cells and B cells.

738 HELPER T CELLS HAVING I-J SUBREGION GENE PRODUCT. - Ko Okumura, Makoto Nonaka, Kyoko Hayakawa and Tomio Tada, Department of Immunology, Faculty of Medicine, University of Tokyo, Tokyo, Japan.

We have recently reported that some but not all carrier-specific Lyt-1^+ helper T cells express determinants controlled by a locus mapped I-J subregion of the mouse major histocompatibility complex. This locus has been shown to be a separate one from Ia-4 locus which is expressed on Lyt-2^+,3^+ suppressor T cells. Ia^+ helper T cell (Th_2) showed a number of different characteristics from those of conventional Ia^- helper T cell (Th_1). Whereas Th_1 can cooperate with hapten-primed B cells only via the cognate or linked recognition of the carrier-hapten conjugate, Th_2 can do so upon stimulation with unlinked carrier and haptenic determinants. Th_2 has been shown to be extremely sensitive to ionizing radiation contrasting to the relative radioresistance of Th_1. Th_2 is unable to cooperate with B cells having low Ia-antigen, whilst Th_1 can stimulate B cells that are resistant to anti-Ia treatment. The avidity distribution of anti-hapten antibody-secreting cells with the Th_1 help has a shift toward the higher avidity than that with the Th_2 help. The effect of Th_2 is directly suppressible by carrier-specific suppressive T cell factor, whereas Th_1 effect is not. These results indicate that the mode of help given by Ia^+ and Ia^- helper T cells is different.

739 DOMINANT VERSUS RECESSIVE INHERITANCE OF HIGH RESPONSIVENESS IN THE IMMUNE RESPONSE TO TNP-MSA, Linda S. Wicker, Walter J. Urba and William H. Hildemann, Department of Microbiology & Immunology, University of California, Los Angeles, CA 90024

The thymus-dependent antibody response to trinitrophenyl conjugated mouse serum albumin (TNP-MSA) in inbred strains of mice is regulated by a gene(s) in the I-B subregion of the H-2 complex. This well-defined system has now been analyzed by radioimmunoassay, isoelectric focusing and detection of TNP-antibody producing cells in a hemolytic plaque assay. The parathymic nodes of high ($H\text{-}2^{b,d,o2}$) and low ($H\text{-}2^{a,k}$) responder mice after secondary immunization contained equivalent numbers of IgM TNP-specific plaques ($0\text{-}50/10^6$) while high responder mice made substantially more IgG plaques ($200\text{-}1500/10^6$) than low responders ($0\text{-}100/10^6$). A possible role for non-H-2 linked genes was investigated by examining the anti-TNP responses of mice of different non-H-2 backgrounds but identical H-2 haplotypes. No substantial background effects were observed among C3H, A, and B10 sources as all H-2 identical mice produced similar numbers of plaques regardless of background. Low X high responder F1 hybrids on the C3H and B10 backgrounds were examined for inheritance of responsiveness to TNP-MSA. $H\text{-}2^{b/a}$ and $H\text{-}2^{b/k}$ F1 hybrids were low responders, whereas $H\text{-}2^{d/a}$ and $H\text{-}2^{d/k}$ mice were indistinguishable from the high responder ($H\text{-}2^{d}$) parent. This finding of either recessive or dominant inheritance of high responsiveness depending on the H-2 haplotype combination suggests that allelic interaction in F1 heterozygotes is decisive. The parental Ir alleles or their products do not act independently.

740 HAPTEN-SPECIFIC DELAYED-TYPE HYPERSENSITIVITY (DTH) RESPONSE, Hiroshi Yamamoto and David H. Katz, Scripps Clinic and Research Foundation, La Jolla, CA 92037

Hapten (phosphorylcholine, PC) specific delayed-type hypersensitivity responses could be induced in BALB/c mice by immunization with syngeneic peritoneal exudate cells (PEC) coupled with diazotized phenyl-phosphorylcholine which is an active derivative of PC. T-15 is a well-known idiotypic determinant of anti-PC antibody in BALB/c mice. Anti T-15 idiotypic antiserum was raised in A/J mice by immunization with TEPC-15 myeloma protein of BALB/c origin. Repeated injections of this antiserum, both before and after sensitization, into recipient BALB/c mice could inhibit PC-specific delayed-type hypersensitivity responses. The significance of these observations to concepts of shared T-15 idiotypic determinants on T and B lymphocytes will be discussed.

741 T CELL HELPER REQUIREMENTS FOR IDIOTYPE EXPRESSION, Robert T. Woodland and Harvey Cantor, Harvard Medical School-Farber Cancer Institute, Boston, MA 02115

A/J mice immunized with the hapten azophenylarsonate (Ar) on a variety of carriers produce anti-Ar antibodies; 20-70% bear a cross-reactive idiotype (Id). We have used this system to test the premise that the expression of predominant or "germ line" idiotypes is not simply a reflection of B cell precursor frequency but is governed, to a large degree, by special regulatory T cell influences. The induction of Id^+ anti-Ar antibody was found to require the participation of two distinct T helper cell populations: one carrying receptors specific for protein or "carrier" determinants and a second bearing receptors for Id (Id-specific helper T cells). Reconstitution experiments suggest that Id-specific T helpers are found in T cell populations prepared from animals that have not been exposed to the Ar hapten, and do not contain detectable levels of Id in their serum. Studies will be discussed which bear on the requirements for the induction, expression and surface phenotype of idiotype-specific T helper cells, and their effect on B cells at different stages of development.

742 COLLABORATION BETWEEN FULLY ALLOGENEIC T AND B CELLS IN THE PRIMARY ANTIBODY RESPONSE TO SHEEP RED CELLS IN *IN VIVO* AND *IN VITRO*, Jose R. Corvalan and Jonathan C. Howard, Agricultural Research Council, Inst. of Animal Physiology, Babraham, Cambridge CB2 4AT, U.K.

We attempted to reconcile the apparently contradictory findings of Herber-Katz and Wilson in rats (J. EXP. MED. 142:928) and Sprent and von Boehmer in mice (J. Exp. Med. 144: 617) on the question whether appropriately negatively selected T cells can collaborate with fully allogeneic B cells in a primary antibody response to sheep red blood cells (SRBC) and found that the difference lies in the choice of assay systems. We find normal collaboration *in vitro* and none *in vivo*.

We depleted specific T help from rat thoracic duct lymphocyte populations by passage through irradiated syngeneic recipients previously injected with large doses of SRBC. Such specifically depleted populations collaborated with syngeneic B cells *in vitro* but not *in vivo* when transferred into F_1 hybrids. Similarly, negative selection through allogeneic animals left a T cell population able to collaborate with allogeneic B cells *in vitro* but not *in vivo*. T cell populations doubly selected against SRBC and alloantigen collaborated normally with both syngeneic and allogeneic B cells *in vitro* and with neither *in vivo*. Preliminary experiments with mouse thoracic duct T cells and splenic B cells suggest a similar lack of specificity at the T cell level *in vitro*.

We conclude that antigen-specific induction of T help in both rats and mice normally entails a marked prejudice against collaboration with fully allogeneic B cells, and that T cell help in primary *in vitro* antibody responses may lack significant antigen specificity. (Supported by grants Medical Research Council G.975/871/C and Nat. Inst. of Health AI 13162.)

743 IN VITRO AND IN VIVO INDUCTION OF EFFECTOR T-CELLS MEDIATING SPECIFIC DTH RESPONSES. Zelig Eshhar, Gideon Strassman and Edna Mozes. The Weizmann Institute of Science, Rehovot, Israel.

In order to get a better insight into the genetic and molecular mechanism of the cellular interactions and the antigen recognition involved in the activation of DTH effector T-cells we have developed an in vitro system for the induction of effector T-cells. Low doses of protein antigens as well as the synthetic polypeptide (T,G)-A--L were bound to splenic macrophages and served as immunogens for the in vitro sensitization of lymphocytes. Such lymphocytes differentiate into effector T-cells capable of mediating DTH responses as monitored by adoptive transfer into recipient mice. The in vitro system was found to be as efficient as an in vivo system where we activated thymocytes in irradiated recipients. In both systems we could also induce specific helper T-cells. The helper effect was manifested by the ability of the educated cells to collaborate with B cells in the production of specific antibodies when adoptively transferred to irradiated recipients. Genetic analysis of the capacity of educated T-cells from various strains to respond to (T,G) A--L suggested that the effector DTH cells is controlled by the same Ir genes as the helper T-cells.

744 CHARACTERIZATION OF SUPPRESSOR T CELLS GENERATED IN CARRIER-PRIMED MICE PRETREATED WITH ANTI-IDIOTYPE ANTIBODIES. Kim Bottomly and Donald E. Mosier, NIAID, NIH, Bethesda, Md. 20041 and ICR, Fox Chase Cancer Center, Philadelphia, PA.

The adoptive secondary anti-phosphorylcholine (PC) response to PC-KLH requires KLH-primed T cells and is inhibited by pretreatment of the T cell donors with anti-idiotype antibodies In BALB/c mice, where the response to PC is dominated by the T15 idiotype, administration of rabbit anti-T15 idiotypic antibodies prior to KLH-priming results in the generation of Lyl^-, $Ly2^+$ suppressor T cells which suppress the anti-PC plaque-forming cell response without expression of alternate idiotypes. We examined the specificity of suppression and found that suppressor T cells generated by anti-T15 pretreatment and KLH-priming will only suppress the response to the appropriate hapten (PC) on the homologous carrier, KLH. The suppressor T cells fail to interfere both with the antibody response to PC on a heterologous carrier and with the response to a different hapten (TNP) on the same carrier, KLH. Preliminary experiments indicate, however, that T cells from anti-T15 pretreated KLH-primed donors will suppress a response to PC on a heterologous carrier if the cells are also exposed to unconjugated homologous carrier. These findings suggest that the activation of the suppressor cell population during the in vivo assay requires recognition of both hapten or idiotype and the appropriate carrier, and thus mimicks the conditions under which these cells were originally primed. Possibly this restriction is due to the existence of two interacting populations of suppressor cells, one specific for carrier and the other specific for hapten or idiotype. If both are challenged during the adoptive transfer, the efficiency of suppression may be greatly enhanced.

745 CELL-CELL INTERACTIONS IN THE T-CELL PROLIFERATIVE RESPONSE, Harley Y. Tse, Ronald H. Schwartz and William E. Paul, Laboratory of Immunology, NIH, Bethesda, MD 20014.

T cells are not only functionally heterogeneous, but in many instances, a given measureable response also requires the interaction of more than one T cell subset. In the T-cell proliferative response, it has been firmly established that antigen-specific T cell activation signals are provided by a Mϕ-like antigen-presenting cell. However, the number of responding T cell subpopulations involved has not been clearly delineated. We addressed this problem by employing the mathematical model used by Mosier and Coppleson (PNAS 61,542 (1968)) to demonstrate the interaction of T, B and accessory cells in antibody responses. In essence, if one plots the logarithm of the response against the logarithm of the cell dose, the slope of the resulting straight line indicates the number of interacting populations required to give the response. Thus, antigen-primed lymph node cells were passed through nylon wool and cultured at several different concentrations. The proliferative responses were measured four days later by monitoring labeled thymidine incorporation. A log-log plot gave a slope of 3, indicating three interacting populations present at limiting concentrations. The slope was not changed when normal nylon wool-passed thymocytes were used as fillers to keep the total cell number in each well constant. However, the use as fillers of either normal irradiated spleen cells OR lymph node cells primed to another antigen converted the slope to 2, indicating that one of the interacting populations provided by the filler population was now present in excess of the other two. Furthermore, the slope became 1 when BOTH the above filler populations were present in the same well. Therefore, it is likely that the cell types provided by spleen and lymph node filler populations are different. The identity of the interacting cells will be discussed.

746 POSSIBLE IDIOTYPIC RESTRICTIONS BETWEEN T SUPPRESSOR AND B CELLS OF THE SAME SPECIFICITY, Clifford J. Bellone and Yael G. Alevy, St. Louis University School of Medicine, St. Louis, MO 63104

The anti-trimethylammonium (TMA) response in A/J mice is characterized by a major cross-reactive idiotype (CRI) which is linked to the Ig-1^e allotype. This finding makes it attractive to look for a CRI on T cells reactive to the same TMA determinant. Thus a suppressor T cell (T_s) assay specific for L-tyrosine-p-azophenyl-TMA, tyr(TMA), was developed.

A/J mice were primed with either tyr(TMA) in CFA, tyr(ABA) in CFA or with CFA alone. Six weeks later all mice were inoculated with TMA_9BSA in CFA, boosted with soluble TMA_9BSA three weeks later, and plaqued 7 days after the soluble boost. Priming with tyr(TMA) in CFA resulted in 70% suppression of anti-TMA PFC as compared to control groups primed with tyr(ABA) in CFA or CFA alone. The suppression was shown to be mediated by T_s cells as only T cells but not B cells from suppressed animals transfer the suppression in adoptive cell transfer into lethally irradiated recipients. The profile of the anti-TMA PFC in the suppressed and non-suppressed animals was examined via incorporation of anti-idiotypic sera (specific for CRI-TMA) into the plaquing medium. The results of these experiments indicate that the suppression of the major CRI^+-TMA PFC was virtually complete while the CRI^--TMA PFC are left intact.

When A/J mice are primed with anti-idiotypic sera or normal rabbit serum (NRS) rather than with the antigen or CFA alone, and the same protocol is followed thereafter, the anti Id inoculated mice are suppressed by 63% when compared with the NRS primed controls.

The possibility that the antigen-induced idiotype suppression may result from idiotypic restrictions between interacting CRI^+-T_s and CRI^+-B cells will be discussed.

747 MURINE MODEL OF PROFOUND B-LYMPHOCYTE IMMUNE DEFECT. James J. Mond, C. Bona, S. Kessler, I. Scher, and W.E. Paul. USUHS, NMRI, NIH, Bethesda, MD 20014

Analysis of mice with genetically determined immune defects have provided valuable insights into the heterogeneity and function of normal lymphocytes. We report here breeding experiments which have resulted in a new, and potentially powerful, immune deficiency model. Female CBA/N mice, which have a discrete X-linked B lymphocyte defect, were mated with male C3H/HeJ mice, which have an autosomal defect which precludes responsiveness to LPS. F_1 female mice were then crossed to male C3H/HeJ mice to yield backcross-1 (BC.1) mice. Approximately half of the male mice of this type displayed a profound immune defect characterized by unresponsiveness to thymus-independent (TI) antigens, including both TI-1 and TI-2 antigens, and failure to proliferate in vitro to LPS, and Nocardia water soluble mitogen. These defects are more profound than those of either parent; the CBA/N is unresponsive to TI-2 antigens, but gives good responses to TI-1 antigens and to B cell mitogens, while the C3H/HeJ mouse responds well to TI-1 and TI-2 antigens and gives good proliferative responses to B cell mitogens other than LPS. Spleen cells of defective BC.1 male mice have approximately 30% Ig^+ lymphocytes. These B lymphocytes have an exceptionally abnormal ratio of SIgD:sIgM (0.27) compared to that of CBA/N (0.56) or C3H/HeJ (1.25). Despite these abnormalities, defective BC.1 male mice give good responses to TNP-KLH. BC.1 female mice and the "non-defective" BC.1 male mice give essentially normal responses to all TI antigens tested and do B cell mitogens.

748 REGULATION OF ANTIBODY RESPONSES BY FEEDBACK SUPPRESSION. Diane D. Eardley, Harvey Cantor, and Richard K. Gershon. Laboratory of Cellular Immunology, Howard Hughes Medical Institute, Pathology Dept., Yale Medical School, and the Sidney Farber Cancer Institute, Harvard Medical School.

Feedback suppression is a mechanism for preventing or shutting-off antibody synthesis by B cells and is mediated by a network of T cell interactions. We have identified a circuit in which an immune inducer T cell (cell surface phenotype Lyl^+, IJ^+, Qal^+) in concert with another resting non-immune T cell (cell surface phenotype Lyl^+, $2,3^+$, IJ^+, Qal^+) produces potent Ly $2,3^+$ suppressor activity. Increasing the activity of this latter Ly $2,3^+$ cell feedsback and inactivates the suppressor inducer and other cells involved in the primary in vitro antibody response to SRBC. When the two T cells come from mice of different Ig-1 haplotypes of Balb/c congenic mice, efficient induction of this circuit fails to occur. Preliminary genetic mapping studies using BAB-14 mice suggest the relevant determinant governing this interaction is not the allotype marker per se.
Our results indicate that feedback suppression possesses restrictions at the Ig-1 locus. These restrictions appear to be at the level of T cell interactions although we cannot exclude the possible role of B cells. These results are compatible with the notion that there are functional homologies between the Ig-1 and MHC loci in that some interactions of immunologically competent cells require haplotype identity at one or the other (or perhaps both) loci.

Mediators of Cell-Cell Interactions

749 A RECIPROCALLY-ACTING LYMPHOCYTE PROLIFERATION HELPER (RALPH) MEDIATES SYNERGY BETWEEN T CELLS AND B CELLS RESPONDING TO 2-MERCAPTOETHANOL (2-ME), Michael G. Goodman and William O. Weigle, Scripps Clinic and Research Foundation, La Jolla, California 92037
Investigation of the mechanism by which B and T lymphocytes collaborate in the proliferative response to 2-ME as a mitogen was undertaken using cultures of C3H/St spleen cells. Synergistic interaction between these cells was not mediated by the release of a soluble factor into the culture supernatant, but required physical contact between the collaborating cell types. Sonicates prepared from spleen cells which had been activated with optimal concentrations of 2-ME for 24 hr and then washed extensively, stimulated the uptake of $[^3H]TdR$ as well as morphological blast transformation of fresh, unstimulated spleen cells. The active principle was found to reside within the soluble fraction of the activated cells, and to stimulate spleen cells optimally at a ratio of 1 naive cell : 1 activated cell-equivalent. Such factors were generated by B cells as well as by T cells, and each of these evoked a kinetic peak at 48 hr of culture. Biologically active factor was produced by viable but not by nonviable cells incubated with 2-ME, and was non-dialyzable. It could not be induced by the B cell mitogens LPS, Poly IC, or PPD, or by the T cell mitogen Con A. The reciprocally-acting lymphocyte proliferation helper (RALPH) activity induced by 2-ME in T cells activated B cells exclusively, whereas that induced in B cells acted predominantly upon T cells. While this may well be the full extent of such recruitment, it is also conceivable that a chain of reciprocal interactions between T and B cell subsets culminates in the generation of a positive feedback loop which is ultimately responsible for the synergistic response to 2-ME.
Supported by NIH Grants AI-15284 and AI-07007.

750 SOLUBLE SUPPRESSOR FACTOR RECOGNIZES DNP PLUS H-2K AND/OR H-2D ON DNFB-IMMUNE T CELLS. John W. Moorhead, Depts. of Medicine and of Microbiol. and Immunol., Univ. of Colo. Med. Sch., Denver, Colo. 80262.

Lymph node cells from mice tolerized with 2,4-dinitrobenzene sulfonate and then painted with 2,4-dinitrofluorobenzene (DNFB) release a soluble suppressor factor (SSF) when cultured in vitro. This factor suppresses the passive transfer of immunity by DNFB-immune LN cells. SSF is hapten specific and is an I region product. However, for SSF-mediated suppression to occur, the donor of SSF and of the DNFB-immune LN cells must share either the H-2K or H-2D locus of the major histocompatibility complex (MHC). I region compatibility is not required. Thus, acceptor sites for SSF appear to be coded for by genes in the H-2K and H-2D loci. I have investigated the nature of these acceptor sites. It was found that purified immune LN T cells are suppressed by SSF indicating that T cells express the acceptor molecules. Adsorption experiments showed that the only cells capable of adsorbing SSF are DNFB-immune T cells from donors expressing the proper H-2K or H-2D gene products. This adsorption is blocked by pretreating the immune cells with antibodies directed against H-2 antigens or against the hapten DNP but not by antibodies against Ia or θ antigens. In addition, treatment of immune T cells from (AxB)F_1 mice with anti-$H\text{-}2^A$ blocks adsorption of SSF from parent A but not from parent B and vice versa. Collectively, these results indicate that the acceptor molecules on DNFB-immune T cells for SSF are histocompatibility antigens, i.e., products of H-2K and/or H-2D genes, associated with the hapten DNP. Supported by NIH Grant AI-12993.

751 ANTIGEN-SPECIFIC SUPPRESSOR AND HELPER SUBSTANCES OF T-CELLS FOLLOWING THE FEEDING OF ANTIGEN. Jerome A. Mattingly and Byron H. Waksman, Yale University, School of Medicine, New Haven, Connecticut 06510.

The introduction of antigen by the oral route induces a decreased ability of an animal to respond with a delayed-type hypersensitivity reaction or with IgG or IgM to the same antigen upon a later intravenous or intraperitoneal challenge. Using sheep red cells (SRBC) as antigen and inbred Wistar rats, this phenomenon was previously shown to be due to antigen specific suppressor T cells (T_s) (Mattingly, J.A., and B.H. Waksman, J. Immunol. 121:1878, 1978). These T_s migrate from the Peyer's patches to the spleen during the first week of feeding. If the spleen cells of fed rats are cultured for 48 hours with SRBC, the supernatant of these cultures will suppress a primary in vitro SRBC plaque forming cell (PFC) response, while having no effect upon a horse red cell PFC response. Upon Sephadex G-100 fractionation of the 48 hour supernatant, the suppressing substance (T_sF) moves as a single peak with a MW of approximately 60-75,000 d. This fractionation reveals that the same supernatant contains an antigen specific enhancing fraction (T_HF) with a MW of approximately 40-45,000 d. T_sF exerts its maximum effect when added to culture during the first 24 hours while T_HF has its maximum effect when added anytime within the first 60 hrs of a 5 day culture. These findings thus indicate that antigen feeding induces both helper and suppressor T-cells which mediate their activity through release of soluble antigen specific substances, and the suppressor substance is dominant both in vivo and in vitro.

752 TWO T CELL SIGNALS ARE REQUIRED FOR THE B CELL RESPONSE TO SOLUBLE PROTEIN ANTIGENS, Daniel M. Keller, John Kappler and Philippa Marrack, University of Rochester, Rochester, New York 14642

We will present evidence for a two signal model of T cell helper function in the B cell response to the soluble protein antigen TNP-keyhole limpet hemocyanin (TNP-KLH). Our results suggest that antigen-specific and non-specific helper signals may be involved in different stages of the B cell response. Signal 1 appears to be antigen-specific and is required for the initiation of B cell proliferation in response to antigen. It can be provided in culture by KLH-primed T cells, but thus far we have been unable to produce a cell free product which can deliver this signal. Preliminary results suggest that macrophages preincubated with KLH and KLH-primed T cells can provide this activity to cultures. This signal, therefore, may be presented to B cells indirectly via T cell - macrophage interaction.
Signal 2 is present in a 24 hour supernatant of Con A stimulated spleen cells, which suggests that it is non-antigen specific. Both signal 1 and signal 2 are required for a response. Whereas signal 1 must be present from the beginning of the response, signal 2 may be added to cultures as late as 36 hours after initiation of the response.
These results are in contrast to the response of B cells to red blood cell antigens (RBC). In the response to RBC, antigen alone is sufficient for the initiation of the response, and only signal 2 is required thereafter. This non-specific helper signal may be delivered by direct T cell - B cell interaction or by addition of Con A supernatant.

753 A SPECIFIC HELPER FACTOR THAT ENHANCES THE CYTOTOXIC RESPONSE TO A SYNGENEIC TUMOR, Douglas G. Kilburn and Julia G. Levy, Department of Microbiology, University of British Columbia, Vancouver, V6T 1W5, Canada.

Cell free extracts of spleens from mice bearing the mastocytoma P815 contain factors which either enhance or suppress the generation of cytotoxicity to P815 in cultures of normal DBA spleen cells with mitomycin C treated P815. These activities are antigen specific and are usually present simultaneously in an extract although in differing proportions depending on the time after tumor injection. They can be separated on the basis of size by gel filtration. Further purification of the helper factor has been affected by adsorption and elution from columns of P815 membrane fragments linked to Sepharose. This material does not influence an anti-$H2^d$ MLR or the in vitro cytotoxic response of DBA spleen cells to L 1210. It enhances the response to P815 by about 4 fold in terms of cytolytic units at concentrations down to 0.005 spleen equivalents per culture. The properties of this factor are similar to those of helper factors active in the antibody response. It bears I region coded markers but not constant region determinants of mouse Ig. On gels it appears as a monomer of 65000 daltons.

754 ABSENCE OF MEASLES-INDUCED LYMPHOCYTE SUPPRESSION AND INTERFERON PRODUCTION IN MULTIPLE SCLEROSIS, P. Andrew Neighbour and Barry R. Bloom, Albert Einstein College of Medicine, Bronx, NY 10461.

Lymphocytes from normal adult donors (18/32) exposed in vitro to inactivated measles virus were found to exert significant suppression (33.9%) of the Concanavalin A responses of cryopreserved, autochthonous responder cells. In marked contrast, lymphocytes from multiple sclerosis (MS) patients exhibited significantly reduced suppression (1.5%), and 29/36 patients failed to suppress at all. The degree of suppression increased slightly with age, but did not vary with clinical stage of disease. There was no apparent genetic restriction of suppressor activity. While specificity of this phenomenon for measles virus has not yet been definitively established, no differences in the responses of normal or MS patient donors were found with subacute sclerosing panencephalitis,Sendai, distemper, mumps, or influenza viruses.

Supernates of measles-treated lymphocytes from normal donors possessed both suppressive and antiviral activities. Both activities were resistant to pH 2 treatment and were neutralized by an anti-human leukocyte interferon serum, strongly suggesting that interferon, probably type I, was the mediator of suppression. Consistent with their inability to suppress Concanavalin A responses, lymphocytes from MS patients failed to produce significant amounts of interferon in response to measles challenge in vitro. These results extend previous observations that MS patients are unable to respond appropriately to measles virus antigen in vitro.

755 PURIFICATION AND B-CELL TRIGGERING PROPERTIES OF ANTIGEN SPECIFIC AND NONSPECIFIC T-CELL DERIVED HELPER FACTORS. C. Shiozawa, S. Sonik, B. Singh, and E. Diener, Dept. of Immunology & MRC Group on Immunoregulation, Univ. of Alberta, Edmonton, AB Canada.

Soluble carrier specific and nonspecific helper factors extracted from in vivo primed mouse T-cells to Rabbit gamma globulin (RGG), have been purified on Sephadex G-100 and DEAE-cellulose columns. The requirements for B-cell stimulation in vitro by these factors, using monomeric antigen (TNP-RGG) as well as polymeric antigen (TNP-RGG-Ficoll) were established. Allo geneic restriction was observed with antigen specific helper factors but not with nonspecific factors. The specific factors have different antigen dose requirements for triggering of the B-cells, while nonspecific factors, induced at the same time, do not require any antigen.

Specific and nonspecific factors were separated into three fractions, A, B, and C on a Sephadex G-100 column, and were shown to have different isoelectric points. Fractions B and C contained most of the specific helper activity and required a different antigen dosage for optimum stimulation of the B-cells. The factors were also fractionated over a DEAE-cellulose ion exchange column with pH 8 buffer-gradient. Two of five fractions contained antigen-specific activity and were found to have different optimum antigen dosages for triggering. Most of the nonspecific helper activity was not bound by the DEAE-cellulose column under these conditions.

756 MOLECULAR WEIGHT DETERMINATION AND SUBUNIT COMPOSITION OF HELPER T CELL REPLACING FACTOR. Marilyn Thoman and James Watson, Department of Microbiology, University of California, Irvine 92717.

The molecular size and subunit composition of a helper T cell replacing factor (TRF) has been analyzed by polyacrylamide gel electrophoresis of iodinated factor. Purification of the factor from culture supernatants of Concanavalin A-stimulated spleen cells by Sephadex G-100 chromatography revealed active material had a Stokes radius corresponding to a globular protein of 30,000-35,000 daltons molecular weight, and a pI ranging from 4-5 by isoelectric focusing (IEF). Aliquots of the active IEF fractions were pooled and ^{125}I-iodinated. Iodinated factor run on 7.5% native polyacrylamide gels revealed one radioactive peak of approximately 52,000 daltons. Eluted and reelectrophoresed under reducing conditions on SDS-containing gels, this material still demonstrated only a single peak of 68,000 daltons molecular weight.

To determine whether this 68,000 dalton material had T cell replacing activity, IEF-purified TRF was electrophoresed on native gels. Gel slices were eluted and tested for the ability to restore a Con A mitogenic response. The active fractions were iodinated and reelectrophoresed on SDS gels. A 68-70,000 dalton material was present in the active fractions.

These data suggest that a T cell replacing factor (TRF) derived from Con A-stimulated spleen cells appears to be composed of a single polypeptide chain of approximately 68-70,000 daltons.

757 AUTO-REGULATION OF THE GROWTH AND DIFFERENTIATION OF T LYMPHOCYTE, Alan M. Wu[1] and Gerald E. Price[2], Dept. of Anatomy(Histology)[1] and Dept of Medical Biophysics[2], University of Toronto, Toronto, Ontario, Canada.

Differentiation of T lymphocytes is achieved by a series of interactions between differentiating T cells and their regulatory proteins. The regulatory proteins are produced from regulatory cells. To elucidate the mechanisms underlying this cell-factor interaction, a suspension culture system which allows long-term growth of T lymphocytes was used (Morgan et al., Science 193: 1007,1976). This culture system requires T lymphocyte-grwoth stimulatory activity (TL-GSA). TL-GSA are isolated from conditioned medium prepared from pure T lymphocytes stimulated by PHA. They are glycoproteins with a MW of 13,000 which is different from that of thymopoietin and that of granulocyte-macrophage CSA.TL-GSA selectively support the growth of human T cells. After several monthsof grwoth in culture, the cultured cells maintained normal karyotype and were able to form theophylline resistant and 37°C stable non-immune rosettes with sheep erythrocytes. Some cultured cells were able to respondspecifically to allogenic stimulators in a mixed lymphocyte reaction and some able to form T cell colonies in semi-solid medium containing PHA. In addition, some cultured cells were able to help B lymphocyte differentiation and some able to kill cultured human melanoma cells and transplantable mouse mastocytoma cells in vitro. The number of killer cells reached a maximum at the third to rourth week of culture and maintained for several months. In conclusion, these data show that the differentiation of T progenitor cells which give rise to various lineages of functional T lymphocytes are regulated by specific regulatory factors produced from other T lymphocytes.

758 ANTIGEN-SPECIFIC SOLUBLE MEDIATOR OF FEEDBACK SUPPRESSION, Rudolf Zubler, Ronald Germain and Baruj Benacerraf, Harvard Medical School, Dept. of Pathology, Boston, Mass. 02115

Spleen cells educated *in vitro* with sheep red blood cells (SRBC) according to the protocol developed by Eardley and Gershon for induction of feedback suppressor cells have been found to release a soluble suppressor factor (SF) into the culture supernatant. This SF is antigen specific in function, binds to antigen, and is between 50 - 100 x 10^5 daltons. SF equivalent to 10^6 educated cells suppresses both primary IgM and IgG plaque-forming cell responses $\geq$ 80% compared to control SF from cells educated *in vitro* in the absence of SRBC, and significant activity is detectable at 5 - 10 x 10^4 cell equivalents. This soluble material acts efficiently across H-2 barriers, but appears to be at least partially restricted in its action by non-H-2 linked genes. Characterization of the factor itself and of the cell(s) required for its production is currently in progress.

759 BIOCHEMICAL CHARACTERIZATION OF MOLECULE(S) WHICH ACTIVATE A HUMAN MACROPHAGE-DERIVED CONTINUOUS CELL LINE IN VITRO James W. Larrick, Steven J. Anderson and Hillel S. Koren, Div. Immunology, Duke Medical Center, Durham, N.C. 27710

During the course of biochemical studies of major histocompatibility antigens of lymphoblastoid cell lines we failed to detect Ia-like antigens on U937 a recently characterized human monocyte-like cell line (Int. J. Cancer 17: 565, 1976). Because B cell alloantigens have been reported to occur on early myeloid cells, an attempt was made to produce the differentiation and expression of these cell surface molecules. Cells were cultured in medium conditioned by mixed lymphocyte culture (MLC). Although the U937 cells failed to express new Ia antigens they demonstrated remarkable morphological and functional changes. The activated appearance of the cells is accompanied by increased numbers of Fc and complement receptors, marked elevation of antibody-dependent cellular cytotoxicity (ADCC) and phagocytosis. Kinetic studies of the ADCC activity (utilizing several red cell targets as well as nucleated targets such as K562 and HSB) show stimulation of killing as early as 3 hours, a peak after 3-5 days with subsequent decline toward unactivated levels after two weeks. Aggregated ^{125}I-IgG binding and EA rosette formation follow similar rise-plateau-decline kinetics. The molecules mediating activation are active at low concentration--less than 1% MLC conditioned medium produces activation. They are stable at 56°C for 30 minutes and have a MW greater than 100,000 daltons. Preliminary biochemical studies suggest that these molecules differ from other molecules reported to modulate macrophage function such as migration inhibition factor (MIF), macrophage activating factor (MAF) and complement components.

760 PRODUCTION OF HELPER FACTOR BY MURINE T CELLS STIMULATED WITH THE POKEWEED MITOGEN PA-2. T.Y. Basham, S. Toyoshima, F. Finkelman*, and M.J. Waxdal, LI/NIAID/NIH, Bethesda, MD 20014 and *Uniform Services University Medical School, Bethesda, MD 20014.

Pa-2 is a potent mitogen for murine T cells but suppresses murine B cell mitosis and also inhibits immunoglobulin production. We now wish to report that cell free supernatants from murine spleen or thymus cells which have been stimulated by a pulse of Pa-2 contains a B cell stimulating factor(s) (BSF). When added to fresh spleen cell cultures, BSF induced both cell division, as measured by ^{3}H-thymidine incorporation, and an increase in the number of immunoglobulin producing cells, as measured by the reverse plaque assay. BSF appears in the culture supernatant a few hrs after stimulation. Maximal activity is reached at about 12 hrs and no further production is found after 24 hrs of culture. Cell division is not required for BSF production. Loss of activity is sporadic and does not appear to be due to the later activation of suppressor T cells. Pa-2, itself, inhibits the helper activity. BSF appears to be made by T cells as determined by anti-theta plus complement killing and separation of Ig positive and negative cells by FACS. BSF needs to be present in culture for only the first 6 hrs to achieve full stimulation of cell division and non-specific immunoglobulin production.

761 SOLUBLE PRODUCTS FROM STIMULATED LY I T-CELLS TRIGGER ANTIGEN-PRIMED LY 23 T-CELLS TO CELL PROLIFERATION AND CYTOLYTIC ACTIVITY, M. Röllinghoff and H. Wagner, Institute of Medical Microbiology, 6500 Mainz/Germany

Upon polyclonal (Con A), or antigen specific (MLC) stimulation, Ly 1 T-cells release a factor, which in turn triggers alloantigen primed Ly 23 T-cells to proliferation and cytolytic activity. The "secondary T-lymphocyte inducing factor" (SCIF) is produced within 24 hours. For its production no DNA metabolism, but an intact protein metabolism is required. Once induced, the functional activity of SCIF is non specific and not H-2 restricted. SCIF allows exponential growth and long term propagation of cytolytic Ly 23 T-cells with specificity to alloantigens used for primary sensitisation. SCIF induced activation of alloantigen primed Ly 23 T-cells does not require the presence of alloantigens. The results therefore reveal a process by which Ly 1 T-cell derived non-specific factor (s) induce autonomously Ly 23 T-cell mediated, antigen specific, cytotoxic T-lymphocyte (CTL) responses.

Network of Regulation

762 IMMUNOREGULATION OF ANTIGEN-BINDING MYELOMA CELLS BY T CELL DERIVED ANTIGEN-SPECIFIC AND IDIOTYPE-SPECIFIC FACTORS: EFFECT OF SIMULTANEOUS PRESENTATION OF HELPER AND SUPPRESSOR SIGNALS. Howard M. Gebel, James W. Rohrer, and Richard G. Lynch, Washington University, St. Louis, MO 63110

MOPC-315, a TNP binding (IgA,λ_2) myeloma, undergoes progressive differentiation during in vivo growth in diffusion chambers (DC) implanted (IP) into normal BALB/c mice. Differentiation was shown to be regulatable by carrier-specific presentation of TNP to MOPC-315 cells in carrier-primed hosts. Mice that were carrier-primed to induce helper or suppressor activities effected promotion or suppression of MOPC-315 cell differentiation, respectively. Myeloma cell help and suppression can be adoptively transferred to normal mice with distinct subsets of purified T cells from carrier-primed mice. In separate studies DC-enclosed MOPC-315 cells in M315-immunized mice were specifically inhibited from secreting M315 (secretory blockade) and expressing cell surface membrane M315 (sIgA^{315}). Subsequent studies have demonstrated that secretory blockade is dependent on idiotype-specific (Id^{315}) T cells whereas suppression of sIgA^{315} is dependent on anti-Id^{315} antibodies. In the present studies we monitored the growth and differentiation of MOPC-315 cells in Id^{315}-immunized hosts that had also been immunized with a help-inducing dose of carrier. Preliminary results indicate that both Id^{315}-specific suppressing, and carrier-specific promoting immunoregulatory signals are focused onto the target B cells and simultaneously modify their growth and differentiation. These observations imply a dual regulation of the malignant B cell clone by distinct T cell-derived immunoregulators one of which is focused onto the target B cell via the surface TNP-receptor, and the other via idiotypic determinants which are located in the TNP-receptor.

763 POSSIBLE ROLE OF ANTI-RECEPTOR ANTIBODIES IN THE REGULATION OF CONTACT SENSITIVITY TO DNFB IN MICE. Sy, M-S., Moorhead, J. W., and Claman, H. N., Dept. of Microbiol. & Immunol. and Medicine, University of Colo. Med. Center, Denver, Colo. 80262.

When mice are sensitized with optimal doses of 2,4-dinitrofluorobenzene (DNFB), maximum delayed hypersensitivity develops in 4 to 5 days as measured in vivo by ear swelling. The intensity of this reaction declines rapidly, and by 9 days post sensitization significant immunity is no longer detectable. We have investigated this rapid loss of immunity and have found that serum taken from animals 9 to 15 days post sensitization will block the ability of DNFB-immune LN cells to passively transfer immunity. Affinity chromatography studies have revealed that the suppressive molecules are immunoglobulins. These antibodies do not have anti-DNP activity and are not associated with DNP as antigen-antibody complexes. Further studies have shown that the serum is antigen specific, i.e., it blocks DNFB-immune LN cells but not TNCB- or oxazolone-immune cells, and lacks strain specificity in inhibiting passive transfer. The serum can be adsorbed only by DNFB-immune LN cells (both syngeneic and allogeneic), and pretreatment of the DNFB-immune LN cells with an auto-anti-receptor antiserum prepared against DNFB-immune LN cells in adjuvant blocks the adsorption and vice versa. This suggests that the blocking antibodies obtained from optimally sensitized mice recognize the same determinants as the anti-receptor serum. From these results, we conclude that the blocking antibodies possess anti-receptor activity and represent part of the immunoregulatory elements within a complex network of idiotype/anti-idiotype interactions. We believe these antibodies are in part responsible for the transient nature of the contact hypersensitivity reaction. Supported in part by NIH Grants AI-12685 and AI-12993.

764 ROLE OF ANTIGEN-ANTIBODY COMPLEXES IN INDUCING AUTO-ANTIIDIOTYPIC IMMUNITY G. G. B. Klaus, National Institute for Medical Research, London, U.K.

We have shown that antigen-antibody (Ag-Ab) complexes are very effective in inducing auto-antibodies to the idiotype (Id) of M315, a DNP-binding murine myeloma protein (Nature, 272, 265, 1978). Subsequent studies have extended these observations to other idiotypes. In addition, it has been shown that Ag-Ab complexes can induce immunity simultaneously to both Ag and Ab, and that cooperation between anti-idiotypic B cells and antigen-specific T helper cells can generate an anti-Id antibody response via complexes. The possible relevance of these findings to network control of the antibody response will be discussed.

765 ALLOTYPE SUPPRESSION IN THE CHICKEN. Michael J.H. Ratcliffe and Juraj Ivanyi, The Wellcome Research Laboratories, Beckenham, Kent BR3 3BS, England.

In heterozygous (Ml^a/Ml^b Gl^a/Gl^e)B14 line chickens, antibodies directed against the IgMlb allotype produced a suppression of serum IgMlb levels with a compensatory increase in IgMla levels as quantitated by single radial immunodiffusion. This suppression was induced (a) by passive injection of anti-Mlb serum into 13 day old embryonal or neonatal recipients or, (b) by egg-yolk transmitted antibody in chickens hatched from homozygous (Ml^a Gl^a) hens immunised against IgMlb of the homozygous (Ml^b Gl^e) rooster. IgMlb suppression was associated with a concomitant suppression of the genetically linked IgGle and a compensatory increase in IgGla allotype levels. Suppression of the IgMlb allotype could be induced in heterozygotes which inherited the Ml^b allele either maternally or paternally. However there was no suppression of either IgMlb or IgGle levels when (Ml^b Gl^e) homozygous chickens were injected with anti-Mlb. Hence allotype suppression only occurred in Ml heterozygous chickens which had an alternative source of B cells available. Preliminary results indicate that suppression is also demonstrable on a cellular basis, i.e. suppressed chickens had low numbers of surface IgMlb positive cells in the peripheral blood, spleen and bursa as detected by indirect immunofluorescence. This is the first demonstration of allotype suppression in the chicken and its features reveal more similarities with the phenomenon in rabbits than with the murine model.

766 REGULATION OF THE IMMUNE RESPONSE BY AUTO-ANTI-IDIOTYPIC ANTIBODY, A.F.Schrater, E.A. Goidl, G.W. Siskind, & G.J. Thorbecke, NYU Med. Cent. & Cornell Univ. Med. Coll., New York, NY 10016. The immune response to trinitrophenyl-lys-ficoll (TNP-F, 10 μg i.v.) was studied in AKR/J mice by assay of splenic plaque-forming cells (PFC). The response peaked at 150,000 anti-TNP PFC/spleen (day 4) and was only 15,000 PFC on day 7. A decrease in affinity and heterogeneity determined by hapten inhibition (HI) was also noted. To study the regulatory mechanism responsible for the rapid decline in PFC, day 7 immune spleen cells were transferred i.v. with 10 μg TNP-F into normal mice and anti-TNP PFC were assayed 4 days later. Experimental mice had a 40-90% suppression of observed PFC as compared to responses of normal-cell recipients. In the HI assay of PFC from immune-cell recipients, as hapten concentration increased, there was an unexpected rise in observed PFC especially at 10^{-8}-10^{-7} M TNP-ε-amino caproic acid (EACA), which brought the PFC level to values of control mice. It was hypothesized that auto-anti-idiotypic (Id) antibody (ab) on the surface of potential PFC was displaced in vitro by hapten. Thus, the putative anti-Id should be present in day 7 immune sera and also should be elutable from spleen cells of immune-cell recipients by in vitro incubation with 10^{-8} M TNP-EACA. Indeed, hapten reversible stoichiometric inhibition of day 4 anti-TNP PFC was obtained in vitro with immune sera or cell eluates. The inhibitory factor could be adsorbed onto anti-mouse Ig or anti-TNP ab columns but not onto antigen columns. Further studies showed the presence of hapten augmentable PFC after day 7 in intact immune mice. Such PFC were not detected in nude mice nor was sera from nude mice suppressive. We conclude that a T-dependent auto-anti-Id regulation was responsible for the rapid decline in observed PFC during the immune response to TNP-F in AKR/J mice. Supp. AI-05196, AG-00681, CA-20075, & AI-3076.

767 SUPPRESSOR CELL NETWORKS IN MICE TOLERIZED WITH DNP-MODIFIED SYNGENEIC SPLEEN CELLS. Stephen D. Miller and Henry N. Claman, Univ. of Colorado Med. Ctr., Denver, Co. 80262

We investigated the organ distribution, kinetics and mechanism of action of Ts induced by i.v. injection of syngeneic DNP-SC on DNFB contact sensitivity. 4-7 days post tolerization both lymph node (LN) and spleen contained Ts (Ts-I) capable of inhibiting efferent sensitivity as assessed by their ability to block passive transfer of sensitivity by DNFB-immune LNC (T_{DH}). 14 days post tolerization only spleen retained significant suppressor activity as assayed by adoptive transfer, at 21 days both LN and spleen were negative in this regard. These latter Ts (Ts-II) were found not to inhibit T_{DH} in co-transfer experiments, but did block afferent sensitization by inhibiting antigen-induced proliferation in draining LN's of recipient mice. Transfer of 7 day efferent-blocking tolerant LNC (Ts-I) to normal recipients also gave rise to afferent-blocking Ts-II in both the spleen and LN or the recipients. As opposed to Ts-I which are genetically non-restricted, Ts-II were found to be genetically restricted. Ts-II were also found to be antigen-specific and their precursors were sensitive to pretreatment with high doses of cyclophosphamide. Inhibition experiments showed that co-transfer of Ts-II with Ts-I + T_{DH}, prevented Ts-I from suppressing T_{DH}, perhaps indicating an anti-receptor mechanism. A network model is proposed whereby a genetically non-restricted, efferent-blocking Ts-I, gives rise to a genetically restricted, afferent-blocking Ts-II which may play an important role in maintenance of the tolerant state.

768 REGULATION OF A SMALL FAMILY OF V_H GENES, Eric Enghofer, Melvin Bosma and C. P. Glaudemans, The Inst. for Cancer Research, Philadelphia, Pa. 19111 and the National Institute of Arthritis Metabolism, and Digestive Diseases, NIH, Bethesda, Md. 20014.

We recently described a mouse idiotype (U10-173) that is inherited in Mendelian fashion, is closely-linked to C_H allotype genes, is found on IgG, IgA and IgM classes, is representative of ∿1% of normal Ig in U10-173$^+$ strains and appears to correspond to a few V_H germline genes (J. Exp. Med. 146: 1041, 1977 and ICN-UCLA Symp. on Mol. and Cell. Biol. 6: 99, 1977).

Five inbred strains (C3H, C3H·SW·Ig^a (CWA), CBA, PL/J and AKR) do not express detectable U10-173. We asked whether this reflects the absence of U10-173 structural genes or the presence of regulatory mechanisms that prevent U10-173 production? Evidence suggesting the latter is as follows: First, injection of bacterial levan (15 μg) into CWA and its congenic partner, C3H·SW·Ig^b (CWB) elicits comparable levels of anti-levan one week later; CWA anti-levan is U10-173$^-$, whereas virtually all of CWB anti-levan is U10-173$^+$. However, many CWA mice will produce U10-173$^+$ antibodies if they are x-irradiated prior to being stimulated with levan antigen. Second, U10-173 can be produced (60-400 μg/ml) in nude AKR but not in normal AKR mice following levan stimulation.

As U10-173 can be considered the equivalent of a V_H allotype, our results seem relevant to the phenomenon of hidden allotypes; i.e., the transient production of allotypes in animals not thought to contain the corresponding genes. The present results suggest that regulatory T cells may be controlling some allotype polymorphisms rather than allelic structural genes. (This work was supported by USPHS grants AI-13323, CA-04946, CA-06927 and RR-05539 from the National Institutes of Health and by an appropriation from the Commonwealth of Pennsylvania.)

769 COMMON IDIOTYPIC DETERMINANTS DETECTED ON LYSOZYME SPECIFIC SUPPRESSOR CELLS AND ANTIBODIES. Michael A. Harvey*, Luciano Adorini+, Christopher Benjamin, Alexander Miller, and Eli E. Sercarz. University of California, Los Angeles, California 90024.
Most antibodies from any strain of mouse immunized with hen egg-white lysozyme (HEL) are directed against the N-terminal, C-terminal peptide of the molecule (NC residues 1-17:Cys 6-Cys 127:120-129). Immunization of C57BL/10 mice, which are genetically non-responsive to HEL, with HEL, NC, or the N terminal fragment of NC induces T suppressor cells. Anti-idiotypic antisera were raised in the guinea pig against several restricted anti-HEL antibody populations from B10.A mice and adsorbed extensively over normal Ig immunoadsorbents. These sera react with nearly all the anti-HEL produced by B10.A mice as well as antibody from mice representing 7 different Ig-1 loci, but not with antibodies of different specificity or normal Ig. These sera, at least in part, recognize site-related determinants in that they interfere with the binding of idiotype populations to either HEL or NC. In an in vitro anti-HEL system, C57BL/10 T suppressor cells, induced by HEL, can be killed by these anti-idiotypic sera plus rabbit C. Priming with a fragment of HEL which lacks the NC region (L-II, residues 13-105) induces T-helper cells which are unaffected by anti-idiotypic treatment. Thus, these antisera have allowed a partial definition of a cellular network to a multideterminant antigen.
*American Cancer Society Postdoctoral Fellow. +Cancer Research Institute Postdoctoral Fellow.

770 IMMUNOGLOBULIN RELATED SPECIFICITY OF CARRIER PRIMED T CELLS, Maurice Zauderer, Humberto Cosenza, Michael Imperiale, Joseph Sproviero, Columbia University, N.Y., N.Y.
A number of workers have suggested that helper T cells may show specificity for immunoglobulin determinants in their cooperation with B precursors. In recent reports it has been suggested that this is a function of a special subset of T cells distinct from those helper T cells which are carrier specific and H-2 restricted. In a limiting dilution analysis we have obtained evidence for a helper T cell function with immunoglobulin related specificity. We observe that a single limiting carrier primed helper T cell in the presence of excess B cells primed to two independent haptens, phosphorylcholine and dinitrophenol, will cooperate with B precursors to give rise to antibody forming cells of one specificity only. Since the frequency of either hapten specific response remains the same in the presence or absence of the second hapten-carrier conjugate, we believe that this cannot represent competition for the helper function and must reflect a restriction of specificity. We are presently examining whether we can detect in this type of analysis a variable region related specificity among helper T cells which cooperate with T15 idiotype positive or negative B precursors specific for phosphorylcholine.

In the course of these experiments we have noted that under certain conditions we detect a hapten related feedback inhibition. This feedback is hapten related for its induction but non-specific at the effector level. When mice of about seven months of age or older are used as B cell donors such effects are considerably reduced. Because in cell mixing experiments the feedback signal appears to be dominant, it does not seem that lymphoid cells of older mice have lost the receptor for this signal. We are investigating whether the signal might be due to contaminating sub-population of T cells which are eliminated in older mice.

771 IMMUNE LYMPHOCYTES CAPABLE OF TRANSFERRING ALLOTYPE SUPPRESSION CONTAIN CYTOTOXIC T CELLS SPECIFIC FOR ALLOTYPIC DETERMINANTS. H. Ralph Snodgrass, Melvin J. Bosma, and Darcy B. Wilson.
BALB/c (Ig^a) mice immunized with serum immunoglobulin (Ig^b) from C57Bl mice generate T cells which have the capacity to inhibit the production of Ig-1b when transferred to sublethally irradiated C.B-17 mice. C.B-17, an allotype congenic, is a BALB/c mouse with the Ig heavy chain gene complex derived from the C57Bl strain. These suppressor and/or cytotoxic T cells ($T_{c/s}$ cells) are allotype specific since they have no effect on Ig-1a nor Ig-4b. When Ig-1b containing spleen cells are cultured in vitro with irradiated CB101 myeloma cells (CB101 is an Ig-1b producing myeloma derived from C.B-17 strain) cytotoxic T cells are generated which lyse isotopically labeled CB101 targets in a standard 4 hour chromium release assay. These killer cells are specific for Ig-1b in that they do not lyse UPC-10, a BALB/c Ig-1a producing myeloma, nor do they lyse C.B-17 Con A or LPS blasts. Furthermore, the killing of CB101 targets can be blocked with anti-Ig-1b antiserum. These experiments strongly support the idea that in this system the target of the T cells mediating the in vivo Ig-1b suppression may in fact be the allotype bearing B cell. The data also suggests an as yet undiscribed mode of T cell regulation of B cells and may be a reflection of allotype dependent networks.

772 RECIPROCAL EXPANSION OF THE ANTI-IDIOTYPIC CLONE FOLLOWING ANTIGEN STIMULATION OF THE IDIOTYPIC CLONE, G. Kelsoe and J. Cerny, Harvard University, Boston, MA 02115.

Jerne's proposed idiotype network would shape, buffer, and "remember" perturbations of a particular (idiotypic) clone by idiotopic or paratopic interactions with regulatory clones. While much phenotypic diversity exists within the anti-idiotypic clone (e.g., helping or suppressing the idiotypic response), the importance of the network theory lies in its emphasis of the reciprocal relationship that should exist between the clonal members of the network. We demonstrate here reciprocal and periodic expansions of both the idiotypic and anti-idiotypic clones following a single antigenic challenge.

Utilizing the T-15/phosphorylcholine/BALB/c system, we measured changes in the antigen activated T-15 clone (by radio-hapten binding and enumeration of specific PFC) and the anti-idiotypic clone(s) (by specific radio-idiotype binding) over a period of 14 d. following antigen challenge. Cohorts of mice were sequentially immunized with 20 µg of R36a vaccine. Controls were unimmunized mice. On d.14 all mice were killed and their spleen cells assayed as described.

Antigen specific PFC followed a cyclic pattern with peaks on d.5 and 12. Hapten binding curves were identical except their maxima were reached 1d. earlier than the PFC. Serum (idiotypic) antibody titers did not cycle but reached a peak on d.7-8 and thereafter declined. Idiotype binding was also cyclic and out of phase with the PFC curve. Peaks (2-3 fold increases above controls) of idiotype binding occurred on d.3,11, and 14. The close association between the kinetics of these two clones suggest that their cyclic behavior may result from mutual interactions.

773 CELLULAR BASIS OF NETWORK REGULATION OF CELLS SECRETING ANTI-TNP ANTIBODIES CARRYING MOPC460 IDIOTYPE. C. Bona and W.E. Paul, NIAID, NIH, Bethesda, Maryland 20014

An idiotype of the DNP- and TNP-binding myeloma protein MOPC460 was expressed on a small but significant proportion of anti-TNP antibodies after in vivo immunization of BALB/c mice with three T-independent TNP antigens. In vitro experiments show that the fraction of anti-TNP response carrying idiotype of MOPC460 myeloma protein (460 Id) is regulated by 460 Id specific suppressor T cells. The suppressor activity was ablated in BALB/c mice by treatment of 460 Id specific cells with anti Lyt 2.2 antibodies. The Qal phenotype of these 460 Id specific suppressor T cells was studied in (C58j x BALB/c)F_1 mice.

A significant increase in the 460 Id^+ component of the anti-TNP response was observed in BALB/c mice which had been actually immunized with anti-460 Id antibodies or injected with anti (anti 460 Id) antibodies. In these mice, no 460 Id specific suppressor T cells were detected.

Our data indicate that 460 Id specific suppressor T cells which regulate the 460 Id^+ component of anti-TNP response are the target of anti (anti-460 Id) antibodies. Elimination of these cells by anti (anti-460 Id) antibodies leads to an increase in 460 Id^+ component of the anti-TNP response.

774 DIFFERENT SPECIFICITIES OF ISOLOGOUS AND HOMOLOGOUS ANTI-IDIOTYPIC ANTIBODIES, W. Schuler & E. Weiler,University of Konstanz,Konstanz,FRG

Homologous anti-idiotypic antibodies raised in mice of strain A/J against the α-1,3-dextran (α-dex) reactive BALB/c myeloma protein J558 induce a long lasting total suppression of the anti-α-dex immune response when given neonatally to BALB/c mice. In contrast, isologous BALB/c-anti-J558 idiotype antibodies fail to induce immune suppression when given to neonatal mice as well as in those animals making anti-J558 themselves. In addition, immunization of BALB/c mice against a second α-dex reactive BALB/c myeloma protein, MOPC 104E, causes only in few cases a reduction of the anti-α-dex titer after subsequent α-dex immunization. The different suppressive capacity of homologous and isologous anti-idiotypic antibody may be due to different specificity:Homologous A/J-anti-J558 idiotype antibodies react both with J558 and MOPC 104E protein.In contrast, the isologous BALB/c anti-J558 idiotype antibodies only react with J558 but not with MOPC 104E or other BALB/c myeloma proteins and,conversely,BALB/c anti-MOPC 104E idiotype antibodies solely react with MOPC 104E but not with J558 protein.Moreover, in the case of isologous anti-idiotype antibody the idiotype/anti-idiotype reaction is completely inhibited by 10mM nigerose or nigerotriose(i.e.α-1,3-linked di- and trisaccharide) whereas with homologous anti-idiotypic antibody the idiotype/anti-idiotype reaction is only partially inhibited under these conditions.

775 IMMUNOREGULATORY ALTERATIONS IN THE MRL MOUSE. M.Horowitz, J. Kemp, D. Murphy, E. Murphy, J. Roths and R. Gershon. Laboratory of Cellular Immunology, Howard Hughes Medical Institute, Department of Pathology, Yale University and Jackson Laboratories.

We have examined the T-cell immunoregulatory network of the new inbred autoimmune strain of mouse designated MRL/mp lpr/lpr. These mice posess a single autosomal recessive gene (lpr-lymphoproliferation) which controls the development of a severe autoimmune syndrome. The spleens of MRL mice contain both a chronically activated T-suppressor cell and a helper activity which is largely insensitive to the suppressor and which may be responsible for the constant induction of the suppressor cell. It is likely that this dysfunction in feedback regulation contributes to the development of autoimmune disease in these mice.

Role of Macrophages in Initiation and Regulation of the Immune Response

776 Ia ANTIGENS ON T CELLS, ACT AS RECEPTORS FOR Ig-ANTIGEN COMPLEXES FORMED WITH 6 HOURS AFTER IMMUNIZATION. F. Paraskevas, S.T. Lee, Department of Medicine, University of Manitoba, Winnipeg, Man. Canada

Within 6 hours after immunization of mice antigen is detected in the serum of the animals complexed with Ig (6 hour complexes). It has been shown that the 6 hour complexes are taken up by 20-25% of splenic T cells. These cells belong to the subpopulation bearing Fc receptors (Fc+). However the Fc receptors on Fc+ T cells is not necessary for the uptake of the 6 hour complexes although it is necessary for the uptake of ordinary antigen-antibody complexes. The receptor for 6 hour complexes is labile and disappears after culture of T cells for 4 hours in vitro. In this respect it behaves in a similar way to the Fc receptors and Ia antigens of T cells. Macrophage supernates which can reconstitute both the Fc receptors and Ia antigens on T cells cultured in vitro for 4 hours, were found capable of reconstituting also the receptor for 6 hour complexes. Removal of Fc receptors from the macrophage supernates did not abolish their ability to reconstitute the receptor for 6 hour complex although removal of Ia antigens from the supernates did. These results suggest that Ia antigen released from macrophages are taken up by T cells and act as receptors for complexes of Ig and antigen formed within 6 hours after immunization.

777 T CELL PROLIFERATION: ROLE OF MACROPHAGE SURFACE Ia AND FACTORS, Kwok-Choy Lee, Andrew Wilkinson and Mabel Wong, The University of Alberta, Edmonton, Alberta, Canada T6G 2H7

The proliferation of Mycobacterium-primed murine lymph node T cells to PPD (purified protein derivative of tuberculin), as measured by the uptake of tritiated thymidine, requires the obligatory participation of macrophages which stimulate the T cells either directly with antigen in association with cell surface Ia (I region-defined antigens), or indirectly by means of soluble factors. We have examined the possibility that this functional dichotomy is due to heterogeneity within the macrophage population. Since the maturation of macrophages from the precursor monocytes is associated with cell enlargement, macrophage subpopulations differing in developmental stage are obtained by cell fractionation according to size by velocity sedimentation. Nylon wool purified T cells which have been depleted of macrophages and B cells are stimulated with PPD either in a free form or bound to macrophages which have been incubated for a short time (i.e. pulsed) with PPD. We found that for PPD-pulsed macrophages, only the smallest (and probably the most immature) are capable of inducing T cell proliferation. This antigen presentation function is mediated by cell surface Ia since it is abolished by pre-treatment of the macrophages with anti-Ia serum and complement. On the other hand, all macrophages, irrespective of sensitivity to anti-Ia serum, secrete factors which will stimulate T cell proliferation in the presence of free PPD. Thus the maturation of macrophages is accompanied by a shift from Ia-dependent to Ia-independent mechanisms of immunostimulation.

778 MARROW MACROPHAGES ARE A PRIMARY TARGET FOR INFECTION AND TRANSFORMATION BY MURINE SARCOMA VIRUSES AND SHOW ALTERED PRODUCTION OF HUMORAL MEDIATORS OF HEMOPOIESIS FOLLOWING INFECTION WITH MURINE LEUKEMIA VIRUS, Joel S. Greenberger, Diane Donahue, and Mary A. Sakakenny, Joint Center for RadiationTherapy, Department of Radiation Therapy and Sidney Farber Cancer Institute, Harvard Medical School, Boston, MA 02115

Bone marrow cultures which select for hemopoietic stem cell differentiation in the granulocytic series with no detectable lymphocytes, provide a test system for the interaction ofmacrophages and pluripotent stem cells without lymphocyte regulation. NIH/Swiss mouse marrow cells containing no ecotropic endogenous virus did not generate leukemia in vitro spontaneously. Following infection of NIH/Swiss cultures with each of several RNA type-C leukemia virus (retroviruses) with long latent periods for leukemogenesis in vivo, increased numbers of granulocytic cells were detected compared to uninfected controls. No morphologic alteration was observed in macrophages. When purified macrophage populations were infected in vitro, increased production of CSF was detected. In contrast, infection of either whole bone marrow or purified macrophages with Kirsten murine sarcoma virus pseudotypes of each of several murine leukemia viruses, produced morphologic macrophage transformation, decreased CSF production and blocked macrophage support of hemopoiesis. Bone marrow macrophages supported replication of endogenous or exogenous leukemia viruses as well as sarcoma viruses; however, the former virus class transformed hemopoietic stem cells while the latter transformed the macrophages themselves. The absence of lymphocytes from these cultures indicates that leukemogenesis does not require lymphocytes as obligatory target or helper cells.

779 INDUCTION OF SPECIFIC T CELL-DEPENDENT PROLIFERATIVE RESPONSE IN VITRO TO LEISHMANIA PARASITES. J. Louis, E. Moedder, R. Behin and H. Engers. WHO Immunology Research and Training Centre, Institute of Biochemistry and Department of Immunology, Swiss Cancer Institute, 1066 Epalinges, Switzerland.

Leishmania parasites are obligate intracellular protozoa which preferentially infect macrophages.In order to assess the T cell response to these parasitesin the mouse,we have adapted the method recently described for the study of T lymphocyte activation by protein antigens(J.Immunol.1977,119,1048) to the study of Leishmania tropica (LT)-induced T cell proliferation.Periaortic and inguinal lymph node cells obtained from mice previously injected at the base of the tail with LT parasites emulsified in complete Freund's adjuvant exhibited intense proliferation when cultured in the presence of LT parasites.Proliferation was measured by incorporation of (^{3}H) thymidine using a 16h pulse.The response was antigen-specific,was demonstrable from 4 to 75 days after in vivo priming,and was T cell-dependent since,(a) treatment of lymph node cells with rabbit anti-mouse MTLA serum and complement greatly reduced the magnitude of the response, (b) lymph node cells from nu/nu mice injected with LT parasites showed no proliferation, and (c) purified T cells responded as efficiently as unfractionnated lymph node cells provided irradiated normal peritoneal exudate cells (PEC) were also added to the culture. Finally, normal PEC pulsed with LT were able to induce a proliferative response of primed T cells in the absence of extracellular parasites.These results indicate that macrophages infected with LT are able to induce a specific response of primed T cells, in vitro. The nature of the interactions between antigen presenting cells and primed T cells is currently being investigated.

780 THE REQUIREMENT FOR MACROPHAGES IN THE Fc FRAGMENT INDUCED PROLIFERATIVE RESPONSE OF MOUSE LYMPHOCYTES, Edward L. Morgan and William O. Weigle, Scripps Clinic and Research Foundation, La Jolla, California 92037

Fc fragments of human gamma globulin have been shown to induce mouse splenic bone marrow derived (B) cells to proliferate. The proliferative response was found to be dependent upon adherent cells since Sephadex G-10 filtered spleen cells lost their ability to respond to Fc fragments whereas the response to lipopolysaccharide was unaffected. The response to Fc fragments was restored by the addition of the Sephadex G-10 filtered cells to a monolayer of plastic adherent spleen cells. In addition, irradiation or treatment of the cells with anti-T cell serum prior to adherence had no affect on the ability of adherent cells to restore the response.

The C3H/HeJ mouse has been shown not to respond to Fc fragments but the C3H/St and C3H/HeN strains were found to respond. The inability of the C3H/HeJ to respond did not appear to be due to Fc concentration or cell concentrations. The defect appeared to be in the adherent cell population since the addition of Sephadex G-10 filtered C3H/HeJ spleen cells to C3H/St or C3H/HeN adherent cell monolayers allowed the C3H/HeJ cells to proliferate but the addition of Sephadex G-10 filtered C3H/St or C3H/HeN cells to C3H/HeJ adherent cell monolayers did not restore the proliferative responses of these strains.

(Supported by NIH Grant AI-07007 and Damon-Runyon Fellowship DRG-239-F)

781 EVIDENCE FOR TWO SERUM FACTORS REQUIRED FOR INDUCTION OF ARGINASE IN MACROPHAGES, James P. Jakway and David W. Talmage, The University of Colorado Medical Center, Denver, CO 80262

Peritoneal macrophages (Mϕ) are potent stimulator cells for the induction of an allogenic immune response in vitro. However, if the concentration of Mϕ's exceeds 10^5/ml, complete suppression of the response is obtained. We have previously shown that this suppression is due to induction of the enzyme arginase in the Mϕ's which depletes the medium of the essential amino acid arginine. Peritoneal Mϕ's obtained from normal mice contain no detectable arginase. Culturing these cells in MEM + FCS causes a dramatic rise in arginase concentration which increases for at least 4 days. Arginase induction requires a minimum of 1% serum and is maximal with 30% serum. Arginase production requires ongoing protein synthesis, as demonstrated by its inhibition by cycloheximide. Indomethacin, an inhibitor of prostaglandin synthesis, inhibits arginase induction by 85%. Addition of PGE_2 or DBcAMP to cultures reverses indomethacin inhibition, but only in the presence of 3% serum. Mϕ's make PGE_2 upon culture, a process which also requies serum. Addition of DBcAMP to cultures reduces the requirement for serum by 10-fold, but does not abolish it, indicating that there are two factors in serum required for arginase induction. One of these requires 30% serum for maximum activity and may be replaced by PGE_2 or DBcAMP. The other factor requires only 3% serum for maximum activity. Both factors are found in mouse, human and calf serum but not in ovalbumin.

782 BIOLOGICAL AND CHEMICAL STUDIES OF GLUCOCORTICOSTEROID RESPONSE MODIFYING FACTORS PRODUCED BY CULTURED MURINE CELL LINES. Stanley M. Shiigi, Yu-hua Una Chen, Kenneth H. Grabstein, David A. Lee, Barbara B. Mishell and Robert I. Mishell. University of California, Berkeley, CA 94720

Glucocorticosteroid response modifying factors (GRMF) are macromolecules which block the immunosuppressive effects of glucocorticosteroid hormones on cultured mouse spleen cells. They are most conveniently assayed by comparing the humoral immune responses of mouse spleen cells cultured with 10^{-6}M dexamethasone in the presence or absence of the factors. GRMF protected cultures generate responses in the range of 3,000-20,000 plaque forming cells compared to unprotected steroid suppressed controls of less than 100. Under these conditions, allogeneic conditioned media which have strong T cell replacing activity do not provide significant protection against the steroids. Initially GRMF were obtained from medium conditioned by resident peritoneal accessory cells of normal mice, which had been cultured in the presence of bacterial adjuvants. This report presents data on the production of GRMF and other mediators by several murine accessory cell lines: WEHI-3, PU-5.1, WEHI-274 and $P388D_1$. Steroid protecting factors are produced by each of the cell lines. WEHI-3, $P388D_1$ and WEHI-274 require induction with LPS, peptidoglycan or another adjuvant for GRMF production, but preliminary studies indicate that PU-5.1 produces GRMF constitutively under serum free conditions. Comparative studies of the relative effectiveness of several chemically characterized bacterial substances in inducing the production of GRMF by the adjuvant dependent cell lines are in progress and results will be reported. WEHI-274 produces two distinguishable mediators, one with GRMF activity and one without. Studies comparing the properties of these factors will be reported.

783 ANTIGEN PRESENTATION BY GUINEA PIG KUPFFER CELLS. T.M.Rogoff & P.E.Lipsky, Southwestern Medical School, UTHSC at Dallas, Texas 75235. To better understand the role of Kupffer cells (KC) in immune surveillance, isolated guinea pig (GP) KC were assessed for their ability to take up antigen (Ag) in vitro and present it to primed T cells for the induction of proliferative responses. KC were isolated from inbred GP by sequentially exposing liver to collagenase and trypsin, followed by differential centrifugation and adherence to glass. Resulting KC preparations consisted of >95% mononuclear phagocytes (MP). Oil-induced peritoneal exudate macrophages (PEM) treated in identical fashion were used for comparison. KC and PEM from unprimed GP were exposed to Ag (TNP-ovalbumin or PPD) in vitro for 30 minutes at 37°C, mitomycin C-treated, and washed repeatedly. The capacity of each MP population to present Ag for recognition by sensitized T lymphocytes was tested by mixing Ag-pulsed KC or PEM with Ag-primed peritoneal exudate lymphocytes (PEL) and assaying for lymphocyte proliferative responses. PEL were prepared from GP 4-6 weeks after immunization by passing oil-induced peritoneal exudates over nylon wool columns. Varying numbers of Ag-pulsed KC or PEM were combined with PEL in the wells of microtiter plates and cultured for 72h at 37°C. Ag-specific PEL proliferation was then assayed by ^{3}H-thymidine incorporation. Both Ag-pulsed KC and PEM were capable of inducing significant DNA synthetic responses in primed PEL. To determine whether KC-lymphocyte interaction was genetically restricted, the histocompatibility requirement for Ag presentation by KC was tested. Ag-pulsed strain 13 KC were able to induce responses in strain 13 PEL (ΔCPM=24,460) but were unable to do so in strain 2 PEL (ΔCPM=436). The data indicate that KC are capable of presenting Ag to primed lymphocytes, and that the induction of T cell proliferation by Ag-pulsed KC is restricted by determinants coded for by the major histocompatibility complex.

784 REGULATION OF PROLIFERATIVE AND CYTOTOXIC RESPONSES TOWARDS ALLOANTIGENS IN VITRO AND IN VIVO BY NON-SPECIFIC SUPPRESSOR CELLS, A. Matter, F. Hoffmann-La Roche & Co.Ltd Basle, Switzerland

A non-specific suppressor cell can be isolated from the spleens of tumor-bearing mice or from M-locus stimulated mixed lymphocyte cultures. This cell is capable of abrogating non-specifically a proliferative and cytotoxic response of fresh, syngeneic cells towards a wide variety of alloantigens. This cell is adherent, non-phagocytic, anti-Thy-1 insensitive and anti-Ig insensitive (A.M., Cell Immunol. 37: 107, 1978). It carries F_C-receptors and is Ia positive. In albumin-density gradients it can be found enriched in the intermediate layers. Supernatants of suppressor cell cultures are inactive. The cell is distinct from natural killer cells since it is adherent, F_CR positive and also because it does not affect growth of a variety of tumor cells including the cell line YAC-1. It is distinct from macrophages because of the lack of phagocytosis. The hypothesis was examined that this cell might be an ADCC cell acting via alloantibody-antigen complexes or via antiidiotype antibody.

785 MACROPHAGE-LYMPHOCYTE INTERACTION IN RESPONSE TO NONSPECIFIC MITOGENS & LYMPHOKINES. Abby L. Maizel and Richard J. Ford, M.D. Anderson Hospital and Tumor Institute, Houston, Texas 77030. Utilizing human peripheral venous monocytes (macrophages) and T-cells prepared to greater than 98% purity revealed that proliferation of the T-cells to non-specific nonimmunogenic mitogens (PHA) is markedly dependent upon macrophage interaction. This interaction involves a contact component which has been visualized early after stimulation utilizing scanning electron microscopy. The proliferative response of the T-cell has also been examined in response to putative lymphokinetic agents derived from PHA stimulated lymphocytes (Lycm). The T-cells are mitogenically responsive to the Lycm lymphokine for periods of time ranging up to 6-8 weeks. The rate of proliferation continually declines throughout the incubation period. T-cell proliferation to the crude lymphokine may be augmented when the lymphocytes are co-cultured with syngeneic macrophages. In addition, the duration of T-cell kinetic response to the lymphokine may be prolonged by adding macrophages to T-cell cultures as these cultures begin to decline in proliferative capacity. The optimal number of macrophages to T-cells, as with PHA exposure, is on the order of 5 T-cells for each macrophage. The augmentation of the proliferative response is most easily demonstrated when there is direct cell to cell contact between macrophage and T-cell. A somewhat diminished response, yet greater than T-cells alone, may also be observed when the cells are prevented from contact by a permeable membrane. (Supported by NIH grant number CA 21927 and CA 16672).

786 MURINE MACROPHAGE-LYMPHOCYTE BINDING - SEPARATION OF B CELL SUBSETS ON MACROPHAGE MONOLAYERS, Margot O'Toole and Henry H. Wortis, Tufts University School of Medicine, Boston, Massachusetts 02111

Spleen and lymph node cells from normal adult mice contain cells that adhere to macrophage monolayers. These "binder cells" are B cells as shown both by staining with fluoresceinated rabbit anti mouse Ig antisera and by testing the binding of purified T and B cells. B cell binding to macrophages is antigen independent. A population of non-binding B cells can be isolated by allowing the binder cells to adhere to macrophage monolayers, and harvesting the non-adherent cells. These cells will not subsequently bind to macrophages and are called "non-binder" cells. "Non-binder" cells comprise about 42% of normal adult CBA splenic B cells. Young mice lack macrophage binding B cells. CBA/N mice, which lack a subset of B cells, also lack splenic "binder" cells. Antiserum (provided by B. Huber) raised in deficient mice against normal B cells, reacts with 46% of normal B cells and fails to react with isolated non-binder cells. These data show that B cell subsets can be defined on the basis of their macrophage binding properties. The "binder" population includes that subset which is deficient in CBA/N mice. We have also investigated the macrophage binding properties of T cells. We found, as have others, that thymocytes bind to macrophages. However, few if any T cells isolated from spleen or lymph node bind. These non-binding T cells can be shown to disrupt macrophage-B cell binding, but do not effect the macrophage thymocyte interaction.

We conclude that a subset of B cells, which corresponds to a known functional subpopulation, can bind to macrophages and that this interaction can be influenced by T cells.

787 REQUIREMENT FOR RECOGNITION OF MHC DETERMINANTS EXPRESSED ON ACCESSORY CELLS BY UNPRIMED HELPER T CELLS, Richard J. Hodes, Karen S. Hathcock, and Alfred Singer, NIH, National Cancer Institute, Bethesda, MD 20014.

It has been demonstrated that unprimed helper T cells are required to recognize H-2 determinants expressed on non-T, non-B adherent accessory cells for antibody production. This was determined by experiments in which helper T cells were obtained from three different $(AxB)F_1 \rightarrow Parent_A$ radiation bone marrow chimera situations. Two types of experiments were performed: (1) chimera spleen cells were depleted of accessory cells and were analyzed for their ability to cooperate with accessory cells from $Parent_A$, $Parent_B$, or third party allogeneic strains; (2) three cell mixing experiments were performed in which $(AxB)F_1$ chimera helper T cells were mixed with $(AxB)F_1$ B cells and titered for their ability to cooperate with accessory cells from $Parent_A$, $Parent_B$, or A/B recombinant strains. It is concluded that (1) $(AxB)F_1 \rightarrow Parent_A$ helper T cells are restricted in their ability to recognize and cooperate with accessory cells expressing the K or I-A region encoded H-2 determinants of $Parent_A$ and not $Parent_B$; (2) such restrictions on recognition exist prior to antigen exposure; and (3) therefore, a strict requirement exists for helper T cell recognition of H-2 determinants expressed on adherent accessory cells.

T Cell Hybridomas with Biological Function

788 T cell hybridomas with antigen-specific suppressor function

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Laboratories for Immunology, School of Medicine, Chiba University, and
Department of Immunology, Faculty of Medicine, Tokyo University*, Japan

The establishment of T cell hybridomas with specific function provides ideal tool to analyse the suppressor molecule and the mechanism of suppression. The hybrids were made by fusion of a AKR Thymoma cell line, BW5147, with KLH-specific enriched suppressor T cells of C57BL/6 mice. Ig negative spleen cells from KLH-primed mice were adsorbed to and eluted from Petri dishes coated with KLH. The fusion was made with polyethylene glycol of M.W.2000. The fused cells were selected in the HAT medium and I-J positive hybrid cells were separated by FACS-II after staining with anti-$I\text{-}J^b$ antiserum (B10A(5R)anti-B10A(3R)). The cell free extracts were obtained from $I\text{-}J^+$ hybrid cells. One of the hybrids produced a large quantity of ascites when transplanted i.p. into (C57BL/6 x C3H)F1 mice. The suppressive activity of the extracts and ascites was examined by adding them into the culture of spleen cells primed with DNP-KLH or DNP-EA. Both ascites and extracts suppressed anti-DNP-IgG PFC response to DNP-KLH but did not affect the response to DNP-EA. The suppressive effect was completely removed by absorption with immunoadsorbents of KLH_L or $anti\text{-}I\text{-}J^b$ but not with those composed of unrelated antigen (Asc), anti-Fab and $anti\text{-}I\text{-}J^k$ antiserum. The results indicate that the suppressor molecule from hybridomas has both antigen-binding capacity and determinants coded for by genes in I-J subregion. The molecular weight of the active principle was estimated to be between 64000 and 46000.

789 LONGTERM GROWTH OF SPECIFIC HUMAN T CELL BLASTS, James T. Kurnick, Richard Robb, Arthur Kimura, Olof Sjöberg, Valdemar Skoog, J.B.Lindblom and Hans Wigzell, Uppsala University, Uppsala, Sweden.

Human T lymphoblasts purified after antigen activation were maintained in longterm dividing cultures using the approach of adding supernatants from PHA-activated human lymphocytes. We could confirm that cytolytic T blasts (here with specificity for HLA-A or B) were maintained. We could extend the system to include an HLA-D restricted proliferative response by T blasts specific for PPD or tetanus toxoid. The T blast driving factor in the supernatants was not the lectin itself, but could be shown to be a trypsin-sensitive agent with approximate size of 17-20,000 daltons. This factor could be absorbed to and eluted back from T blasts, but not to B blasts or resting T lymphocytes. In agreement with the binding data, a lectin-free supernatant had no ablility to activate small resting T cells but was able to keep the T blasts in proliferation. Studies on the more detailed features of the growth factor(s), its cell source, the receptor for such factor(s) on the T blasts, and the defined subsets of T blasts which can be maintained are underway.

790 FUNCTIONAL PROPERTIES AND CELL SURFACE MARKERS OF MURINE CYTOLYTIC T-CELL LINES.
Markus Nabholz, Marcel North, Andreas Conzelman, Richard Pink, Dino Collavo and Howard Engers, Swiss Institute for Experimental Cancer Research, CH-1066 Epalinges and Depts. of Pathology, University of Geneva, CH-1211 Geneva, Switzerland.

Several continuously proliferating murine T-cell lines which maintain their specific cytolytic activity have been established. These lines can be cloned with a very high efficiency. The phenotypic stability of their functional characteristics as well as their expression of T-cell differentiation markers has been investigated. The potential of these cells as material for an analysis of T-cell functions by means of somatic cell genetics is being explored.

791 THE GENERATION OF MONOCLONAL T-CELL LINES. Raimund Di Pauli and Rod Langman, The Salk Institute for Biological Studies, San Diego, Ca. 92112.

A collection of T-cell lines with known specificity and effector function would obviously be invaluable. Attempts to generate such cell lines have so far met with very limited success, and the promising avenue suggested by cell-cell hybridization techniques has proven surprisingly unsuccessful. Our approach to this problem has been to apply the methodology of carcinogenesis to tertiary *in vitro* mixed lymphocyte cultures, followed by cloning at limiting dilution. The initial protocol used CB.20(H.2^d) anti-B6 (H.2^b) tertiary MLC cultures treated with nitrosoguanidine and maintained by constant stimulation with irradiated B6 cells. When mutagenized MLC cells were plated in microtiter plates at 10 cells per well 5/800 wells showed growth and of these two produced cytotoxic T-cells (T^k): one clone was specific for H-$2K^b$ antigens, the other was specific for H-$2D^b$ antigens. Of the remaining three clones, one will kill only in the presence of 10 µg/ml ConA, another two have no detectable cytotoxic activity. All cell lines require irradiated B6 spleen cells for continued proliferation and in the case of H-$2K^b$ and H-$2D^b$ killer clones stimulator cells carrying only the corresponding H-$2K^b$ and H-$2D^b$ type respectively are sufficient to maintain growth. These lines have been in continuous culture for 8 months with doubling times in the order of 2 days.

792 ANTIGEN SPECIFIC SUPPRESSOR FACTOR FOR DELAYED-TYPE HYPERSENSITIVITY PRODUCED BY T CELL HYBRIDS. Foo Y. Liew and Jane Hewitt, Department of Experimental Immunobiology, The Wellcome Research Laboratories, Beckenham, Kent, England.

Spleen cells from CBA mice injected intravenously (i.v.) with $1x10^9$ sheep red blood cells (SRBC) contain specific T suppressor cells for delayed-type hypersensitivity (DTH) to SRBC. Antigen specific suppressor factor can be obtained by incubating these spleen cells *in vitro*. A cell fusion technique was used to produce hybridomas between the primed spleen cells and the AKR T lymphoma BW5147. Supernatants from hybridomas were tested for suppressor activity by injecting i.v. either into sensitized mice (suppression of expression of DTH) or into normal mice which were challenged immediately with antigen (suppression of induction of DTH). In two fusion experiments a total of 12 functionally active cultures were obtained: 8 produced suppressor activity for the expression of DTH, 4 produced suppressor activity for the induction of DTH. All those supernatants suppressing the expression of DTH were antigen specific, whilst only one supernatant active in suppressing induction of DTH was specific. These cultures were passaged in normal AKR mice and the serum obtained had activity up to 10^{-6} dilution. These cultures were also successfully cloned in soft agar. Immunochemical analyses of the suppressor factor indicate that it is similar if not identical to the suppressor factor produced by the primed spleen cells. The factor bears I-J^K determinant but not Ig determinants. It has a M.W. of <50,000 daltons. It acts across the H-2 barrier. Further analyses of the hybridomas and the suppressor factor are in progress.

793 T CELL HYBRIDOMAS SECRETING COLONY-STIMULATING-FACTORS. M. Howard, A. Burgess, and D. Metcalf. The Walter and Eliza Hall Institute, Melbourne, Australia.
When mouse spleen cells are cultured with pokeweed mitogen, the activated cells produce specific factors able to stimulate granulocyte-macrophage, eosinophil, megakaryocyte, and erythroid colony formation in agar cultures of marrow or fetal liver precursor cells. These factors appear to be synthesised or released by T lymphocytes. For this reason, spleen cells were stimulated with pokeweed mitogen and the activated cells were fused with the murine T lymphoma BW5147 to produce hybridomas. A stable cloned T hybridoma has been isolated which secretes one or more of the colony-stimulating-factors (CSFs). The hybridoma expresses the H-2 antigens of both fusion parents, has a median chromosome number of 56, and secretes a factor(s) which stimulates the growth of granulocyte-macrophage and eosinophil colonies. The maximum amount of CSF is produced when the hybridoma is maintained for several days at high cell concentration in serum-free medium. This line has existed in continuous cell culture since May, 1978

794 EXPRESSION OF ANTIGEN BINDING ACTIVITY IN T LYMPHOCYTE HYBRIDS
Nancy H. Ruddle, Diane D. Eardley, Stephen O'Connor, and Robert E. Cone.
Yale University, New Haven, Connecticut 06511

Murine T cells educated in vitro to sheep red blood cells (SRBC) which form rosettes with such erythrocytes have been hybridized with BW 5147, a T lymphoma. Hybrid clones express isozymes, thy-1 and H-2 antigens of both parents. One clone, Hyb 29P, forms rosettes with SRBC. The realization that rosette forming activity is dependent upon physiological parameters and culture phase has permitted enrichment of Hyb 29P cells to 70-100% purity rosette forming activity. This has facilitated analysis at the biochemical level of cell synthesized molecules which bind antigen. Lysates from ^{3}H-leucine labelled Hyb 29P contain polypeptides with molecular weights (determined by SDS-PAGE under reducing conditions) of 68,000 and 45,000 daltons which bind specifically to SRBC.

795 USE OF T AND B CELL HYBRIDS IN THE CHARACTERISATION OF HUMAN LYMPHOCYTE SUBSETS. P.C.L. Beverley and R.E. Callard, The Human Tumour Immunology Group, University College Hospital Medical School, University Street, London WC1E 6JJ.
Antibody producing hybrids. Spleens from mice immunised with human lymphocyte fractions were hybridised to the NS-I myeloma. Immunogens were either peripheral blood lymphocytes, thymocytes, thymocyte membrane glycoproteins or human x mouse T - T hybrids (see below). Supernatants from the resulting hybrids were analysed using a sandwich 1^{125} antiglobulin binding test. A number of specific antisera have been developed. T - T hybrids. Activated human T cells were hybridised to the AKR T lymphoma, BW 5147. Resulting hybrids have been analysed karyologically and for a number of surface markers. Function has been assessed by ability to respond to mitogens and to produce supernatants capable of modifying other in vitro immune responses.

796 SOMATIC CELL HYBRIDS PRODUCING MONOSPECIFIC ANTIBODIES AGAINST HUMAN LYMPHOCYTE SURFACE ANTIGENS.

R. S. Accolla, S. Carrel, J.-P. Mach, J.-C. Cerottini. Unit of Human Cancer Immunology, Lausanne Branch, Ludwig Institute for Cancer Research, Epalinges s/Lausanne, Switzerland

Somatic cell hybrids have been produced which synthesize monoclonal antibodies against human lymphocyte surface antigens. Membrane enriched fractions from DAUDI cells have been used as immunogen in mice. The protocol of fusion between spleen cells of immunized mice and the BALB/C myeloma cell line P3-X63Ag8 was carried out as originally described by Kohler and Milstein by using polyethylenglycol as fusing reagent.

From 2 fusion experiments we obtained 74 hybrids. 14 of them produced antibodies with binding activity for DAUDI cells as detected by radioimmunoassay. 8 of the antibodies were also cytolytic against the same target cells in the presence of rabbit complement. The specificity of the cytolytic antibodies was analyzed against a large pannel of target cells including B, T, non-B, non-T cell lines as well as normal PBL, thymocytes and tonsil lymphocytes.

The results showed that hybrid products could be divided in 4 major groups according to their pattern of reactivity: 1) against all lymphoid cells; 2) restricted to B cells; 3) restricted to some B and some T cells; 4) against DAUDI cells only.

797 THE USE OF ANTIGEN AND POLYCLONAL ACTIVATORS TO GENERATE ANTI-ARS PRODUCING HYBRIDS,

S.M.Robertson, J.D.Capra,& J.R.Kettman. Southwestern Medical School, Dallas, TX 75235

Hybrid cells producing anti-ARS (ā-ARS) antibodies of four isotypes were generated by the fusion of A/J spleen cells with MPC-11 myeloma cells. Spleen cells from mice hyperimmunized to p-azophenylarsonate-KLH (ARS-KLH)were cultured for 4 and 7 days in the presence of dextra sulfate and LPS with or without ARS-KLH. Both cultured and uncultured cells were hybridized with MPC-11 cells using PEG. After 3 to 6 weeks the wells were scored for anti-ARS binding activity in a solid phase radioimmunassay. Hybrids derived from uncultured A/J cells produced ā-ARS Ab in 11% of the wells plated. No ā-ARS hybrids were detected from fusions of mitogen treated cultured spleen cells. The frequency of ā-ARS hybrid wells produced from fusions of mitogen stimulated cells was 3-5% and the frequency of fusions from mitogen + antigen stimulated cells was between 10-33%. Of the ā-ARS hybrid wells, the ten which grew successfully were all derived from mitogen or mitogen + antigen stimulated spleen cells. All hybrids displayed the parent isotype (γ2b) plus an additional isotype, with a preponderance for μ. Upon subsequent transfer ā-ARS hybrids were identified which produce only one type of heavy chain (by gel diffusion, μ, γ2b, γ3, γ1). Other hybrids are possibly double or triple producers. The two hybrids which appear to be secreting molecules which bear the ā-ARS Ab cross-reacting idiotype are under study. Data from analysis of the A/J spleen cell populations used for hybridization indicate that the cells which were successfully hybridized and grown may have originated from a population of blast cells which were antigen sensitive but were not ā-ARS antibody secreting cells.

798 HUMAN LYMPHOID CELL SURFACE ANTIGENS AS DEFINED BY MONOCLONAL ANTIBODIES, Levy, R. Lampson, L.A., Dilley, J., and Fox, R., Stanford University, Stanford, CA. 94305

A series of antibody producing hybridomas have been derived from animals immunized with homogeneous human cell populations. Immunizing cells have included B lymphoblastoid cell lines, T cell lines, freshly isolated CLL cells and T cell type ALL cells. Among the most interesting antibodies produced are some with specificity for human B cell antigens, including those of the MHC D locus, and some with specificity of various subpopulations of T cells, including one defining a population of cells found only in the cortex of the normal thymus. Data will be presented on the cellular distribution and chemical characterization of these antigens.

799 HYBRIDS OF B LYMPHOCYTE CELL LINES. William C. Raschke, The Salk Institute for Biological Studies, San Diego, California 92112
The objective of this study is to determine the expression of immunoglobulin in hybrids made between various types of B lymphocytes. The following results were obtained: 1) Hybrids of B cell lymphomas with plasmacytomas have the properties of the plasmacytomas with the immunoglobulins of both parents secreted. The 7S cell surface IgM of the B lymphoma which is not secreted in the parent is secreted in the hybrid as 19S IgM. 2) B lymphomas fused with other B lymphomas or with normal B lymphocytes retain the phenotype of B lymphomas with regard to immunoglobulin expression. Both parental immunoglobulin products are expressed on the cell surface and not secreted. In the case of a Syrian hamster B lymphoma fused with a normal mouse B cell, the hybrid expresses mouse IgM, IgD and Ia molecules on the cell surface. 3) Hybrids of Abelson virus transformed lymphocytes with plasmacytomas or B cell lymphomas have the same immunoglobulin expression as the plasmacytoma or B lymphoma parent with no new immunoglobulin detectable. Abelson virus transformed lymphocytes do not synthesize detectable levels of immunoglobulin. To determine if the level of synthesis is too low to detect, if a dominant shutoff mechanism for immunoglobulin synthesis is operating in these cells or if the immunoglobulin genes can be turned on if supplied with the biochemical machinery for immunoglobulin synthesis, the hybrids with immunoglobulin synthesizing cells were made. Since no new immunoglobulins were produced in the hybrids and the synthesis of the plasmacytoma or B lymphoma immunoglobulin was unaltered, none of the above possibilities are valid and the Abelson lymphoma lines can be considered cis dominant for the lack of immunoglobulin expression.

Genetics and Cell Interactions in Cell-Mediated Lympholysis

800 ANALYSIS OF THE ANTI H-Y CYTOTOXIC RESPONSES IN CHIMERIC MICE, Takeshi Matsunaga, Elizabeth Simpson, Clinical Research Centre, Watford Road, Harrow, Middlesex, England
Anti H-Y (male specific antigen) cytotoxic T cell responses in mice are H-2 restricted in the same manner as anti virus responses. In addition, genetic studies have revealed that H-2K/D as well as I region loci determine the responsiveness. For the dominant H-2^b haplotype Ir gene(s) mapping in the IA subregion determine responsiveness. In certain non-responder haplotypes, complementation Ir genes have been mapped to the IC subregion (1). To shed more light on the mechanisms of MHC control over anti H-Y responses, we have studied two kinds of mouse chimeras (allophenic chimeras and irradiation bone marrow chimeras) in which lymphocyte components were of two non-responder strains (BALB/C and C3H/He). The results showed that those chimeras responded to H-Y antigens in association with H-2^k (C3H/He) haplotype with cytotoxic cells mainly being BALB/C type (2). We present a possible model to explain this kind of "H-2 complementation" in chimeras, and emphasize the essential importance of the association between H-Y antigens and the MHC haplotypes for responsiveness.

(1) Hurme, M. et al, 1978 J. Exp. Med. 147, 758

(2) Matsunaga, T. et al, Proc. Nat. Acad. Sci. (in press)

801 THE REPERTOIRE OF CYTOTOXIC PRECURSORS TO TNP-MODIFIED SELF AND TNP-MODIFIED ALLOANTIGENS, Hung-Sia Teh, Department of Microbiology, University of British Columbia, Vancouver, Canada V6T 1W5
A limiting dilution assay for precursors of cytotoxic lymphocytes (CLP) was used to measure the frequency of CLP to TNP-modified self and TNP-modified alloantigens. It was found that the frequency of CLP to TNP-modified self antigens is small compared to the frequency of CLP to alloantigens. Thus, the number of lymphocytes in C57BL/6J (B6) lymph nodes responding to DBA/2J (D2) and B6-TNP are 1199 and 40 per 10^6 cells respectively. About 40% of the cytotoxic clones, produced as a result of stimulation of B6 lymphocytes with B6-TNP, can lyse both B6-TNP and D2-TNP targets. A current controversy exists as to whether CLP for TNP-modified alloantigens exist in normal animals. My clonal analyses data are consistent with the interpretation that for every ten precursors in B6 mice that respond to D2 alloantigens, a precursor exists that can respond uniquely to D2-TNP.

Cytotoxic T lymphocytes (CL) to TNP-modified self antigens can also be generated by stimulating syngeneic cells with either unmodified allogeneic cells or with Con A. Thus, B6 anti-D2 CL or Con A-activated B6 CL will kill B6-TNP but not unmodified B6 targets. About 16% of the B6 anti-D2 clones can kill both B6-TNP and D2 targets. In other words, there is a high degree of cross-reaction between allodeterminants and TNP-modified self determinants. These data are consistent with the hypothesis that cytotoxic responses to TNP-modified self antigens may be similar to responses to alloantigens, the allodeterminant being created as a result of modification of H-2 antigens with TNP. (Supported by the NCI of Canada)

802 INDUCTION OF SECONDARY CYTOTOXICITY BY CELL-FREE FACTOR PRODUCED BY I OR D REGION PRIMED T-CELLS, Shogo Kano and Kazuo Oshimi, Jichi Medical School, Tochigi, JAPAN 329-04

Cell cooperation in the generation of secondary cytotoxic responses has been studied, in analogy with T-B cell cooperation in antibody production, by selectively priming in vitro with I or K/D region differences and by combining the primed lymphocytes in the secondary mixed lymphocyte culture. (B10.A x A.TL)F_1 spleen cells were sensitized in vitro with either A.TH or B10.A(2R) cells. A.TH-primed and B10.A(2R)-primed cells gave secondary proliferative responses only to the original stimulating cells. Restimulation of B10.A(2R)-primed cells with B10.A$(2R)_m$ resulted in the generation of strong cytotoxic responses against EL-4($H\text{-}2^b$) target cells. A.TH-primed cells, although by themselves unable to develop cytotoxicity against $H\text{-}2^b$ target, could induce secondary cytotoxicity from B10.A(2R)-primed cells when both cells were mixed and restimulated with A.TH_m in the absence of B10.A$(2R)_m$. Cell-free supernatants from secondary MLC obtained 24 hr after restimulation of A.TH-primed cells with A.TH_m induced cytotoxic responses against EL-4 target when added to B10.A(2R)-primed cells. Both elaboration of active cell-free factor by A.TH-primed cells and generation of cytotoxic activity from B10.A(2R)-primed cells are T-cell dependent. Furthermore, active supernatants were also obtained when B10.A(2R)-primed cells were stimulated with B10.A$(2R)_m$. Taken together, these results suggest that cell cooperation between I region primed and K/D region primed T-cells plays a role in the generation of secondary cytotoxic responses, that cell-free factor(s) can substitute for such T-T cell cooperation, and that cell cooperation may be involved in the development of cytotoxic responses to K/D region differences alone.

803 PRECURSOR FREQUENCY ANALYSIS OF CYTOTOXIC T CELLS IN H-2 MUTANT STRAINS SENSITIZED AGAINST THE STRAINS OF ORIGIN, Meade Pimsler, Richard Ciavarra, Jo Ann Trial, and James Forman. Dept. of Microbiology, Univ. of Texas Health Sci. Ctr., Dallas, TX

Lymph node cells from intra-H-2 recombinant and mutant congenic strains were sensitized in vitro in limiting dilution cultures to estimate the number of cytotoxic lymphocyte precursors (CTL.P) against different regions of the H-2 gene complex. When cells from the recombinant responder strains differed from the stimulator at both the H-2K and I regions, the number of CTL.P was 60% of that observed when the responder cells differed from the stimulators at the entire H-2 gene complex. When the responder and stimulator cells differed at the D-region only, the number of CTL.P was 10% of that activated across the entire H-2 complex. However, B10.D2(M504-H-2^{da}), an H-2 mutant strain derived from B10.D2 and carrying a mutation in an H-2D region gene, when sensitized against B10.D2 had a CTL.P frequency that was identical to that observed when the responder differed from the stimulator at the H-2D region by intra-H-2 recombination. When we tested 4 $H\text{-}2K^b$ mutants of C57BL/6 (B6) in limiting dilution against B6, a very high CTL.P frequency was observed. Relative to completely H-2 incompatible strains sensitized against B6, B6.C-$H\text{-}2^{ba}$ and B6.C-$H\text{-}2^{bgl}$ averaged approximately 40%, B6.C-$H\text{-}2^{bd}$ 50%, and B6.C-$H\text{-}2^{bh}$ 120%. Since these mutant gene products show minimal structural differences and no qualitative serological differences from the wild type molecule, and in the case of $H\text{-}2K^{ba}$ the difference from $H\text{-}2K^b$ may be limited to a single amino acid, these results indicate that the number of CTL.P against allogeneic H-2 molecules does not increase as serological and structural similarities diverge. This may suggest that the T cell receptor repertoire recognizes those H-2 molecules or determinants closest to self.

804 SURFACE MARKERS ON NATURAL KILLER CELLS OF MICE. - Sonoko Habu, Kyoko Hayakawa*, Ko Okumura* and Tomio Tada*, Department of Pathology, Tokai University School of Medicine, Bosei-dai, Isehara-shi, Kanagawa, and *Department of Immunology, Faculty of Medicine, University of Tokyo, Tokyo, Japan.

No positive cell markers which define natural killer (NK) cells have been reported both in the mouse and human. We have studied cell surface phenotype characteristic to NK cells of CBA/J and nude mice with some hetero- and allo-antisera. The rabbit antiserum against mouse brain tissue (anti-BAT) was found to be capable of completely eliminating splenic NK cells as detected with a Moloney virus lymphoma (YAC-1) and a radiation induced leukemia (RL♂1) cells. The same antiserum could abolish various T cell functions, e.g., helper and suppressor functions and responsiveness to Con A, but not B cell functions, e.g., immunological memory for the secondary antibody response and responsiveness to LPS. The absorption of the anti-BAT with thymocytes completely removed its ability to react with T cells leaving the activity to kill NK cells intact. None of other heterologous and isologous antisera, i.e., rabbit anti-mouse thymocyte serum, goat anti-ThB, anti-Thy-1.2 and anti-Ia could eliminate NK function regardless their definite reactivity with T or B cells. The results indicate that the absorbed anti-BAT can distinguish NK cells from other known subsets of T and B cells.

805 THE EFFECT OF *IN VIVO* PRIMING AND SUPPRESSION ON THE MAGNITUDE AND SPECIFICITY OF CYTOLYTIC T LYMPHOCYTES, Robert Finberg, Steven Burakoff, Mark Greene, and Baruj Benacerraf, Harvard Medical School, Boston, Mass. 02115

Cytolytic T lymphocytes (CTL) were induced after a primary *in vitro* culture of normal spleen cells with irradiated, trinitrophenyl (TNP) modified syngeneic spleen cells. If mice received TNP modified syngeneic cells subcutaneously 7 days prior to *in vitro* culture, cytolytic activity was markedly enhanced. This *in vivo* priming was also observed to affect the specificity of these CTL. Specifically, CTL from mice bearing k alleles in the H-2K and IA loci (*e.g.* A/J mice) have been shown to lyse TNP modified syngeneic targets but revealed little if any cross-reactive lysis of TNP modified allogeneic targets. However, if A/J mice were primed *in vivo* with TNP modified syngeneic cells prior to *in vitro* culture, CTL from these mice revealed the same degree of cross-reactive lysis observed for other strains.

The increase in cytolytic activity observed after *in vivo* priming could be eliminated by the intravenous administration of antigen specific suppressor T cells. These suppressor cells also eliminated the cross-reactive lysis observed after *in vivo* priming of A/J mice. Preliminary evidence suggests that the target of these suppressors are radio-resistant helper cells induced by *in vivo* priming.

806 INHIBITION OF CYTOTOXIC T CELL ACTIVITY BY ANTI-INFLUENZA HYBRIDOMA PROTEINS, Rita B. Effros, Mark E. Frankel, Peter C. Doherty, and Walter U. Gerhard, The Wistar Institute, Philadelphia, PA 19104

The lysis of ^{51}Cr-labeled target cells by T cells from virus-immune mice involves recognition of both viral antigens and molecules coded for by the MHC. One approach to analyzing the specificity of this recognition is to attempt to inhibit the cytotoxicity with serological reagents. Anti-H-2 antisera have been used successfully, but anti-viral antisera have been generally unsuccessful. We report here that the cytotoxic activity of influenza-immune T cells can be inhibited by pre-incubation of the infected target cells with monoclonal anti-influenza antibodies. The system we have investigated utilizes P815 mastocytoma cells infected with A/WSN/33/HON1. Following 4 hours of incubation to allow for surface expression of the viral antigens, the target cells are exposed to one of a panel of anti-WSN hybridomas. A 4-5 hour microcytotoxicity assay with these targets, using either spleen or thoracic duct lymphocytes (TDL) from immune mice indicates that killing can be inhibited from 20-50% by certain hybridomas. Several aspects of the inhibition are of interest and are currently being analyzed. Although the hybridomas we have used are all directed against the hemagglutinin of the viral surface, they differ markedly in their ability to inhibit T cell cytotoxicity. Furthermore, spleen cells and TDL from equivalently immunized mice appear to be differentially inhibitable by these hybridomas. Finally, the H-2 type of the mice and the target cells may be factors in the inhibition phenomena. (Supported by NIH Grants AI 13989 and AI 14162).

807 STUDY OF THE RESPONSE PATTERN REGULATION TO K AND D REGION ANTIGENS IN TNP-SELF AND ALLOGENEIC CYTOTOXICITY, Robert B. Levy and Gene M. Shearer, NIH, National Cancer Institute, Bethesda, MD 20014.

Spleen cells from H-$2^{k,d,and\ b}$ inbred mouse strains were sensitized in vitro to syngeneic cells modified with trinitrobenzene sulfonate (TNP-self). Cytotoxic effector cells assayed on TNP-modified, H-2 matched targets in the presence of cold target blocking cells exhibited three distinct patterns of response: (a) cells from H-2^k mice generated responses predominantly to K^k-TNP; (b) cells from H-2^d mice responded predominantly to D^d-TNP; and (c) cells from H-2^b mice generated equivalent responses to K and D region TNP-self. F_1 hybrids between these parental haplotypes were similarly investigated to analyze their responses to K and D region TNP-self. When the k parental haplotype was expressed in the responder and stimulator cell population, D region TNP responses were weak or undetectable, irrespective of the D region allele. Thus, when the k haplotype was expressed in the F_1, the presence of the b or d did not result in a D region TNP response. However, (responder x non-responder) F_1 lymphocytes did possess the ability to respond to D-TNP self, as assessed by stimulation with the non-k parent. These results are consistent with the regulation by the k haplotype of D region TNP-self cytotoxic responses and a similar regulation of allogeneic cytotoxic responses is under study.

808 INVOLVEMENT OF H-2L GENE PRODUCTS IN CYTOTOXIC T-CELL RESPONSES, T. H. Hansen, R. B. Levy, W. E. Biddison, P. C. Doherty, and G. M. Shearer, NIH, National Cancer Institute, Bethesda, MD 20014 and The Wistar Institute, Philadelphia, PA 19104.
The function of the L antigen, a second D region product, was compared to that of the K and D antigens in various in vitro T-cell cytotoxic (CTL) responses. Results were obtained using alloantisera against L, K or D target cell antigens to block cytolysis by sensitized spleen cell effectors. Our findings indicated: (1) in primary responses to products of the D region, CTLs against L alloantigens were generated in addition to those against D alloantigens; (2) in the response to TNP-modified cells, cytotoxic effectors against L-TNP were not demonstrable under conditions when CTLs against D-TNP and K-TNP were readily detected, suggesting a more limited involvement of L in the recognition of TNP-modified cells; and (3) when responders and targets were D region matched in secondary responses to influenza virus-infected cells, CTL subsets were found which independently recognized L and D in association with virus, indicating an H-2L-restricted component of this response. Therefore, these in vitro cytotoxic experiments demonstrate that L antigens function analogously to K and D antigens in allogeneic and influenza virus-immune CTL responses.

809 FAMILY STUDIES DEMONSTRATE HLA-LINKED GENETIC CONTROL OF THE SPECIFICITY OF HUMAN CYTOTOXIC T CELL RESPONSES TO INFLUENZA VIRUS, William E. Biddison, Stephen Shaw, and Gene M. Shearer, NIH, National Cancer Institute, Bethesda, MD 20014.
The genetic control of the specificity of human in vitro cytotoxic T cell responses to influenza virus-infected autologous cells has been investigated in studies of large families. Virus-specific cytotoxic effectors were generated by cells of all family members, and the pattern of virus-immune cytotoxicity among siblings demonstrated that the determinants which T cells recognize in association with virus are coded by genes closely linked to HLA. Studies of one large family revealed that many family members consistently generated cytotoxic activity against influenza predominately in association with antigens coded by genes of only one of their HLA haplotypes. The patterns of preferential responsiveness were similar among HLA-identical siblings, indicating that preferential responsiveness is controlled by HLA-linked genes. The failure to demonstrate control by HLA-linked regulatory genes (i.e. trans control, or cis control in a recombinant) suggests that the structural gene which codes for each determinant recognized in association with influenza can control, to a large extent, the magnitude of the T cell response which is generated against virus in association with that determinant.

810 MOUSE ALLOANTIBODIES WHICH BLOCK CML BY REACTING WITH KILLER CELLS, Nobukata Shinohara and David H. Sachs, NIH, National Cancer Institute, Bethesda, MD 20014.
In an attempt to produce alloantibodies to the T cell receptor, hyperimmune antisera were raised in various strain combinations of mice. These sera were tested for their ability to block allogeneic CML in the absence of complement using strain combinations chosen to assess effects on the killer cells. Most of these sera failed to show any significant and reproducible blocking effects. However, among C3H anti-B10.BR antisera, a small percentage were found to be capable of significantly inhibiting CML. This effect was attributable to antibodies reacting with the killer population rather than the target cells, since the sera inhibited B10 anti-C3H CML but not C3H anti-B10 CML. Among mouse strains tested, A/J, BALB/c, B10, and B6 strains were sentitive to the blocking effect whereas AKR, CBA, C3H, and DBA/2 strains were insensitive. This sensitivity of killer cells to the blocking effect correlated well with the strain distribution of the Lyt-2.2 antigen. In the presence of complement, these same sera were toxic to 100% of spleen cells of AKR, BALB/c, B10 and DBA/2 strains, with comparable cytotoxic titers. Thus, the blocking activitiy of the sera could not be explained by a nonspecific effect of high-titered antibodies. In order to study the relationship between the antigen(s) responsible for the blocking effect and Lyt-2-linked genes as well as IgCH-linked genes, killer cells from Lyt-2 congenic and Ig congenic strains were tested. Among B6 ($Lyt\text{-}2^b$, $IgCH^b$), B6.Ly2.1 ($Lyt\text{-}2^a$, $IgCH^b$), C3H·SW ($Lyt\text{-}2^a$, $IgCH^a$) and CWB ($Lyt\text{-}2^a$, $IgCH^b$) strains, only the B6 strain was significantly sensitive. These results thus indicate that the target molecules responsible for blocking of killer cells are encoded by a single genetic locus linked to or identical with Lyt-2.

811 H-2 LINKED GENETIC CONTROL OF MURINE T-CELL MEDIATED LYMPHOLYSIS TO SYNGENEIC CELLS MODIFIED WITH LOW CONCENTRATIONS OF TRINITROBENZENE SULFONATE, Anne-Marie Schmitt-Verhulst, Gene M. Shearer, Carla B. Pettinelli, and Richard P. Polisson, Centre D'Immunologie de Marseille-Luminy, Marseille, France, and NIH/National Cancer Institute, Bethesda, MD 20014. The H-2 complex controls the in vitro generation of cytotoxic T-effector cells to trinitrophenyl-modified syngeneic cells (TNP-self) at two distinct but interacting levels: (a) specificity of the effectors generated involves recognition of K and D region self products; and (b) regulation by genes mapping in the K and I regions control the magnitude of the response to H-2D-TNP. In the present study spleen cells from a number of inbred mouse strains were sensitized in vitro to syngeneic cells modified with different concentrations of trinitrobenzene sulfonate (from 10.0 to 0.01 mM TNBS). Cells from $H\text{-}2^{k,a}$ strains generated good cytotoxic activity to TNP-self when stimulated at concentrations down to 0.05 mM TNBS (responder strains). In contrast, $H\text{-}2^{b,d}$ strains did not respond to TNP-self when the cells were modified at concentrations below 0.5 mM TNBS (non-responder strains). These strain-dependent differences were observed at the sensitization phase, but were not detected at the lytic phase by using different TNBS concentrations for modification of either ^{51}Cr-labelled targets or inhibiting cells. Spleen cells from (responder x non-responder) F_1 mice were non-responders when stimulated with F_1 or parental cells modified with low concentrations of TNBS. Mice primed by intravenous injection with TNP-self and restimulated in vitro exhibited similar genetic patterns: i.e. $H\text{-}2^{b}$ mice were not primed, whereas $H\text{-}2^{k}$ mice were primed to TNP-self. These results suggest that some Ir gene effects which may be obscured at high concentrations of modifying of infecting agents but can be detected at more limiting doses.

812 H-2 NONRESTRICTED CYTOTOXICITY ASSOCIATED WITH THE QA-1 LOCUS, Daniel L. Kastner and Robert R. Rich, The Howard Hughes Medical Institute Laboratory and the Department of Microbiology and Immunology, Baylor College of Medicine, Houston, TX 77030

B10.BR ($H\text{-}2^{k}$, $Qa\text{-}1^{d}$) cytotoxic lymphocytes (CTL) generated in secondary *in vitro* cultures against AKR ($H\text{-}2^{k}$, $Qa\text{-}1^{b}$) stimulators lysed targets of several *H-2* haplotypes, including B10 ($H\text{-}2^{b}$, $Qa\text{-}1^{b}$) targets congenic with the B10.BR responder cells. The activity of these effector cells was totally abrogated by anti-Thy 1.2 plus complement. The clones killing B10 targets were not distinct from the AKR-specific clones, since lysis of ^{51}Cr-labeled B10 targets by B10.BR anti-AKR CTL was inhibited by unlabeled B10 and AKR targets equally well. Experiments using targets from over twenty different strains showed that nonrestricted lysis by B10.BR anti-AKR CTL correlated perfectly with the $Qa\text{-}1^{b}$ allele. The use of B6, B6-Tla^{a}, B6-$H\text{-}2^{k}$, B6.K1, and B6.K2 targets confirmed that the *H-2* nonrestricted activity of B10.BR anti-AKR CTL maps with *Qa-1* in the interval between *H-2D* and *Tla*. Moreover, when AKR anti-B10.BR CTL were tested on these panels of targets, *H-2* nonrestricted lysis of $Qa\text{-}1^{a}$ targets was observed. Cold target inhibition and cross-immunization experiments failed to reveal additional antigenic complexity. Studies of T cell-depleted LPS blasts showed that this *Qa-1* associated antigen is not unique to T cells. Although it is not certain that the determinant recognized by CTL is the same as that recognized by anti-Qa-1 antisera, these data suggest that Qa-1 recognition is bidirectional and that *Qa-1*-encoded determinants are found on B cells. The fact that *Qa-1* associated cytotoxicity is not restricted by the classical H-2 antigens suggests that the Qa-1 antigenic system behaves like a part of the major histocompatibility complex. (Supported by USPHS Research Grant AI 13810.)

813 MECHANISM OF RECOGNITION BY CYTOLYTIC T CELLS, Pierre Golstein, K. Shortman and I.C.M. MacLennan, Centre d'Immunologie INSERM-CNRS de Marseille-Luminy, Case 906, F- 13288 Marseille Cedex 2, France.

Within the framework of a systematic sequential analysis of the mechanism of T cell-mediated cytolysis, we recently found that cytochalasin A sensitivity as well as Mg^{++} requirement could be used as metabolic markers for the recognition stage. We then "mapped" these two markers, and further analyzed the metabolic requirements of T cell recognition using as an experimental end-point either "conjugate" formation or ^{51}Cr release. The results can be summarized as follows : (1) The recognition stage includes a first, cytochalasin-A sensitive step, followed by a second, Mg^{++}-requiring step; (2) the metabolic requirements are more for recognition as assessed by conjugate formation than for recognition as assessed by ^{51}Cr release; (3) specific recognition however occurs in both cases, probably during the cytochalasin-A-sensitive step, while the Mg^{++}-requiring step may correspond to non-specific strengthening interactions necessary to resist the resuspension procedure of the conjugate technique.

Main conclusions from these studies are (1) The metabolic requirements of T cell recognition are not fixed, but are imposed by the experimental conditions under which recognition is tested; (2) Specific recognition, sufficient for subsequent cytolysis, may be of weak affinity, with the T cell receptor having more of a "reading" than a "binding" role; (3) The establishment of more tenacious links between a cytolytic T cell and a target cell requires secondary, non-specific interactions, which are not necessary for cytolysis.

814 TARGET CELL LYSIS BY SUPERNATANTS DERIVED FROM ALLOIMMUNE MURINE CYTOTOXIC T LYMPHOCYTES: POSSIBLE ROLE FOR A LYMPHOTOXIN-T CELL RECEPTOR COMPLEX? John C. Hiserodt, Gale A. Granger, and Benjamin Bonavida, UCLA School of Medicine, Department of Microbiology and Immunology, Los Angeles, Ca. 90024

Lymphocytes or purified T cells obtained from alloimmune C57Bl/6 or Balb/c mice, when placed on monolayers of allogeneic fibroblasts, rapidly release (6-8 hr) into the supernatant antigen specific cell lytic material(s). These supernatants could induce rapid (10 hr) and specific lysis of the sensitizing allogeneic target cells during in vitro ^{51}Cr release assays. Analysis of the lytic supernatant revealed the following properties: a) antisera which could neutralize murine lymphotoxin (LT) activity in vitro could inhibit this effect; b) absorption of supernatants on the specific target cells at 4°C removed both the specific lytic activity and nonspecific LT activity detectable on L-929 cells in vitro; c) polyspecific goat anti mouse Ig sera had no effect on this lytic activity, and removal of T cells by anti θ serum + C' removed the capacity of the remaining cells to release these materials; and d) this material(s) was highly unstable. Because the lytic effect could not be shown to be due to classical Ab + C', and since purified alloimmune T lymphocytes yielded the most active supernatants, we feel the data is consistent with the concept that the short-lived specific cell lytic material in these supernatants is a high MW complex containing LT or LT-like molecules in functional association with specific (T cell?) antigen binding receptor(s) molecules. However, the possibility of ADCC mediated by secreted or cytophilic antibody has not been completely ruled out. Supported by NCI CA12800.

815 APPLICATION OF AN IMPROVED SINGLE-CELL CMC ASSAY TO DETERMINE FREQUENCY OF CYTOTOXIC T LYMPHOCYTES (CTL) AND KINETICS OF THE LYTIC EVENT. Elizabeth Grimm and Benjamin Bonavida, Dept. of Micro. and Immunol., UCLA School of Medicine, L.A., Ca. 90024

A highly reproducible single-cell CMC assay, modified from our previously reported single-cell assay (J.Immunol.119:1041, 1977), has been devised by plating effector-target cell conjugate-containing populations in agarose. Following incubation, single target lysis is measured by trypan blue uptake. We used this assay to determine the absolute CTL frequency and the kinetics of single target lysis. Murine BALB/c anti EL-4 peritoneal exudate lymphocytes (PEL) were used as the source of CTL, of which 15% to 30% were observed to conjugate. Single target lysis is evident in a few cells as early as 15 minutes, and proceeds until shortly after two hours, when approximately 80% of the conjugated EL-4 are lysed. Continued incubation results in no further increase in target death. Results from 0 to 2 hours were subjected to computer analysis for determination of the best-fitting mathematical function with known biological relevance. We found that the first-order enzyme kinetics was able to describe our results in a highly significant manner. During early time points, lysis can be approximated to a linear function, however, attempts to fit zero-order kinetics to the entire 0 to 2 hour interval were unsuccessful, with a lack of fit approximately ten times greater than that of the first-order function. The simplest interpretation of our results is that a single rate-limiting step is operational in CMC, and that measurements of target lysis are best represented as an exponential function. Other data from our laboratory suggests this rate-limiting component to be due entirely to the CTL.

816 AUTOREACTIVITY OF F_1 ANTI-PARENT CYTOTOXIC T LYMPHOCYTES, Ichiro Nakamura, Keiichiro Nakano and Gustavo Cudkowicz, State Univeristy of New York, Buffalo, NY 14214

F_1 hybrid anti-parent cell-mediated lympholysis (CML) is inducible in mixed cultures of splenocytes. The specificity of F_1 cytotoxic effectors is determined by the H-2 type of stimulator cells: $B6D2F_1$ ($H\text{-}2^{b/d}$) effectors that primarily lyse $H\text{-}2^b$ or $H\text{-}2^d$ targets are induced by B6 or DBA/2 cells, respectively. $B6C3F_1$ ($H\text{-}2^{b/k}$) splenocytes generate anti-$H\text{-}2^b$ or anti-$H\text{-}2^k$ effectors depending on whether B6 or C3H stimulators are employed. Target structures are genetically controlled by the H-2D region in anti-parental $H\text{-}2^b$ and $H\text{-}2^d$ CML, and by the H-2K region in anti-$H\text{-}2^k$ CML. The cytolytic reaction does not depend on exposure of target cells to fetal calf serum before or during assay. Target cells must be homozygous at the relevant H-2 region for cytolysis to occur; H-2 heterozygous targets are not lysed at E:T ratios of 20 to 80. H-2 heterozygous and homozygous cells are both capable, however, of competitively inhibiting the specific lysis of parental targets, the former cells being less effective than the latter. Structures of inhibitor and target cells recognized by given cytotoxic effectors are controlled by the same H-2 region. Since F_1 cells syngeneic with F_1 effectors function as inhibitors of cytolysis, the anti-parental response is directed, at least in part, against autologous structures controlled by the MHC. Gene dosage or other genetic interactions may account for the failure of F_1 effectors to lyse F_1 targets. Alternatively, restricting MHC determinants of F_1 cells may be sufficient to compete with the set of restricting plus restricted parental determinants that are fully expressed on homozygous cells. According to this explanation, the requirements for target lysis and inhibition of cytolysis would be different.

817 THE USE OF SOMATIC CELL HYBRIDS TO PROBE EFFECTOR CELL-TARGET CELL INTERACTIONS, Rudolf C. Kuppers and Christopher S. Henney, Fred Hutchinson Cancer Research Center, Seattle, Washington 98104

Somatic cell hybrids produced by polyethylene glycol fusion have been used to probe the antigenic requirements for induction of cytotoxic T cells and to investigate the specificity of the killer cells induced. Hybrid cells bearing a variety of MHC encoded cell surface structures have been used to define antigenic requirements for "triggering" cytotoxic T cell responses and to compare such requirements with those structures that are recognised by cytotoxic T cells. Using cells of different cellular origins, hybrids displaying unique sets of antigens have been produced and tested both as stimulator cells and as targets for effector T cells. In this way we are attempting to dissect antigenic requirements for cytotoxic attack from those cellular parameters which determine the intrinsic susceptibility of the cell to lytic attack.

A variety of techniques to separate somatic cell hybrids have been developed using the fluorescence-activated cell sorter (FACS II); the usefulness of such methodologies will be discussed.

818 EVIDENCE AGAINST Ca^{++} POISONING BY KILLER CELLS: MAST CELLS KILLED BY T LYMPHOCYTES DO NOT SECRETE PRELYTICALLY, Eric Martz, Constantine D. Tsoukas, and William J. Wechter, Harvard Medical School, Boston, MA 02115

The lethal hit in tumor cell killing by murine cytolytic T lymphocytes (CTLs) is markedly calcium dependent. We investigated the hypothesis that CTLs kill target cells by selectively admitting a lethal amount of calcium into the target cell. At concentrations from <10μM to 200μM, the calcium ionophore A23187 induced calcium-dependent lysis (^{51}Cr release) of P815 mouse ascites tumor cells or normal rat mast cells within one hour. In the latter case, lysis was preceded by secretion (^{14}C-serotonin release). In contrast, when mouse CTLs sensitized to BN rat cells killed rat mast cells, ^{14}C-serotonin was not released from the target mast cells until after ^{51}Cr. Moreover, such mast cells, serving as targets of CTL attack, lost the ability to secrete in response to compound 48/80 or A23187 prior to releasing ^{51}Cr. Therefore, these results do not exclude the possibility that calcium influx may contribute to target cell demise during CTL-mediated killing, particularly at late stages. However, the present results argue against selective calcium influx being the sole primary event effected by killer T lymphocytes, since prelytic secretion would then have been expected. On the contrary, some other early event during CTL-mediated killing is able to inhibit the secretory function of target mast cells. Supported by NIH grants CA14723 and AI00233, the Runyon-Winchell Cancer Fund and the Upjohn Company.

819 SPECIFIC AND NON-SPECIFIC HELPER T CELLS IN THE GENERATION OF CYTOTOXIC T LYMPHOCYTES, Ronald B. Corley and Kelly A. Switzer, Duke Medical Center, Durham, N.C. 27710

Helper T (Th) cells participate in the generation of cytotoxic T lymphocyte precursors (CTL.P) into CTL. Nothing is known about the ability of a single Th cell to interact with CTL.P or the relationship of these cells to Th cells which interact with B cells, nor is it even clear if all CTL.P require Th cells for functional expression of their activity. We are studying the function of Th cells in the generation of CTL using limiting dilution analysis in microcultures. Thymocytes are used as responder cells with irradiated allogeneic spleen cell stimulators. CTL.P are not limiting, but their activity is not detected unless a source of Th (sensitive to anti-θ + C') is added. Two types of Th have been detected. "Specific" Th cells syngeneic to the thymus donor are enriched after MLR-priming to the H-2 of the stimulator. CTL.P can also be activated by an allogeneic effect, and the frequency of these "non-specific" Th cells is increased after priming to the H-2 of the thymus donor. Titration of allogeneic Th cells follows a straight line on a Poisson plot, while titration of specific Th cells does not. Several reasons could explain these latter results. First, limitation of a second cell type could account for the multi-hit curve, but our results suggest that it is not a non-T cell. Second, our system could be too insensitive to measure the activity of small numbers of CTL. If true, the generation of CTL by syngeneic Th cells must result in a smaller clone size or in the activation of fewer CTL.P than allogeneic help. Third, suppression might prevent activation at low specific Th cell input. Experiments are under way to investigate these possibilities. Our results to date suggest that the activation of CTL.P by allogeneic and syngeneic Th cells proceeds by different mechanisms or is under different regulatory controls.

820 CTL PRECURSORS IN THYMUS: Lyt PHENOTYPE AND SENSITIVITY TO ANTI-CTL OF THYMOCYTE SUBPOPULATIONS, Michael Mage, Bonnie Mathieson*, Ido Betel, and Susan Sharrow, NCI and *NIAID, Bethesda Md. 20014.

Thymocytes from BALB/c and C57BL/6 mice, separated by peanut lectin (PNA) into agglutinated (PNA+) and non-agglutinated (PNA-) subpopulations, were analyzed for Lyt phenotype by flow microfluorometry, for CTL precursors (CTLp) by culture with nonirradiated semiallogeneic target cells, and for sensitivity to a guinea pig antibody specific for spleen CTL and CTLp (cf Rothstein et al. J. Immunol. 120, 209, 1978).

The PNA- subpopulation was significantly enriched for Lyt 1+2- cells(~50%) compared to unfractionated thymocytes(~10% Lyt 1+2-). This PNA- fraction, responsible for 80 to 100% of the recoverable CTLp activity, also contained Lyt 1+2+ cells. CTLp activity was completely abolished by anti-Lyt 2.2, but only partially abolished by anti-CTL. (Spleen CTLp activity is completely abolished by such treatment).

These findings suggest that thymocytes with a differentiated Lyt phenotype (Lyt 1+2-), as well as the Lyt 1+2+ CTLp responsive to alloantigen in vitro, are found in the PNA- population. Two kinds of CTLp can be distinguished in this Lyt 1+2+ thymocyte population on the basis of sensitivity or resistance to anti-CTL. These cell types may represent two different stages of maturation, or two different lineages of CTL precursors.

Origin of Antibody Diversity

821 EXPRESSION OF LATENT ALLOTYPE IN A STOCK OF SJL MICE, Cornelia Kolb and Eberhardt Weiler, University of Konstanz, D 7750 Konstanz,Germany

Mice of strain SJL (immunoglobulin allogroup Ig^b), originally purchased from Jackson Institute and further bred at Konstanz, were immunized against immunoglobulins from strain BALB/c (allogroup Ig^a). Contrary to expectation, 19 of 49 animals did not produce anti-Ig^a antibodies, but instead the "allotypic" determinant of Ig^a itself, indistinguishable from that of BALB/c. The results of crosses between these mice were not compatible with single-gene, dominant mendelian inheritance of the "allotypic" phenotypes. We have so far observed 11 animals to change their phenotype, from one allotype to the expression of the other or of both during the course of anti-allotypic immunization. We conclude that our stock of SJL contains both structural genes for "allotype" in a cis-configuration, and that it has a labile factor controlling their expression. We are now studying the phenotypic expression of allotype-correlated idiotypes in these mice.

822 HYBRIDOMAS EXPRESSING NEONATAL ANTI-DNP CLONOTYPES, Kathleen A. Denis, Roger H. Kennett and Norman R. Klinman, University of Pennsylvania, Philadelphia, Pa. 19174

Analysis of spleen cell populations from Balb/c mice during the first three days after birth has shown the response to 2,4-dinitrophenyl to be relatively restricted (Klinman and Press,J. Exp. Med.141:1133,1975). These early clonotypes can be presumed to reflect either an expression of germ line genes or an early permutation of germ line information. Since the parameters of stimulation and tolerance induction for these neonatal clonotypes are firmly established, the analysis of the specific interaction of these antibodies with antigen should help elucidate triggering mechanisms. In addition, the study of these neonatal specificities at the nucleic acid, amino acid and molecular levels would provide valuable insight into the process of antibody diversity. The use of suitable stimulatory environments for neonatal B-cells in combination with recently developed techniques for the immortalization of antibody secreting cells now makes it possible to isolate these clonotypes for study.

Spleen fragments derived from neonatal B-lymphocytes were stimulated for three days in vitro with DNP-hemocyanin. Fragments which produced anti-DNP antibody on day nine after antigen exposure were teased apart and fused with the mouse myeloma line 45.6TG1.7 (MPC 11). Several hybridomas producing an IgM anti-DNP antibody have been isolated, and two were selected for further study. SDS gel electrophoresis has been used to examine the chains secreted by the hybrids and amino acid sequencing of the 30 amino terminal residues has been completed (Margolies et al.,Immunogenet.,in press). The binding properties of these monoclonal antibodies for antigen are being explored and the relationship of these antibodies to previously defined neonatal clonotypes is being investigated by isoelectric focusing and idiotype analysis.

823 THE GENETIC BASIS OF ANTIBODY DIVERSITY: SEQUENCE DIVERSITY WITHIN BALB/c VK21 KAPPA CHAINS, D. J. McKean, M. Bell, and M. Potter, Mayo Medical School, Rochester, MN 55901 and NCI, NIH, Bethesda, MD 20014

Mouse BALB/c kappa light chain variable regions of the VK21 group have been examined by amino acid sequence analysis in order to determine the pattern of diversity within these structurally related proteins. Even though these VK21 variable (V) regions have nearly identical amino terminal 23 residue sequences, structural homologies found throughout the V region indicate that they can be divided into at least 5 and possibly 7 subgroups. V regions in different subgroups vary from 6 to 22 amino acids with differences occurring in both framework and complementarity determining regions (CDR). The diversity which is observed within subgroups suggests that substitutions within the variable region segment (residues 1 to 95) are limited in number (0 to 2) and tend to concentrate in the CDR. The differences within subgroups appear to be single base point mutations. Position 96, which is presumed to be the first residue of the joining (J) segment, frequently varies in both inter subgroup and intra subgroup comparisons by two base substitutions. At least 3 different J segments are associated with the VK21C subgroup. This suggests that any J segment can combine with any V segment. Since the J segment makes up part of the third CDR it is possible that the combinatorial association of V with J contributes to generate antibody diversity.

824 SEQUENCE DIVERSITY OF NON-T15 ANTIBODIES SPECIFIC FOR PHOSPHORYLCHOLINE. P. J. Gearhart and L. E. Hood, Carnegie Institution of Washington, Baltimore, Maryland 21210, and California Institute of Technology, Pasadena California 91125.

Phosphorylcholine (PC)-specific B cells in BALB/c mice can be divided into two categories: (1) those that produce antibody with the T15 idiotype, and (2) those that produce antibody without the T15 idiotype. Non-T15 B cells specific for PC are diverse and may produce more than 200 unique antibodies. In order to assess the extent of diversity in the non-T15 repertoire, we have prepared anti-PC antibodies by the hybridoma technique and sequenced the N-terminal residues of the variable regions. One affinity-purified antibody (M-1) had the identical sequence in the heavy and light chains for the first 25 amino acids as T15 protein. However, it does not react with A/He anti-T15 idiotype and may be structurally different from T15. Another antibody (G-1) was identical to MOPC 460 for the first 40 amino acids of the heavy chain except for one substitution in the first hypervariable region. It does not have the T15 idiotype and binds dinitrophenyl to some extent. These preliminary analyses indicate that non-T15 antibodies are heterogeneous and can belong to different subgroups than those defined by PC-binding myeloma proteins. More antibodies are being sequenced to determine the extent of diversity in the framework and hypervariable regions.

825 EXPRESSION OF THE V_{k21} VARIABLE REGION ISOTYPE AMONG INFLUENZA-SPECIFIC ANTIBODIES. Judith A. Owen and Michael P. Cancro, University of Pennsylvania Medical School, Dept. of Pathology, Phila., Pa. 19104

The kappa light chain variable regions of murine myeloma proteins have been extensively analyzed by amino acid sequence and serologic techniques. Such studies have revealed several discrete sets of closely related sequences, or subfamilies. Light chains bearing determinants characteristic of the V_{k21} subfamily comprise approximately 5% of kappa chains in normal BALB/c serum, but until now, no antigen-specific immune responses have been described which contain antibodies bearing V_{k21} determinants. We have examined monoclonal influenza-specific antibodies which were produced either by limiting dilution splenic fragment culture or by hybridoma cell lines for the presence of V_{k21} determinants with two antisera: an antiserum raised against the V_{k21} myeloma protein, PC2880; which defines V_{k21} subgroups a,d,e, and f; and an antiserum raised against the V_{k21c} myeloma, PC3741, which recognizes members of V_{k21} subgroups b and c. The results indicate that both antisera detect antibodies present in the BALB/c influenza-specific response. Initial results from reactivity pattern analysis indicate that the distribution of the PC2880 marker is associated with a restricted group of antibodies, suggesting that V_{k21} is required for the generation of particular influenza-binding specificities in the BALB/c mouse.

826 HETEROGENEITY OF λ LIGHT CHAINS IN THE RABBITS. Siegfried Weiss[1], Irene Garcia-Girol[2], Jean-Claude Jaton[2], Dietmar G. Braun[3] and Andrew S. Kelus[3], [1]The Salk Institute, San Diego; [2]Department of Pathology,University of Geneva, Geneva, Switzerland; [3]Basel Institute for Immunology, Basel, Switzerland.

A recently established strain of rabbits, called BASILEA, lacks the expression of κ light chains. This lack is completely compensated by λ chains. When the immune response of BASILEA rabbits against streptococcal A-variant carbohydrate (A-v CHO) was analyzed by isoelectric focussing (IEF), a high degree of heterogeneity was found. This heterogeneity was of the same order as that from standard κ rabbits. Similar results were obtained with different antigens like pneumococcal vaccines and DNP. To reveal the contribution of the light chains to this heterogeneity, light chains from anti-A-v CHO antibodies were isolated and analyzed by IEF. Both types of chains, κ and λ, showed a comparable degree of heterogeneity. This is good evidence that the repertoire of κ and λ chains is of similar size in the rabbit. Unblocked light chains of anti-pneumococcal antibodies from individual BASILEA rabbits were subjected to partial amino acid sequencing. Up to five different amino acids per position in the first five N-terminal residues were identified. Since only up to 40% of the λ chains are unblocked, it suggests that the number of v_λ subgroups and, therefore, the number of v_λ genes is not smaller than that of v_κ. The low contribution of λ chain to the immune response in standard rabbits (5-10%) is therefore not due to a smaller repertoire or a lower number of v_λ germline genes.

827 CHARACTERIZATION OF ALTERED HEAVY CHAINS PRODUCED BY VARIANTS OF THE MPC-11 CELL LINE, Barbara K. Birshtein, Tova Francus, Richard Campbell, Miriam L. Greenberg and Amy Kenter, Albert Einstein College of Medicine, Bronx, N.Y. 10461.

We have isolated several variants of the MPC 11 IgG_{2b}-producing mouse myeloma cell line which synthesize altered immunoglobulin heavy chains, some of which are shorter than the parent, while others have serological, peptide, and assembly characteristics of a new subclass, IgG_{2a}. This latter group is especially interesting since it represents the expression of a previously silent constant region gene. We have shown that the several IgG_{2a} variant proteins retain the parental idiotype, yet differ from each other by peptide maps, charge, and assembly parameters. These same parameters have enabled us to subgroup the variants and lead to the hypothesis that each subgroup will reflect certain common structural features that are related to their genetic origins. A comparison of the partial primary sequence of the MPC 11 Fc region to known sequences of γ2a and γ1 heavy chains has shown considerable differences in the C-terminal CNBr fragment in all three subgroups while in two other stretches, γ2a and γ2b share more identical residues than either shares with γ1. This observation suggested that the γ2a variant chains might have arisen in the MPC 11 cell line by a process of recombination at various sites and that the different γ2a proteins might be hybrid chains containing different lengths of γ2b and γ2a sequences. A study of the Fc region of one γ2a variant heavy chain has shown that it seems completely "γ2a-like" while the Fc of a second γ2a variant seems to be a γ2b-γ2a hybrid with the cross-over point located in CH_2. Partial amino acid sequence studies of a variant synthesizing a short heavy chain of 40,000 MW has shown a major deletion commencing at the junction between CH_2 and CH_3 domains.

Helper and Suppressor Epitopes

828 ALLOHELP STIMULATED BY K OR D ALLOANTIGENS DEPENDS UPON A MATURE Lyl23 CELL. Susan L. Swain, Richard W. Dutton and Peter R. Panfili. University of California, San Diego, La Jolla, California 92093

We have recently found that the Ly phenotype of helper T cells, stimulated by alloantigens, depends on the intra MHC region to which they respond. When T cells and responding B cells differ at whole H-2, I or Mls, the allohelp depends only on Lyl cells in the T population. However, when the MHC difference is restricted to H-2K or D, help depends on the presence of Lyl23 cells. It was not possible in these experiments to determine if another cell type, in addition to the Lyl23 cell,was necessary for the development of help to K/D or whether both precursor and effector helper cell was from the Lyl23 subclass. Since it is possible that the Lyl23 cell is an immature cell playing either a precursor or amplifier role, we have investigated whether allohelp to K and whole H-2 depends on T_1 cells, T_2 cells or both. Our results suggest that allohelp directed against both K and whole haplotype difference depends primarily on T_2 cells. Populations of T_1 cells from ATS injected mice were depleted of both types of helpers and did not synergize with T_2 cells. T_2 populations from adult thymectomized mice contained fewer Ly2 positive cells but retained full helper activity. The help they gave to K but not whole H-2 was still sensitive to anti Ly2 treatment. These results indicate that the Lyl23 cell involved in allohelp to K/D is a mature T_2 cell and suggest that it is neither an amplifier nor an *immature* precursor cell. The difference in the Ly phenotype of T cells which have the same function (allohelp) but respond to different alloantigens (K/D versus I) emphasizes the fundamental dichotomy of the functional role of these two sets of MHC antigens in the immune response.

829 IMMUNOGENIC AND SUPPRESSIVE DETERMINANTS IN THE T CELL PROLIFERATIVE RESPONSE TO LYSOZYME. R.M.Maizels*,J.A.Clarke,M.A.Harvey+,B.A.Araneo',R.Yowell,A.Miller and E.E.Sercarz. Dept. of Microbiology, UCLA, Los Angeles, California 90024.

Hen eggwhite lysozyme (HEL) is immunogenic in all but H-2^b strains of mice. In responder mice there is extensive crossreactivity at the T cell level between native and denatured forms of HEL. Hence fragments of HEL which are in various states of denaturation may legitimately be compared for immunogenicity. In the C57BL/10 (B10) nonresponder mouse there is a similar degree of crossreactivity between HEL and denatured (reduced,carboxymethylated)RCM-HEL in the generation of suppressor T cells. We have analyzed the T cell proliferative responses of B10 and its H-2 congenic partner, B10.A, to HEL,RCM-HEL and to a number of peptide fragments derived from this antigen. Primed lymph node cell responses to these antigens, in conjunction with data on the responsiveness to other sequenced avian lysozymes, allow us to define and delineate the prominent immunogenic epitopes. B10.A mice can be primed for a subsequent proliferative response by native HEL(129 residues),RCM-HEL the fragment NC(1-17,cys6-cys127,120-129),and the major CNBr peptide L_{II} (13-105). B10 mice, unresponsive to HEL or RCM-HEL, do not respond to NC but are primed by the L_{II} peptide for an in vitro challenge with L_{II} itself, HEL or RCM-HEL. Furthermore, B10 PETLEs primed to L_{II} can be suppressed by a small proportion of RCM-HEL-primed cells, if co-cultured in the presence of RCM-HEL. Thus,associated with residues 1-12 or 106-129 there is a determinant which specifically stimulates suppressor T cells in the B10 mouse which can result in the ablation of a proliferative T cell response to the immunogenic portions of the molecule.(Supported by postdoctoral fellowships from *Damon Runyon-Walter Winchell Cancer Fund;+American Cancer Society; 'Naional Institutes of Health)

830 EFFECTOR AND SUPPRESSOR ANTIGENIC DETERMINANTS FOR AUTOIMMUNE ENCEPHALOMYELITIS, Robert H. Swanborg, Wayne State University Medical School, Detroit, MI 48201

Myelin basic protein (BP) is the autoantigen responsible for experimental autoimmune encephalomyelitis (EAE). Structurally, BP is a single polypeptide chain consisting of 170 amino acid residues. In guinea pigs the major encephalitogenic determinant of BP is located within residues 114-122, and synthetic peptides corresponding to this sequence elicit EAE when injected with complete Freund's adjuvant. Guinea pigs can be rendered unresponsive to EAE by pretreatment with BP in incomplete adjuvant (which is nonencephalitogenic) prior to challenge with BP and complete Freund's adjuvant. Peptide 114-122 is not suppressive when employed for pretreatment. However, significant suppression of EAE can be induced with the relatively nonencephalitogenic peptide 44-89 isolated by pepsin digestion of BP. Control experiments render unlikely the possibility that suppression induced with peptide 44-89 is due to trace contamination with peptide 114-122.

Although the mechanism underlying unresponsiveness to EAE in guinea pigs has not yet been elucidated, we have shown that suppressor T lymphocytes mediate suppression of this autoimmune disease in Lewis rats.

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831 ANALYSIS OF ANTIGENIC DETERMINANTS IN THE LYSOZYME MOLECULE INDUCING HELPER AND SUPPRESSOR T CELLS. Luciano Adorini, Michael A. Harvey, Alexander Miller and Eli Sercarz, University of California, Los Angeles, California 90024.

Hen egg-white lysozyme is a small protein (129 a.a.) which, when injected i.p. in CFA, induces a predominant suppression in B10 (non-responder) mice and help in the congenic strain B10.A (responder). We have examined the ability of purified peptide fragments derived from HEL to induce antigen-specific suppression and help in both strains. The L_I peptide (a.a. 1-12) induces suppressor cells in B10 and helper cells in B10.A. The L_{II} peptide (a.a. 13-105) induces helper cells in both strains. Priming with the L_{III} peptide (a.a. 106-129) has no effect in B10 and induces only marginal help in B10.A. These results demonstrate that L_I represents the major antigenic determinant inducing suppressor cells in B10 mice. The activity of this suppressive determinant nullifies the existence of helper cells directed against other determinants on the molecule. If generalized, these findings indicate that: 1) in the response to T-dependent antigens, helper T cells are directed against certain antigenic determinants and suppressor T cells against others. 2) The activity of helper cells, directed against helper epitopes, can be antagonized by suppressor T cells induced by suppressive epitopes on the same molecule. 3) The T cell repertoire reactive with suppressor-inducing epitopes appears to be more restricted than the helper-reactive repertoire. 4) Both helper and suppressor T cells can be triggered by small linear peptides (MW = 1000), obtained from a globular protein antigen. In conclusion, the genetic non-responsiveness of the B10 strain to HEL seems to be a consequence of the activation of suppressor T cells by a small fragment of this antigen. L.A. is supported by a Cancer Research Institute Fellowship.

832 ANTIGEN-SPECIFIC ACTIVATION OF SUPPRESSOR T-CELL SUBPOPULATION AND ITS APPARENT ROLE IN AUTOIMMUNITY. George A. Hashim, St. Luke's Hospital Center, and Columbia University, New York, NY 10025.

Studies have shown that the amino acid sequence of specific regions of the encephalitogenic myelin basic protein are responsible for inducing experimental allergic encephalomyelitis, a cell-mediated, autoimmune and demyelinating disease of the central nervous system. Modification of the amino acid sequence of these determinants rendered them non-encephalitogenic in the respective species. The determinants for the guinea pig are:

H-Phe-Ser-Trp-Gly-Ala-Glu-Gly-Gln-Lys-OH Encephalitogenic

H-(Phe-Ser-Trp-Gln-Lys)$_4$-Gly-OH Non-encephalitogenic

Daily administration of the modified sequence reversed an ongoing fatal disease without altering the immunocompetency of the treated guinea pigs. Further, lymphocytes from animals previously primed with analogous sequences failed to transfer disease but protected recipient animals when the latter were challenged with encephalitogenic emulsions.

These results suggest that the analogous sequences are capable of paralyzing a specific clone of T-cells responsible for demyelination and animals primed with these sequences are capable of transferring suppressor cell-immunity to normal recipients. Recognition of the determinants is species-specific for cell-transfer experiments in the EAE susceptible Lewis rats failed to confir protective immunity in normal recipients.

833 SUPPRESSOR T CELLS IN HUMORAL AND CELL MEDIATED IMMUNITY, Ian A. Ramshaw, University of Saskatchewan, Saskatoon, Sask. S7N OWO

Humoral and cell-mediated immunity are inversely related. This inverse relationship is controlled by differing populations of suppressor T cells (Eur. J. Immunol. 1976, 6, 674 - 1977, 7, 180). Thus, T cells induced under conditions of humoral immunity suppress DTH responses whilst T cells induced under conditions of CMI repress humoral responses. The phenotype of the T cells suppressing humoral immunity are $Ly1^-Ly2^+Ia^+$ whilst the suppressor T cells of CMI are $Ly1^+Ly2^-Ia^-$ (Cellular Immunology 1977, 31, 364). In vitro experiments confirm our in vivo findings, thus intermediate concentrations of antigen preferentially provoke an antibody-forming cell response whilst high and low doses of antigen induce T cells which mediate DTH. The high concentrations of antigen also suppress the antibody response due to the induction of suppressor T cells (Cellular Immunology in press).

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

834 GENETIC CONTROL OF MURINE T CELL PROLIFERATIVE RESPONSE TO COLLAGEN. Lanny J. Rosenwasser and John D. Stobo, Sec. Rheumatology-Clin. Immunol., Dept. of Med., UCSF, San Francisco, CA. 94143

In previous studies it has been demonstrated that the genetic control of the murine T cell proliferative response to insulin is dependent on an Ir gene mediated determinant selection process expressed by macrophages. We have now employed a similar in vitro murine T cell antigen recognition assay, utilizing T cells derived from mouse peritoneal exudates, to gauge the proliferative response of sensitized cells to denatured beef type II collagen. We find that H-2^b mice will respond to collagen, while H-$2^{k,a,d}$ mice are nonresponders. Furthermore, sensitized H-2^b beef collagen immune T cells can be cross stimulated in vitro by highly purified native and denatured rat type II collagen. These immune T cells can also be cross stimulated by the synthetic polypeptides (Gly-Pro-Sarc)n and (Gly-Sarc-Pro)n suggesting that the antigenic determinant in collagen is dependent on Gly and Pro residues. Additionally, the following experiments are now in progress; 1) utilizing other synthetic polypeptides with collagen like structure and properties, we are mapping the molecular determinants (1^o, 2^o, 3^o structure) contributing to T dependent antigenicity in collagen. 2) Using MHC recombinant mouse strains, we are mapping the location of the Ir gene(s) controlling the response of H-2^b to collagen to a specific subregion of H-2.

835 RECOGNITION OF MODIFIED-SELF BY T CELLS REGULATES THE GENERATION OF ANTIBODY BY B CELLS. John J. Jandinski and David W. Scott, Division of Immunology, Duke University Medical Center, Durham, N. C. 27710

Injection of mice with TNP-modifed syngeneic spleen cells produces hapten-specific B cell tolerance, without significantly affecting the generation of cytotoxic T cells. Similarly, in vitro culture of spleen cells with TNP-conjugated spleen cells leads to H-2 restricted T cell cytotoxicity concominant with B cell unresponsiveness to T-dependent and T-independent antigens. Evidence that suppressor T cells (Ts) are involved in this process has already been published. We have now examined the phenotype of these cells and the antigens recognized by them. Briefly, the Ts are Lyl+,2+,3+ in contradistinction from cytotoxic T cells (Lyl$^{+}_{-}$2$^{+}_{-}$3$^{+}_{-}$).

Recognition of modified H-2K-end molecules by T cells (in H-2^{k} mice) results in the generation of Ts cells, whereas recognition of modified-Ia induces the generation of helper T cells. The balance between these activities can be upset by blocking the appropriate TNP-modified MHC-encoded molecules with specific antisera and measuring the subsequent in vitro PFC response. These data reflect the specific genetic requirements which are necessary for the induction of T cell help or suppression by recognition of modified-self cell surface antigens.

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836 SPECIFICITY AND IDIOTYPY OF RAT ANTIBODIES REACTIVE WITH AN ENCEPHALITOGENIC PEPTIDE. Robert B. Fritz, Anne E. Desjardins and Raymond Shapira, Emory University School of Medicine, Atlanta, Georgia 30322.

Injection of peptide 68-88 of guinea pig myelin basic protein into Lewis rats induces both circulating antibody, and a T-cell response, allergic experimental encephalomyelitis. We examined the fine specificity of anti-peptide 68-88 antisera by means of competitive-inhibition radioimmunoassy using peptides 68-88, 68-85, and 79-88. For about 60% of the sera inhibition by peptide 79-88 was equal to that of peptide 68-88, but peptide 68-85 was non-inhibitory. For the remaining sera peptide 79-88 was somewhat less inhibitory than peptide 68-88, and peptide 68-85 showed significant inhibition. These data imply that a major B-cell determinant is located in the region 79-88 of peptide 68-88. Some animals recognized a second determinant in the region of residues 68-85 of peptide 68-88 as well. In order to investigate idiotypic determinants associated with antibodies to peptide 68-88, rabbits were immunized with antibodies purified from individual rat sera by affinity chromatography. The resultant rabbit antisera were then exhaustively absorbed with pooled Lewis rat immunoglobulin and assayed for the ability to inhibit binding of radiolabeled peptide to the rat antibodies used for immunization. In all cases almost complete inhibition was achieved. The rabbit antisera were then tested in the same manner against a panel of rat-anti-peptide antibodies. The degree of cross-idiotypic reactivity was dependent upon the rabbit antiserum used, and in some animals the cross-idiotypic pattern correlated with the fine specificity of the rat antibodies used for immunization.
Supported by USPHS NIH grants NS10721 and NS11418.

837 MINIMUM AMINO ACID SEQUENCES REQUIRED FOR PRIMING AND TRIGGERING OF CELLS INVOLVED IN ANTIBODY,DELAYED HYPERSENSITIVITY AND T-CELL PROLIFERATIVE RESPONSES IN MICE, Bhagirath Singh and Kwok-Choy Lee,Department of Immunology and MRC Group on Immunoregulation, The University of Alberta,Edmonton,Alberta, CANADA T6G 2H7.

Using sequence defined synthetic peptide antigens , TNP-[Glu-Tyr-Lys-(Glu-Tyr-Ala)$_n$],where n=0,1,2,3, and 5,we have been able to define the minimum amino acid sequences required for the priming and triggering of the immune responses in mice.A peptide of at least nine amino acids (n=2),is required for the in vivo priming both in humoral and cell-mediated immunity, however,it is not large enough to trigger the effector cells involved in these responses. The homologous twelve amino acid peptide (n=3),along with priming could also trigger the antibody forming cells and the effector T-cells involved in the delayed hypersensitivity and the in vitro T-cell prolferation assay.Using these peptides and related synthetic polypeptides of defined sequence and conformation,we have shown that the same Ir-genes in the mouse MHC control the expression of in vitro T-cell proliferation ,delayed hypersensitivity and the antibody responses and that these genes control the immune responses by recognising the amino acid sequences rather than the conformation of these antigens.We also have evidence that the T-cells involved in the in vitro T-cell prolieration can transfer specific delayed hypersensitivity in mice.
(Supported by MRC and NCI of Canada.)

838 "IDIOTYPIC" AND "CONSTANT" REGION DETERMINANTS OF ANTIGEN SPECIFIC HELPER AND SUPPRESSOR FACTORS, S. Kontiainen, I. Todd and M. Feldman, Dept. of Bacteriology and Immunology, University of Helsinki, Finland and Zoology Department, University College London, England.

There is now increasing evidence of shared idiotypes of antigen specific receptors on B and T cells, as well as of idiotypic structures expressed on functional subsets of T cells. To test whether idiotypic structures were present on antigen specific suppressor and helper factors, which mediate the function of the cells they are derived from, syngeneic, allogeneic and hetero-antisera were raised against purified (eluates of antigen columns) suppressor and helper factors. The anti-suppressor factor (αSF) and anti-helper factor (αHF) antibodies obtained by immunizing syngeneic mice inactivated suppressor/helper factors of the original strain and antigen specificity, and some factors of unrelated strains but of the same antigenic specificity suggesting that such mouse αSF (αHF) recognised determinants of or within the antigen combining site, eg. "variable" region or "idiotype like" determinants. Genetic linkage studies of these "idiotype like" determinants to Ig-allotype and/or H-2 are in progress by using allotype congenic and backcross mice.

Unlike mouse anti-factor antisera heteroantisera to factors seem to recognise "constant" regions (shared by all suppressor factors) of the factors. As αSF does not inactivate helper factors and vice versa, the "constant" region determinants seem not to be shared by helper and suppressor factors. These sera may be useful probes to investigate the role of factors of this type in the in vitro immune response.

839 SUPPRESSOR MECHANISMS IN SPECIFIC TRANSPLANTATION TOLERANCE ACROSS HISTOINCOMPATIBLE BARRIERS IN MICE. Shimon Slavin, Department of Medicine A, Hadassah University Hosp. Jerusalem, Israel and Samuel Strober, Department of Medicine, Stanford University School of Medicine, Stanford, California.

Stable bone marrow (BM) chimerism can be established between histoincompatible mice, rats and outbred dogs following pretreatment of recipients with total lymphoid irradiation (TLI) followed by administration of approximately 0.3-1.0 x 10^9 BM cells/kg body weight. In rodents TLI consist of 17 daily fractions of 200 rads delivered to the lymphoid organs including the thymus and spleen. Chimerism was not associated with overt clinical signs of graft versus host disease (GVHD). BM recipients develop specific tolerance to donor type alloantigens. BALB/c ($H\text{-}2^d$) recipient mice maintained C57BL/Ka skin allografts (> 360 days) and Lewis (AgB^1) recipient rats maintained ACI (AgB^4) skin and heart allografts (>360 days) but both rejected third party skin allografts. The specificity of the tolerance was also documented in vitro using a one way mixed lymphocyte reaction (MLR). Specific and permanent (> 360 days) tolerance to allogeneic skin allografts was adoptively transferred with 25 x 10^6 spleen cells from tolerant mice. Specific suppression of the reactivity of recipient against donor-type lymphocytes by spleen cells from tolerant mice was demonstrated in vitro using co-culturing techniques in one way MLR systems. It is suggested that specific as well as non specific suppressor cells are generated following TLI. Specific suppressor cells seem to be the mechanism by which chimeras acquire specific transplantation tolerance since it can be adoptively transferred.

Immune Recognition and Regulation in Syngeneic Tumor Systems

840 PREVENTION OF MURINE SARCOMA VIRUS INDUCED TUMORS IN AGING MICE BY THYMECTOMY IN YOUNG ADULT LIFE. Carol L. Reinisch, Sidney Farber Cancer Institute and Harvard Medical School, Boston, Mass. 02115

The Murine Sarcoma-Leukemia viral complex induces myosarcomas in weanling mice which usually regress 20 days after injection of the virus. Depending on the strain of mouse studied, a certain percentage develop secondary lymphomas late in life. Reasons for the development of tumors associated with aging are unclear although an imbalanced immune response has been proposed as a possible mechanism.

Recent work in our lab has shown that thymectomy (tx) of young mice leads to enhanced cytolytic T cell reactivity against syngeneic tumor cell lines. To explore whether tx would affect the pathogenesis of virus-induced tumors in vivo, C57Bl/6 or Balb/c mice were sham tx or tx at 3-4 weeks of age and inoculated with MSV. Mice were killed at specified intervals after MSV inoculation, and their T cells assessed for the capacity to respond in vitro to syngeneic tumor cells bearing the appropriate viral determinants. We find, for example, that T cells from tx Balb/c mice given MSV have dramatically enhanced reactivity against LSTRA tumor cells. More importantly, in comparing the two groups of mice, we find that 25/30 sham tx mice given virus develop lymphomas by ten months of age, while, in contrast, 4/30 mice tx and given virus develop tumors.

At least two mechanisms of host protection appear to be operative: 1. the removal of suppressor cells by atx results in the enhanced generation of cytolytic T cells directed against syngeneic tumor cells and 2. tx removes a lymphoid organ known to be a site of viral replication. We conclude that tx protects mice against MSV-induced lymphomas associated with aging.

841 EFFECTOR AND SUPPRESSOR MECHANISMS IN ANTITUMOR IMMUNITY, R.A. Daynes, C.W. Spellman, L.K. Roberts and M.K. Schmitt, Univ. of Utah, Salt Lake City, Utah 84132

Normal mice challenged with syngeneic UV-induced tumors will generally reject the tumor implant while mice preexposed to the effects of ultraviolet light (UVB) will grow the tumors progressively. We have established that an effective anti tumor response requires or is directly mediated by a population of Ia^-, radioresistant T-lymphocytes. The development of this response, however, requires the involvement of Ia^+ accessory cells which are probably macrophages as well as a differentiation step which is extremely radiosensitive. Effective anti tumor immunity has proven to involve the presence of effector T-cells with at least two specificities: one which recognized the unique tumor specific transplantation antigens (TSTA) and another which recognized common tumor associated antigens (TAA). The outcome of a primary host-tumor interaction within this system (acceptance or rejection of the tumor implant) is controlled by a suppressor T-cell mechanism. Ultraviolet light causes, in exposed animals, an augmentation of suppressor T-cell activity capable of effectively controlling the expression of anti TAA specific immunity. The cell type responsible for this activity is a T-lymphocyte, Ia^+, extremely radiosensitive and is present within the spleen, lymph nodes, and thymus of UV exposed as well as tumor bearing mice. These studies indicate that anti tumor immunity is extremely complex as evidenced by the plethora of interrelated mechanisms which are capable of operating either synergistically or antagonistically with respect to one another. They also suggest that while an antigenic disparity between the host and tumor must exist for an immunologic response, its nature may be much more dependent upon whether a suppressor or an effector mechanism provides the dominant influence.

842 IMMUNE RESPONESE TO WEAKLY IMMUNOGENIC VIRALLY INDUCED TUMORS. OVERCOMING LOW RESPONSIVENESS BY PRIMING MICE WITH A SYNGENEIC IN VITRO TUMOR LINE OR ALLOGENEIC CROSS-REACTIVE TUMOR. D. Naor and B. Bevens. Lautenberg Center for General and Tumor Immunology, The Hebrew University-Hadassah Medical School, Jerusalem.

The immune response in both humans and experimental animals to many syngeneic tumors is very low. Attempts to overcome the low responsiveness to these tumors have had limited success. This report describes model systems which show low primary in vitro syngeneic cytotoxic responses to Moloney induced YAC tumor (syngeneic in A mice) and Rauscher induced RBL5 tumor (syngeneic in C57Bl/6 mice) and examines different approaches to overcome these defects. Two major findings were obtained: a) Spleen cells from A mice, injected with tumor cells from an in vitro tumor line YAC-1, derived from YAC could generate a significant syngeneic cytotoxic response. In contrast, spleen cells from A mice injected with tumor cells from the in vivo tumor line failed to generate a syngeneic cytotoxic response. Thus, tumor cells from the in vitro line were more immunogenic than tumor cells from the in vivo line. b) Spleen cells from A mice which were injected with the cross-reactive allogeneic tumor RBL5 could generate significant cytotoxic responses to the syngeneic tumors YAC and YAC-1. Similarly, spleen cells from C57Bl/6 mice injected with the cross-reactive allogeneic tumor YAC-1 could generate a significant cytotoxic response to the syngeneic tumor RBL5. Thus, cross-reactive allogeneic tumor could stimulate syngeneic cytotoxicity. The in vivo applications of these findings were also successful. Mice which were primed with the in vitro mitomycin C' treated YAC-1 tumor completely rejected viable challenge of YAC tumor cells .

843 THE PRESENCE OF Fc RECEPTOR-LIKE MATERIAL AND OF ANTIBODIES DIRECTED AGAINST Fc-RECEPTOR POSITIVE T-CELLS IN MURINE SARCOMA ELUATES. Maya Ran and I.P. Witz, Department of Microbiology, Tel Aviv University, Tel Aviv, ISRAEL.

Low pH eluates and short-term culture supernatants of freshly explanted murine Polyoma-virus and methylcholanthrene-induced sarcomas contained antibodies with the capacity to mediate complement-dependent lysis of Fc receptor (FcR)-positive 15178Y T-cell lymphoma, of normal thymocytes and of T-cells residing in normal spleen and lymph nodes. These antibodies, in the presence of complement, also inhibited rosette formation between L5178Y cells or allo-activated T-cells and antibody-sensitized sheep erythrocytes (s-RBC, EA rosettes). Eluates which were not cytotoxic towards T-cells, did not inhibit EA rosettes. Some tumor eluates also contained an FcR-like material detected by haemagglutination (HA) of s-RBC sensitized with mouse anti s-RBC. The possible involvement of antibodies directed against mouse Ig which may be present in the tumor eluates was excluded by HA of DNP-conjugated s-RBC sensitized with a subagglutinating dose of rabbit antibodies directed against DNP. Additional proof for the presence of FcR-like material in tumor eluates was obtained from fractionation experiments. FcR-like material purified from tumor eluates by affinity chromatography on Sepharose IgG gave a similar elution pattern as that of FcR purified from L5178Y culture supernatants. Based on these results we propose a working hypohtesis postulating the involvement of FcR and of an immune response directed against FcR or against FcR-expressing immunocytes in tumor-host immune relations.

844 H-2 LINKED RESISTANCE TO SPONTANEOUS AKR LEUKEMIA: A MECHANISM. Daniel Meruelo *+, Dawn Smith *, Nanette Flieger *, and Hugh O. McDevitt ++. N.Y.U. Medical Center, N.Y. 10016, and ++ Stanford University School of Medicine, CA 94305. At least two distinct genes within the major murine histocompatibility complex, H-2, affect virus-induced leukemogenesis. Studies by Lilly et al. have shown that one of these loci, Rgv-1, maps within the K end of the H-2 complex, comprising the K and I regions. The close or identical mapping of Rgv-1 with the I region has suggested that H-2 linked resistance to virus-induced leukemogenesis may result from a stronger immunological response to a given virus-induced antigen. Although data from several labs have tended to favor such a view, no direct evidence for such a mechanism has been previously obtained. The studies reported herein will provide clearcut data for this interpretation. It will be reported that humoral and not cell-mediated immunity plays a key role in survival from AKR-induced neoplasia, and that such humoral responses are T-dependent. Furthermore, an Ir gene mapping in either the A, B, J, or E subregion of the I region determines high responsiveness and overall survival. The helper T cells have been found to express I-J and Ly-1, but not Ly-2 determinants. High responsiveness is dominant and low responsivenss may result from the action of Ly-2, 3 positive suppressor lymphocytes. This mechanism of resistance is distinct from H-2D linked mechanisms such as those affecting radiation-leukemia virus-induced neoplasia. (Supported by NIH grants no. CA 22247, The American Cancer Society IM-163; +Leukemia Society of America Scholar).

845 HYBRID RESISTANCE TO MPC-11 CONTROLLED BY A LOCUS NOT LINKED TO H-2. Mary Clare Walker and Julia M. Phillips-Quagliata. N.Y.U. Med. Cntr., New York, N.Y. 10016 In studies of hybrid resistance to the BALB/c plasmacytoma MPC-11, hybrids between BALB/c and C57BL/10 (B10), B10 congenic resistant (CR) strains, C57L, C57BL/Ks, DBA/1 and AKR were found to be partially resistant to 3 x 10^5 viable MPC-11 cells, a tumor dose which kills 100% of BALB/c mice. Hybrids between BALB/c and A, A CR strains, DBA/2 and SJL were as susceptible as BALB/c themselves. Experiments with (B10 x BALB/c) F_1 hybrids backcrossed to BALB/c indicate that a single, dominant, autosomal gene or gene complex of B10 origin controls resistance to MPC-11. This gene, which we call Rtp (for resistance to transplantable tumor) is not linked to H-2; in its presence slight differential effects of various H-2 haplotypes on resistance can be seen. In hybrids between BALB/c and the 7 Bailey recombinant inbred strains Rtp has the same strain distribution pattern as Ly-4 and Ea-4, thus it could be linked to one of these genes. The susceptibility of BALB/c and hybrids between BALB/c and BALB/c CR strains to MPC-11 is attributed to their lack of the positive allele of Rtp (Rtp^-). The susceptible hybrids between BALB/c and SJL, DBA/2, A and A CR strains could also be Rtp^-. However, they express the G_{IX} determinant also present on MPC-11 but absent from normal BALB/c mice. Experiments with hybrids between BALB/c and $G_{IX}+$ and $G_{IX}-$ congenic strains suggest that the expression of G_{IX} or another product of the Gv-1 gene, even in the presence of Rtp^+, may render certain hybrids susceptible to MPC-11. The mechanism whereby Gv-1 exerts this effect is unknown but may involve the induction of tolerance, suppression or enhancing antibody. Supported by ACS grant VC-253 and in part by NIH CA 20045 and CA 16247.

846 EFFECTOR T LYMPHOCYTE GENERATION AND SV40-SPECIFIC TUMOR TRANSPLANTATION IMMUNITY. Linda R. Gooding, Duke University Medical Center, Durham, NC 27710 SV40 transformed cells express a strong virus-specific transplantation antigen. Immunization with syngeneic, allogeneic or xenogeneic SV40 transformants confers resistance to a challenge of SV40-induced tumor cells. Sensitization of mice with syngeneic SV40 transformants also induces cytotoxic T lymphocytes which specifically lyse syngeneic, SV40-transformed target cells. If these cytotoxic cells are involved in in vivo tumor resistance, they should be generated under conditions which induce transplantation immunity. We have therefore investigated the effect of immunization with allogeneic and xenogeneic SV40 transformants on generation of T cells cytotoxic for syngeneic SV40 target cells. Production of "syngeneic-restricted" cytotoxic spleen cells in this system, assayed by 4-6 hr ^{51}Cr-release, requires both priming in vivo and secondary restimulation in vitro with syngeneic SV40 transformants. Neither primary in vivo nor secondary in vitro stimulation is sufficient to produce detectable levels of cytotoxicity. Sensitization by one or more in vivo injections of allogeneic or xenogeneic SV40 transformants followed by in vitro stimulation with the same cells failed to generate effectors capable of lysing the syngeneic SV40 target cells. However, priming with allogeneic or xenogeneic cells led to a potent syngeneic-restricted cytotoxic T cell response when syngeneic SV40 cells were used in the secondary in vitro sensitization. The mechanism of priming is unknown, and it may reflect expanded pools of either a helper cell population or cytotoxic cell precursors in vivo. In either case, immunization conditions which produce SV40-specific transplantation immunity also prime for an enhanced cytotoxic response to syngeneic tumor cells, and assay for priming may provide an in vitro correlate of in vivo tumor immunity.

847 USE OF CONTINUOUS CYTOTOXIC T CELL LINES TO STUDY THE ROLE OF THE MAJOR HISTOCOMPATIBILITY COMPLEX IN THE T CELL RESPONSE TO MURINE PLASMACYTOMA ANTIGENS, Janis V. Giorgi and Noel L. Warner, The University of New Mexico School of Medicine, Albuquerque, NM 87131

As an approach to the characterization of the target cell specificities against which tumor specific cytotoxic T cell (Tc) responses are directed, we have generated continuous lines of cytotoxic T cells reactive against syngeneic plasma cell tumors (PCTs). The continuous lines are initiated by priming spleen cells in vitro with the tumor cells of interest, and are maintained by cultivation in supernatants from Concanavalin A activated spleen cells. Such lines retain specific cytotoxicity for the PCT against which they were generated, but are not cytotoxic for certain other PCTs or lymphomas. Continuous Tc lines are now being used in our investigations on the role of the major histocompatibility complex (MHC) in the Tc response to PCT antigens. These investigations involve characterizing the tumor associated antigens found on a series of plasmacytomas which we have induced in several BALB/c.H-2 congenic strains, and evaluating the role of the MHC in the Tc response to these PCT antigens. In order to evaluate the possible relationship of MHC antigens and PCT antigens on the cell surface, continuous Tc lines are being studied for their ability to be inhibited by membrane preparations and solubilized components from syngeneic and allogeneic plasmacytomas.

848 GENETIC CONTROL OF THE CYTOTOXIC T-CELL RESPONSE TO SV_{40} TASA, K.Pfizenmaier and B.B.Knowles, Inst.f.Med.Microbiology, Mainz/Germany and The Wistar Institute,Philadelphia

H-2 restricted T-cell mediated cytotoxic responses to SV_{40} tumour associated specific antigen (TASA) were investigated in B10 congenic mice of the s,k, b, f, and q H-2 haplotypes. Generation of CTL was associated with most, but not all of the respective H-2 K and D molecules: No responses to SV_{40}TASA could be demonstrated with either H-2K^d, K^q or D^k. With the mouse strains tested so far no complementation of the ability to mount cytotoxic responses was observed in congenic recombinant and F_1 (responder x nonresponder) hybrid mice. However inhibition of the response to SV_{40} TASA associated with D^d was obtained in some cis and trans combination, e.g. (B10 x B10.D2)F_1 mice were found to be completely nonresponsive to SV_{40} TASA in association with D^d, though responding very well to K^b and D^b. At least partial inhibition was found in (k x d)F_1 mice and (f x d)F_1 mice. Comparing the cytolytic activity of CTL's generated in AQR ($K^qI^kD^d$) versus B10.A ($K^kI^kD^d$) mice preliminary data suggest that gene(s) located within the K-region of the H-2 complex control the D^d associated response to SV_{40} TASA. Possible mechanisms resulting in the observed inhibition will be discussed.

849 CELL-MEDIATED CYTOTOXICITY TO A RAT GROSS VIRUS-INDUCED LYMPHOMA, Irwin D. Bernstein and Robert Nowinski, Fred Hutchinson Cancer Research Center, Seattle, Washington 98104

In order to define the specificity of the cell-mediated response to syngeneic W/Fu rat Gross virus-induced (C58NT)D lymphomas, we evaluated a secondary in vitro cellular response of spleen cells from rats primed with lymphoma cells or with disrupted murine leukemia virus from AKR mice (AKR-MuLV). In initial studies, spleen cells obtained from rats primed with 10^7 (C58NT)D cells were cultured for a five day period with mitomycin inactivated (C58NT)D cells or AKR-MuLV. Results showed both the lymphoma cells and as little as 0.25 ug AKR-MuLV/ml caused the spleen cells to proliferate and differentiate into effector cells cytotoxic for ^{51}Cr labelled (C58NT)D targets but not allogeneic target cells. Specificity was further shown when spleen cells from W/Fu rats primed to BN alloantigen, upon in vitro exposure to stimulating BN cells but not AKR-MuLV became cytotoxic for the allogeneic and not (C58NT)D targets. Only tumor cells, but not disrupted virus, could competitively inhibit the cytotoxic effector cells in the 4-hour ^{51}Cr release assay. In other experiments, spleen cells from rats primed in vivo with either 10^7 mitomycin inactivated (C58NT)D cells or 40 ug AKR-MuLV in CFA were cultivated in vitro with lymphoma cells or AKR-MuLV. Spleen cells from rats primed with virus proliferated but did not become cytotoxic upon cultivation with virus or tumor; cells from rats primed with tumor proliferated and also developed cytotoxic activity. The results of this study show AKR-MuLV to be associated with the cytotoxic response of the rat Gross virus-induced lymphoma. The results also demonstrate differences in the requirement for cell-associated as compared to cell-free viral antigen in the generation of primary and secondary cytotoxic cell responses.

850 REACTIONS OF MONOCLONAL ANTIBODIES WITH MURINE LEUKEMIA VIRUSES. M.R. Stone, M.R. Tam, M. Lostrom, and R.C. Nowinski, Fred Hutchinson Cancer Research Center, Seattle, WA 98104

Endogenous murine leukemia viruses (MuLV) produce proteins which are expressed on the surfaces of lymphocytes of mice from high leukemic strains. Fusion of the mouse myeloma NS-1 with lymphocytes from strain 129 mice that were immunized with AKR leukemia cells has yielded hybrid B cell lines which produce monoclonal antibodies against the antigens of several of these viruses. Hybrids were initially screened by antibody binding assay on six viruses, including ecotropic, xenotropic, and recombinant amphotropic MuLV. Hybrids producing antibodies against each of the individual viruses were found. After cloning and stabilization of the hybrid cell lines they were reassayed and four patterns of antibody binding were observed: 1) Reactions with group specific antigens on ecotropic, xenotropic and recombinant viruses, 2) reactions with class specific antigens on ecotropic and recombinant but not xenotropic viruses, 3) reactions specific for AKR virus and 4) reactions specific for a virus that was associated with the leukemia cells. Hybrids producing antibodies reacting specifically with xenotropic viruses were initially scored but were unstable. Investigations are in progress to define the viral antigens with which each of these antibodies reacts. The finding of different patterns of reactivity suggests the possibility of a new viral classification method based on the use of monoclonal antibodies.

851 TUMOR ASSOCIATED ALTERATION IN H-2 ANTIGEN EXPRESSION, by W. John Martin, Masahiro Imamura, Barbara Pope, Robert Justice and Andrew Hapel, Bureau of Biologics, FDA, Bethesda, Md. 20014

The K and D regions of the H-2 major histocompatibility complex (MHC) perform an essential role in the development and specificity of T cell mediated recognition of allogeneic and virally infected syngeneic cells. An indication that this phenomenon may reflect a basic immune surveillance function against autochthanous tumors may come from studies indicating altered expression of MHC coded antigens on tumor cells. Specifically we have observed that the tumor associated transplantation antigen on several transplacentally induced lung tumors of C3HfeB/HeN mice (C3Hf) is coded by the K region of the MHC. C3Hf mice have apparently deviated from mice of known $H\text{-}2^k$ haplotype in the expression of an H-2K region coded alloantigen. Several transplacentally induced tumors of both C57BL/6 and DBA/2 strain mice appear to have acquired the H-2K coded antigen cross reactive with the C3H but not C3Hf associated alloantigen. In addition there is evidence that diminished expression of the K region coded alloantigen distinguishing C3H and C3Hf mice is a common, but not invariable finding on tumors derived from $H\text{-}2^k$ and $H\text{-}2^a$ strain mice. Ongoing studies are aimed at investigating a possible relationship between tumor-associated and viral-associated alteration in H-2K antigen expression.

852 *CHARACTERIZATION OF THYMIC SUPPRESSOR CELLS AND A FACTOR WHICH SUPPRESSES THE* GENERATION OF CELLS CYTOTOXIC FOR A SYNGENEIC TUMOR IN DBA/2 MICE. Julia G. Levy, Thomas Maier and Douglas G. Kilburn, The University of British Columbia, Vancouver, B.C., Canada V6T 1W5.

Antigen specific suppressor cells and suppressive extracts obtained from the thymuses of DBA/2 mice bearing small syngeneic P815 mastocytomas, were compared for their immunogenic properties and requirements. The assay for specific suppression involved the ability of either cells or extracts to inhibit the primary in vitro cytotoxic response of normal DBA/2 splenocytes to mitomycin treated P815 cells. It was shown that pretreatment of suppressor cell populations with anti-Ia^d antiserum plus rabbit complement removed the suppressive activity. Similarly, absorption of the suppressor factor with anti-Ia^d antiserum removed the suppressive properties of the material. It was found that the suppressor cells, generated in DBA/2 tumor bearers were capable of specifically suppressing the anti-P815 response of B6D2 F1 radiation chimeras possessing lymphoid cells of the $H\text{-}2^b$ or $H\text{-}2^{bd}$ haplotype equally as well as they could suppress the response of $H\text{-}2^d$ bearing effector cells. This indicates that the suppressor cells are not H-2 restricted with respect to K or D markers on responder cells in this system.

853 DETECTION OF CIRCULATING CELLS FROM SOLID TUMORS, F.S. Ligler, R.G. Smith, J.K. Kettman, E.S. Vitetta, J.B. Himes, E.P. Frenkel, and J.W. Uhr.

We have developed a method for the detection of cells from solid B cell tumors in the peripheral blood of nonleukemic patients. We stained cells from both lymph node and blood with affinity purified anti-light chain reagents and, using the fluorescence activated cell sorter (FACS), counted the number of cells staining with discrete levels of fluorescence intensity. Since neoplastic B cells are monoclonal with respect to light chain type, we expected to find an excess of cells staining for one light chain type ("clonal excess") in a narrow range of fluorescence intensity. In all lymph node samples from patients with malignant lymphocytic lymphomas, we found a marked clonal excess among the brightly stained cells. Seven of 8 peripheral blood samples from these patients contained an excess of bright cells with the corresponding light chain type. This clonal excess was evident in the peripheral blood of lymphoma patients even though the blood cells were normal by morphological criteria and the number of tumor cells detected represented only 0.4-9.3% of circulating leukocytes. Samples from normal individuals and patients with unrelated disorders did not show clonal excess.

854 MATURATION WITHIN MURINE PLASMACYTOMAS, Michael J. Daley and Noel L. Warner, University of New Mexico School of Medicine, Department of Pathology, Immunobiology Laboratories, Albuquerque, New Mexico 87108.

A wide variety of B cell neoplasms in man and mouse have been described which appear to represent a maturational arrest at various stages within normal B cell differentiation. In the murine system, representatives of the pre-B cell, B cell, activated B cell and mature plasma cells have been found. In the present study we have examined a number of BALB/c and BALB/c. H-2 congenic derived plasmacytomas. Specifically, we are interested in the total maturational spectrum that may be found within the individual myeloma populations.We have found these various myelomas to show varying degrees of heterogeneity in terms of their total immunoglobulin (Ig) secreted, % of Ig secreting cells, % of membrane Ig positive cells, % of cells and quantity of B cell differentiation antigens expressed (Ly-4, Ly-7, Pca-1 and Ia) and the % of stem cells, as measured by both in vitro and in vivo colony assays. In some cases the stem cell population has been enriched by biophysical methods and has been shown to segregate from the secretory population. These studies have indicated that many murine myelomas and B cell lymphomas may contain subpopulations of cells at varying stages of differentiation, although the population as a whole may indicate arrest of a particular stage in terms of their maturational phenotype. Current studies are analyzing the mechanism by which this maturation may occur and whether it can be specifically immunoregulated by normal factors.

Learning of H-2 Restriction and Lymphocyte-Virus Interactions

855 NEGATIVE SELECTION STUDIES INVOLVING H-2 GENETIC RESTRICTION OF VACCINIA IMMUNE CYTOTOXIC T CELL RESPONSES, Jack R. Bennink and Peter C. Doherty, The Wistar Institute and University of Pennsylvania, Philadelphia, PA 19104

Negatively selected (removal of alloreactivity) throacic duct lymphocytes have been used to study responses of immunologically naive T cells to vaccinia virus in the context of H-2 alloantigens not encountered during development. Results show that in all cases tested so far (excepting non-responder haplotypes) T cells can respond to vaccinia virus in the context of self H-2 antigens. In some haplotype situations, negatively selected T cells can also be stimulated to kill vaccinia virus in the context of alloantigens not encountered during development. This killing is not reciprocal (i.e., for instance, there is killing of infected H-2K^k targets by H-2^b haplotype cells but not killing of H-2^b target cells by H-2^k effectors) and is apparently independent of concurrent presence of effector populations stimulated by self-H-2 and vaccinia virus. These findings make it extremely unlikely that the T-cell expresses two different and anatomically distinct receptors, one specific for H-2 antigen and the other for virus. Studies of T cell responsiveness to vaccinia at the H-2D^b locus show that "high responder" T cells can be stimulated in either a "high or low responder" environment, whereas "low responder" T cells can only be stimulated in a "high responder" environment. These results may be interpretable as evidence against "low responsiveness" being determined by an effect of thymus mediated solely at H-2D^b.

856 MOLECULAR MODIFICATIONS IN VSV-INFECTED CELLS,Paul L.Black,Ellen S.Vitetta,James Forman, Chil-Yong Kang and Jonathan W.Uhr, University of Texas Health Science Center,Dallas,TX 75235. Lysis of infected target cells by virus-immune syngeneic effector T lymphocytes requires expression of both viral determinants and H-2D or H-2K antigens. However, the molecular nature of the surface modifications which effector cells recognize remains unknown. Three major observations emerged from our studies on molecular changes in P815 cells infected with vesicular stomatitis virus (VSV). 1) Glycosylation of H-2 and/or viral glycoprotein is a prerequisite for lysis of infected cells by VSV-sensitized, H-2 identical killer T cells. Treatment of P815 cells before and during VSV infection with the antibiotic Tunicamycin, which specifically inhibits addition of sugars to polypeptides of glycoproteins, inhibited both glycosylation of proteins and lysis of infected P815 cells by VSV-specific killer cells. 2) H-2 disappears from the surfaces of VSV-infected cells or is antigenically altered. Enzymatic radio-iodination of the surfaces of P815 cells 3 hr. after VSV infection revealed no detectable surface H-2 by immunoprecipitation. However, when cells are radio-iodinated before infection, H-2 is detectable. 3) Viral protein(s) associates with a cellular protein already present on the cell surface before VSV infection. Immunoprecipitation with rabbit anti-VSV serum (α-VSV) of lysates of P815 cells labeled before VSV infection revealed a new labeled molecule, which was not seen in α-VSV precipitates of cells labeled after VSV infection. This molecule (mol. wt. >80,000) does not correspond to known VSV proteins.

857 MEASLES VIRUS INHIBITS ACQUISITION OF LYMPHOCYTE EFFECTOR FUNCTIONS BUT NOT ACTIVITIES OF COMMITTED LYMPHOCYTES, Cornelis J. Lucas, Joep M.D. Galama, Jose Ubels-Postma, and Annemieke Vos, NIH, National Cancer Institute, Bethesda, MD 20014 and Central Lab., Netherlands Red Cross Bloodtransfusion Service, Amsterdam, The Netherlands.
We have previously demonstrated that measles virus inhibits the proliferation of human lymphocytes. This inhibition is the result of infection of the responding lymphocytes; it is not mediated by monocytes. In a follow-up study we have assayed the effect of measles virus infection on effector activities and on the generation of certain effector activities. It was observed that addition of measles virus in an allogeneic mixed lymphocyte culture resulted in markedly depressed cytotoxic activity in a subsequent cell mediated lympholysis assay. Late addition of virus does not abolish cytotoxic effector function, although effector cells are probably infected. Similarly, measles virus infection did not affect the ability of lymphocytes to mediate antibody-dependent cellular cytotoxicity. Addition of measles virus to lymphocytes with or shortly after exposure to the polyclonal activator pokeweed mitogen resulted in diminished in vitro immunoglobulin synthesis. If the virus was added up to 48 hours before assay of the immunoglobulin content of the supernatant little or no effect was detected. Finally, if lymphocytes were left without stimulus in medium supplemented with fetal bovine serum, a population of suppressor cells was generated. Measles virus was able to prevent the generation of suppressor cells. In conclusion, the inhibiting effects of measles virus could be attributed only to inhibition of the acquisition of a particular effector function. Activities of committed lymphocytes were generally not affected.

858 DIFFERENTIAL RESPONSIVENESS OF HUMAN T CELLS TO INFLUENZA VIRUS IN CONJUNCTION WITH SELECTED HLA-A AND -B DETERMINANTS, Stephen Shaw, William E. Biddison, and Gene M. Shearer, NIH, National Cancer Institute, Bethesda, MD 20014.
Genetic control of the specificity of human cytotoxic T cells has been investigated by sensitizing lymphocytes from normal donors to influenza virus and assaying their cytotoxic activity on panels of infected target cells from unrelated donors. The data support four conclusions. First, virus is recognized in conjunction with HLA-A and -B antigens (or determinants which are highly associated with them); studies have documented recognition of influenza in association with HLA-A1, -A2, -B7, -B8, and -BW44. Second, many donors generate cytotoxic T cells which recognize influenza in conjunction with only some of their HLA-A and -B antigens. Third, donors' T cells differ in their ability to recognize influenza in conjunction with specific HLA-A and -B antigens. Among groups of donors who share HLA-A2 or groups who share -B7 some "responder" donors will generate a strong response against influenza in conjunction with that antigen, but other "nonresponder" donors will not (despite a response to influenza in association with other self determinants). Fourth, since virus immune T cells from the "responder" donors lyse virus-infected target cells from the "nonresponder" donors, the unresponsiveness of these "nonresponders" cannot be due to absence of the self determinant which the "responders" recognize, nor to defective presentation of viral antigens in conjunction with that self determinant on the target cell surface. These observations suggest that the absence of cytotoxic T cell responses by some donors to influenza virus in association with HLA-A2 or -B7 is not due to an intrinsic property of these HLA determinants, but rather to regulation by Ir-like genes which act at the level of the responder cell or stimulator cell.

859 MHC ANTIGENS ON THYMIC EPITHELIAL CELLS, Robert V. Rouse, Willem van Ewijk and Irving L. Weissman, Stanford University, Stanford, CA 94305

The distribution of major histocompatibility antigens in the thymus is of relevance because of the role of that organ in the determination of self specificity in associative recognition. Immunofluorescent studies using alloantisera give characteristic staining patterns for I-A and H2-K/D. Both types of antigens are present in the medulla in a confluent pattern. I-A antigens are present in a dendritic array throughout the cortex in a distribution characteristic of thymic cortical epithelial cells. In some haplotypes, H2-K/D antigens are not stained in the cortex; in others, a dendritic pattern is seen, but only in localized regions. Monoclonal anti-I-A^k and anti-H2-K^k confirm these patterns. Immunoelectron microscopy demonstrates I-A antigens on thymic cortical epithelial cells. Analysis of bone marrow reconstituted chimeric mice reveals host type but not bone marrow donor type I-A antigens present in the cortex in a dendritic pattern indistinguishable from the normal non-chimeric thymus.

860 DEFECTIVE PRESENTATION OF VACCINIA VIRAL ANTIGENS IN ASSOCIATION WITH K END PRODUCTS OF THE H-2 GENE COMPLEX, by Andrew J. Hapel, Barbara L. Pope, Masahiro Imamura and W. John Martin, Bureau of Biologics, FDA, Bethesda, Md.

C3H and C3Hf mice differ from each other in one antigenic determinant (unique) coded for by the K end of the H-2 gene complex, but share another antigen (common) coded for by the same region. Each of these determinants may play a role in recognition of virus infected cells by cytotoxic T lymphocytes. Using vaccinia virus as a model it was shown that viral antigens in association with both unique and common antigen, can be recognized when present in macrophage or L929 cell membranes. However, using tumor cell lines sharing common antigen but expressing either C3H or C3Hf unique antigen, vaccinia virus was preferentially recognized in association with the unique determinant indicating a defect in H-2 antigen presentation by tumor cells. C3Hf mice were derived from C3H and seem to have deviated from the parent strain only in expression of the 'unique' K end antigen. Similarly the tumor cell lines used differ in H-2 antigen expression only at this same locus.

861 GENETIC RESTRICTION OF CELL-MEDIATED KILLING OF VIRAL INFECTED TARGETS IN THE SYRIAN HAMSTER. M.J.Nelles and J.W. Streilein, U.Texas H.S.C. at Dallas, Dallas, Texas 75235

Genetic restriction of cell-mediated killing of virus-infected target cells in the Syrian hamster was investigated. Similar analyses performed in the mouse have clearly demonstrated the predominant role played by K and D regions of the murine MHC in restricting T cell-mediated killing of virus-infected cells. We have found that lymphoid cells from vaccinia virus-infected hamsters kill vaccinia infected syngeneic targets. Subsequent analysis has demonstrated that, unlike the mouse, no evidence of genetically restricted cell-mediated killing of virus-infected target cells can be found in the hamster. This lack of restriction exists despite a variety of MHC determined hamster responses, including acute skin graft rejection, MLR, and GVHR among classical inbred strains. In fact, recently captured and subsequently inbred hamster strains also fail to show genetic restriction in killing virus-infected cells from classical inbred strains. We propose two alternative explanations for the apparent lack of restriction seen in this species: 1) All Syrian hamsters so far tested possess in common a K or D region "like" molecule, 2) Syrian hamsters do not utilize MHC associated antigens to restrict cell mediated killing to viruses. We are currently utilizing a variety of techniques to determine the identity of the effector cell(s) involved in this type of cell mediated killing.

862 DEMONSTRATION OF ANTIGEN SPECIFIC H-2 ALLOGENEIC RESTRICTED KILLER CELLS. James Forman, Wayne Streilein, Jo Ann Trial, Meade Pimsler, Richard Ciavarra. Dept. of Microbiology, Univ. of Texas Health Science Center, Dallas, Texas 75235

Mice injected with H-2 congenic semiallogeneic cells (donor cells) within 24 hours after birth were rendered tolerant to the allogeneic H-2 antigens as evidenced by acceptance of appropriate skin grafts. When spleen cells from these tolerant animals were cocultured with the donor cells as stimulators in a cell-mediated-lympholysis (CML) assay, no cytotoxic effector cells were generated. However, in 3 out of 4 combinations cytotoxic activity was observed when the donor cells were derivatized with trinitrophenyl (TNP) and used as stimulators in a TNP-CML assay. The effector cells generated preferentially lysed TNP-target cells that were H-2 identical to the TNP-stimulators. Spleen cells from tolerant animals were also tested for their response to minor histocompatibility (H) antigens by in vivo priming and rechallenge in vitro with cells that contained tolerated H-2 antigens but differed with respect to minor H antigens. Such cells from tolerant animals displayed a cytotoxic response to minor H antigens in association with the tolerated H-2. These results demonstrate that in tolerant animals, recognition of antigen in association with tolerated allogeneic H-2 can occur. Further, the antigen specific cells that are generated are restricted in their effector cell activity by the tolerated H-2 allogeneic determinants.

863 *HLA RESTRICTION OF THE VIRUS-IMMUNE T CELL RESPONSE IN ACUTE MEASLES,* *Hans W. Kreth and Volker ter Meulen, Institute of Virology, University of Würzburg, D-8700 Würzburg*

Peripheral lymphoid cells (PBL) from donors with acute measles were tested in short-term ^{51}Cr release assays against measles virus-infected target cells with known HLA specifities. In acute measles, target cell lysis was dependent on effector and target cells sharing HLA determinants. Only minimal lysis was observed in allogeneic combinations. Specific lysis seems to be associated with both HLA A and B and has been found when HLA, A2, A3, AW24,B8, B12 and BW35 were shared between effector and target cells. This restricted killer cell activity is exclusively confined to the acute stage of the disease and can be characterized as a T cell response by cell separation experiments.

The results show that the MHC requirements and kinetics of virus-specific T cells are similar in man as in other species.

864 THE INTERACTION OF PROTHYMOCYTES AND THE THYMIC MICROENVIRONMENT IN NORMAL AND NEOPLASTIC T-LYMPHOCYTE DEVELOPMENT, Samuel D. Waksal, Tufts University, Cancer-Research Center, 136 Harrison Avenue, Boston, Mass 02111

The critical role of the thymus in the pathogenesis of T-lymphocytic leukemias has been established by many investigators. We have postulated that a special interaction exists between the thymic epithelium and its target cells, the prothymocyte during the induction of leukemia. We have done in vitro and in vivo experimentation using the spontaneous thymic lymphoma models of the AKR/J and HRS/J mice and the radiation induced leukemia model of the C57Bl. Our data show the prothymocyte to be the target cell for in vitro transformation. These experiments were performed by a cocultivation technique, developed by us, using thymic epithelium (TE) derived from preleukemic mice as inducing monolayers. TE monolayers which have transforming potential contain the new class of "polytropic" recombinant leukemia viruses. This has been analyzed by virological techniques as well as peptide mapping. Peptide mapping suggests that recombination occurs within the env region. The prothymocyte population also contains natural killer (NK) cell activity against virus infected cells. The killing may be directed against the viral associated antigen, GP70. It is interesting to note that NK activity is abrogated by the same dose of irradiation which induces lymphoma. Studies are also being performed on in vitro MHC restriction for prothymocyte epithelial interaction.

865 ASSOCIATION OF MURINE LYMPHOID CELL SURFACE ANTIGEN EXPRESSION WITH VIRUS PRODUCTION, Kim S. Wise, Susanne L. Henley, Ronald T. Acton, University of Alabama in Birmingham, Birmingham, AL 35294

Studies have been initiated to compare the surface expression of a number of lymphoid cell antigens in two variants of the T-lymphoblastoid cell line EL4, derived from a lymphoma of C57BL/6 mice. Both variants express H-K^b and H-$2D^b$ histocompatibility antigens, the Thy-1.2 differentiation alloantigen, and the MuLV-related viral envelope component gp70. The variant lines differ in the production of MuLV particles and in the expression of the Gross Cell Surface Antigen, an indicator of viral production. Comparison of these antigens on the MuLV producing (EL4G+) and non-producing (EL4G-) has been undertaken to assess quantitative changes in the expression of cell surface antigens accompanying viral production. Quantitation was based on the absorption of cytotoxic antisera to Thy-1, H-$2K^b$, H-$2D^b$, or gp70. While no difference was observed between these variants in the amount of Thy-1.2 expressed per cell, a marked (20-fold) increase in both H-$2K^b$ and H-$2D^b$ specificities was observed in the EL4 G+ line. As expected, the amount of gp70 on the EL4 G+ line was greatly elevated over that measured on the EL4 G- line. To investigate possible mechanisms of MuLV-H-2 interactions, the relative ratios of antigens expressed in virus preparations to those expressed on intact cells or membrane preparations have been measured. Present results indicate that the specific activity of the Thy-1 antigen in virus preparations exceeds that found in highly purified membranes from EL4G+ cells, and that the relative activity of H-$2D^b$ in virus preparations exceeds that of H-$2K^b$. These data are consistent with an active association of lymphoid surface glycoproteins during virus budding and suggest preferential association with H-2D antigens.

866 SPECIFICITY OF HUMAN CYTOTOXIC T LYMPHOCYTES FOR INFLUENZA VIRUS AND HLA DETERMINANTS. Andrew J. McMichael and Peter Parham.

Cytotoxic T lymphocytes (CTL) were generated by incubating human peripheral blood lymphocytes with autologous influenza virus infected cells. These CTL lysed autologous target-cells infected with influenza virus of the same type, A or B, as that used for sensitisation. There was complete crossreactivity however in recognition of the influenza A virus subtypes suggesting that unlike antibodies, the CTL did not recognise the influenza harmagglutinin (HA). Failure of monoclonal anti HA antibody to inhibit cytotoxicity supports this conclusion.

The CTL also showed specificity for HLA antigens. Lysis of autologous infected target cells was inhibited by appropriate monoclonal anti HLA antibodies. Infected allogeneic cells were only lysed when HLA A or B antigens were shared between killer and target cells. While this result was found with most HLA A or B antigens tested, sharing of certain HLA antigens, e.g. HLA, A2, did not permit lysis of the target cell. As HLA A2 has been shown to be efficient in allowing lysis of target cells by CTL sensitized to TNP (Dickmeiss et al., Nature 270, 526, 1977) or Y antigen (Goulmy et al, Nature 266, 544, 1977), this effect may be specific for influenza virus. If so, this finding illustrates how an HLA antigen could confer disease susceptibility.

867 VIRAL INDUCED CELL SURFACE ANTIGENS AND HISTOCOMPATIBILITY ANTIGENS ARE LOCATED ON DISTINCT MOLECULES, Robert I. Fox, and Irving Weissman, Stanford Univ., Stanford, Cal

Previous studies have demonstrated that cytotoxic T lymphocytes (CTL) recognize specific (e.g. viral) antigens in the context of target cell major histocompatibility antigen (MHC). We have used Moloney virus induced lymphoma LSTRA to study the relationship between viral and MHC antigens by iodinating the cell membrane with lactoperoxidase, solubilizing with detergents, immunoprecipitating with anti-MHC sera that have been absorbed to remove contaminating antiviral antibodies,and gel electrophoresis in one-dimensional SDS gels and two dimensional gels (isoelectric focusing followed by SDS gels). Immunoadsorbent columns and cytotoxicity assays have also been employed. We demonstrate: (i) viral antigens and MHC antigens were carried on separate molecules and that no molecules were detected that possessed viral antigens covalently linked to MHC antigens; (ii) no detergent noncovalent linkage between MHC and viral antigen was detected; (iii)no alteration in either size or isoelectric properties of the MHC antigen was detected; (iv)using anti-β_2 microglobulin sera, no "new" MHC molecules were found as a result of viral transformation. Finally, efficient specific binding of virions to the intact cell membrane was shown to not involve MHC antigen.

In the system studied, it is likely that immune CTL recognize MHC and viral antigens which are independently distributed on the cell membrane. We were able to exclude models which involved chemical alteration of the MHC antigen or models in which the MHC molecule serves as a virus receptor.

868 CHANGES IN CELL SURFACE GLYCOPROTEIN, gp70, DURING AKR THYMIC LYMPHOMA, Larry J. Takemoto and Esther F. Hays, Division of Biology, Kansas State University, Manhattan, Kansas 66506 and Laboratory of Nuclear Medicine, University of California, Los Angeles, California 90024

Substantial evidence indicates that amino acid changes in the envelope glycoprotein (gp70) may determine the leukemogenic activity of C-type, murine retroviruses. It is therefore highly possible that viral infection and subsequent expression of an aberrant, cell surface gp70 may accompany the formation of various murine leukemias such as the spontaneous thymic lymphoma of AKR mice. To test this hypothesis, cell surface proteins of thymocytes from normal and spontaneously leukemic AKR mice were radioiodinated, followed by immunoadsorption and tryptic peptide mapping of gp70. Thymocytes from normal AKR mice of 3.5-11.0 months of age possessed similar cell surface peptides of gp70. These peptides could all be assigned to the gp70 of either the AKR ecotropic or xenotropic virus. In contrast, gp70 peptides of cells from many spontaneous thymic lymphomas exhibited variable patterns including the presence of at least two gp70 peptides that could not be assigned to either the ecotropic or xenotropic virus. These results therefore demonstrate the presence of aberrant peptides of gp70 on the cell surfaces of thymocytes from AKR spontaneous lymphomas.

869 IR GENE CONTROLS OF T CELL RESPONSES TO THE MALE SPECIFIC ANTIGEN, H-Y. Brenan, M., Brunner, C., Hetherington, C., Chandler, P., and Simpson, E. Clinical Research Centre, Watford Road, Harrow, Middx., HA1 3UJ, England

T cell responses of female mice to the male specific antigen, H-Y, are Ir gene controlled. Using F1 female mice with two different independent H-2 haplotypes as responders to H-Y, distinct groups of Ir genes can be described.

The H-2^b haplotype has dominant H-2^b I genes which control rejection of syngeneic male skin and the generation of H-2K/D restricted cytotoxicity. F1 mice with one H-2^b parent give cytotoxic T cell responses to H-Y antigen in association with both parental K/D gene products (Simpson, E. and Gordon, R. D. 1977. Immunol. Rev. 35, (59-75).

H-2^d and H-2^r haplotypes have possible dominant H-2^d or H-2^r I genes which control H-2 restricted cytotoxic responses but not skin graft rejection. F1 mice with one H-2^d or H-2^r parent make cytotoxic responses to H-Y antigen in association with the other parental K/D gene products. Analysis using chimeric mice has shown that the cells responding are those of the presumptive dominant responder haplotype (Matsunaga, T. and Simpson, E. 1978. Proc. Nat. Acad. Sci. (in press)).

H-2^k, H-2^q, H-2^s and H-2^f haplotypes possess complementary Ir genes which permit H-2 restricted cytotoxic responses in certain F1 mice but not skin graft rejection. The parental strains themselves are non responders. Certain combinations e.g. H-2^k x H-2^s, H-2^s x H-2^q, H-2^s x H-2f H-2^k x H-2^q make anti-H-Y cytotoxic responses whereas others do not, e.g. H-2^f x H-2^k, H-2^f x H-2^q. Thus complementary Ir genes may fall in different complementation groups.

870 H-2 RESTRICTION OF T CELLS MEDIATING LETHAL GRAFT-VERSUS-HOST DISEASE AGAINST MINOR HISTOCOMPATIBILITY ANTIGENS, Robert Korngold and Jonathan Sprent, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104.

Fatal graft versus host disease (GVHD) occurs when unsensitized CBA/J (H-2^k) T cells are transferred to lethally irradiated (750R) H-2-compatible B10.BR mice. Studies with a variety of strain combinations have shown that GVHD is mediated by mature T lymphocytes reactive to minor histocompatible (H) determinants of the host. To investigate whether the T cells mediating GVHD show H-2-restricted specificity, unsensitized CBA/J LN cells were transferred to heavily irradiated B10.BR (H-2^k), B10 (H-2^b), and B10.D2 (H-2^d) mice. The donor cells were then recovered from thoracic duct lymph of the recipients 1-2 days later and transferred to irradiated B10.BR mice. With filtration through irradiated B10.BR mice, the CBA/J T cells lost their capacity to kill B10.BR mice. By contrast, filtration through either B10 or B10.D2 mice did not affect the capacity of the CBA/J cells to kill B10.BR mice. Thus, with this entirely in vivo system, it appears that T cells reactive to minor H determinants have no capacity to recognize these determinants presented in the context of a foreign H-2 background. The possible role of macrophage-processing in the system and the relevance of the data to the phenomena of "cross-priming" will be discussed.

871 H-2 RESTRICTION OF CELL-MEDIATED CYTOTOXICITY (CMC) TO TISSUE-SPECIFIC ALLOANTIGEN(S) EXPRESSED BY EPIDERMAL CELLS (EC), David Steinmuller, John D. Tyler and Chella S.David, Department of Immunology, Mayo Clinic, Rochester, MN 55901

EC obtained by trypsinization of tail skin of H-2^k haplotype-identical mouse strains AKR, B10.K, CBA, C3H and RF were used as stimulators and targets of CMC in 3-hr ^{51}Cr-release assays. Hosts were primed with an ip inoculation of 10^7 EC; 1-2 weeks later their spleen cells were mixed in culture with 4,000-rad irradiated EC, harvested 5 days later and then assayed with donor strain EC and lymph node cell (LNC) targets. When C3H was the donor strain and any of the other 4 strains, the host, little CMC generally was registered with either type of target cell. However, when C3H was the host and any of the other strains the donor, high CMC was registered with the EC targets but low CMC with the LNC, indicating that C3H mice respond to alloantigen(s) preferentially expressed by EC as opposed to LNC. The high anti-EC CMC evoked in C3H hosts by EC from any of the H-2^k identical strains reacts with EC from all of them, but not with EC from H-2 different strains. As seen in the Table, the restriction is dependent on antigens mapping in the H-2K region because C3H anti-AKR EC effectors lysed B10.K and B10.A but not B10 EC, and among the recombinants tested, B10.A(4R) but not B10.AQR EC. The H-2D end is not involved because B10.0L and B10.W7R EC were not lysed.

C3H anti-AKR(EC) Effectors (specific % lysis at E:T = 25:1)			
Target strain	H-2 haplotype	Target cells EC	LNC
AKR	k	58.0	3.1
C3H	k	5.2	-3.0
B10.K	k	67.8	3.5
B10.A	a(K^kD^d)	54.2	3.9
B10	b	2.6	-2.2
B10.A(4R)	h4(K^kA^k)	67.0	1.0
B10.AQR	y1(K^qA^k)	3.2	-1.6
B10.0L	o1(S^kD^k)	2.3	-1.2
B10.W7R	w7(D^k)	4.8	-3.5

872 HIGH INCIDENCE OF B CELL LYMPHOMAS IN CHICKENS INFECTED WITH SUBGROUP A BUT NOT SUBGROUP E AVIAN LEUKOSIS VIRUSES, Harriet L. Robinson and Margot N. Pearson, Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545

Avian leukosis viruses are classified into subgroups on the basis of their envelope antigens. Chickens which are infected with subgroup A viruses have a high incidence of B cell lymphomas. Neoplastic diseases have not been reported in subgroup E virus infected birds. Differences in the oncogenic potential of A and E viruses are of particular interest since genes of normal chickens code for E viruses.

K28 chickens which we have bred for susceptibility to A and E viruses were infected with A (RAV-1) or E (RAV-60s) viruses. At 3 months after infection, A and E virus infected birds had comparable levels of serum viremia. By 5 months, 7 out of 15 A virus infected birds had B cell lymphomas. 5 out of 108 E virus infected birds had erythroid cell neoplasias. Thus we find that subgroup E viruses can cause neoplastic disease but that the incidence and spectrum of disease is different from that observed for subgroup A viruses.

The following abstracts were inadvertently ommited during the initial preparation of this volume.

Molecular and Cellular Characterization of Antigen Binding Receptors

873 THE SYNTHESIS AND TURNOVER OF IgG-LIKE IMMUNOGLOBULIN FROM RAT T-CELLS, Charles T. Ladoulis and Gurmukh Singh, University of Texas Medical Branch, Galveston, TX 77550 and University of Pittsburgh, Pittsburgh, PA 15261

Purified rat thymic T-cells synthesize and secrete in vitro immunoglobulin-like molecules serologically related to the IgG_{2a} subclass of rat immunoglobulins. This is associated with a lack of surface IgG-like immunoglobulin using immunofluorescence or immunoperoxidase immunohistochemical techniques or the identification of any IgG subclass by surface radioiodination. Double antibody immunoprecipitation of radioactively labeled supernatants from T-cell cultures showed that secreted immunoglobulin-like molecules amounted to approximately 5% of the total labeled protein in 20 hours. This immunoglobulin has a molecular weight of approximately 165,000 after reduction with 2-mercaptoethanol, consists of a heavy and light chains of approximately 60,000 and 23,000 daltons, respectively. The heavy and light chain molecular weight estimates in sodium dodecyl sulfate polyacrylamide gel electrophoresis are similar to those of normal rat serum IgG immunoglobulin chains. Specific antisera for rat immunoglobulin subclasses indicate that T cell immunoglobulin product is primarily IgG_{2a} specificity. Isolated plasma membranes of rat T-cells contain an IgG-like immunoglobulin detectable by immunodiffusion analysis of solubilized membrane proteins. The results indicate that rat T-cell immunoglobulin is synthesized, membrane-associated and relatively rapidly released from the cell surface.

Genetics and Cell Interactions in Cell-Mediated Lympholysis

874 DISTRIBUTION OF LY 6.1 AND LY 7.2 ANTIGENS ON THE HELPER AND SUPPRESSOR T CELLS THAT REGULATE CYTOTOXIC T CELL RESPONSES. Linda M. Pilarski and Ian F.C. McKenzie, Department of Immunology, University of Alberta, Canada, and Department of Medicine, University of Melbourne, Australia.

In vitro systems have been developed to generate antigen-specific helper and suppressor T cells which are assayed by their ability to regulate the induction of a cytotoxic T cell response to alloantigens (1,2). We have determined the distribution of both Ly 6.1 and Ly 7.2 antigens on the precursor and effector cells of the helper, suppressor and cytotoxic T cell lineages. Helper T cell effectors (radioresistant function) were found to be Ly 6 + Ly 7 +. Helper cell precursors however were Ly 6 + Ly 7 + indicating that in this differentiation pathway, Ly 7 is acquired only at the effector stage. This finding provides a valuable tool for selectively removing helper effector cells from a given population. Suppressor T cells (radioresistant function) are Ly 6 + Ly 7 +. Suppressor precursor cells are also Ly 6 + Ly 7 +. This distribution of Ly 6 allows selective removal of both suppressor precursor and effector cells. The cytotoxic T cell is only marginally affected by treatment with anti-Ly 6.1 and is Ly 7 +. The cytotoxic T precursor is Ly 6 + Ly 7 +.

(1) Baum, L., and Pilarski, L., J. Exp. Med., in press.
(2) Al-Adra, A., Pilarski, L., Eur. J. Immunol. 8:504, 1978.

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Immune Recognition and Regulation in Syngeneic Tumor Systems

875 CROSS REACTIVITY OF ALLOANTIGENS AND TUMOR ANTIGENS *IN VIVO*. IMPLICATION FOR POLYMORPHISM, Mark I. Greene, Linda L. Perry and Baruj Benacerraf, Harvard Medical School, Boston, MA 02115.

The relationship between alloreactivity and reactivity to antigen and self determinants has been studied. We have analyzed whether immunogenic tumor antigen expressed on the methylcholanthrene induced sarcoma S1509a ($H\text{-}2^a$) cross react with murine alloantigens coded for by the H-2 complex. A/J mice primed to BALB/c cells develop delayed type hypersensitivity (DTH) reactivity to BALB/c cells and S1509a tumor antigen and vice versa. Furthermore if A/J mice are primed to S1509a or to BALB/c alloantigens, and simultaneously receive suppressor T cells specific for discrete antigens expressed on S1509a, DTH to S1509a is inhibited. Attempts to identify the antigen shared by BALB/c cells and S1509a tumor involved priming A/J mice with cells from congenic resistant strains. It was found that B10.D2 ($H\text{-}2^d$) but not B10 ($H\text{-}2^b$), B10.Br ($H\text{-}2^k$) or B10.A ($H\text{-}2^a$) cells primed A/J mice for DTH when challenged with S1509a tumor cells. We conclude that defined alloantigens expressed by genes in the K end of the $H\text{-}2^d$ haplotype resemble tumor specific antigens expressed on S1509a tumor cells. The implication of these findings are that there is an intimate relationship between the polymorphism of tumor antigens and H-2 coded products and moreover that alloreactivity may be due to the resemblance of alloantigens to self structures associated with antigen.

876 MONOCYTE FACTORS SUPPORT THE GENERATION OF POKEWEED MITOGEN (PWM)-INDUCED IMMUNOGLOBULIN SECRETING CELLS (ISC) IN THE ABSENCE OF T CELLS, Stuart A. Rosenberg and Peter E. Lipsky, Univ. of Texas Southwestern Medical School, Dallas, Texas 75235

We have previously demonstrated that monocytes (MØ) are required for PWM-induced generation of ISC from human peripheral blood mononuclear cells (PBM). In order to evaluate further the role of MØ in this system, PBM were separated into B cell-enriched (B) and MØ-enriched populations. MØ were cultured with or without PWM for 24 hours at 37°, after which the culture supernatants were harvested and spun free of cells. B cells were then cultured in microtiter plates with or without PWM, and with or without the MØ derived factors. Following a 7 day incubation at 37°, the number of ISC was determined by a reverse hemolytic plaque assay. PWM stimulation of B cells resulted in a negligible increase in ISC compared to controls. However, supplementation of cultures with MØ supernatant produced a 10-20 fold increase in the number of ISC obtained. Additional experiments showed that the MØ factor(s) is released during the 1st 36 hours of culture, that PWM is not required for its generation and that the factor(s) is not a polyclonal B cell activator in that there is no generation of ISC in the absence of PWM. These data indicate that early in culture, MØ elaborate factors with the capacity to provide a requisite helper signal for PWM-induced generation of ISC from human PBM. These preformed MØ factors are active in the absence of T cells. Since it is known that T cells can also provide help in this system, the present findings suggest that PWM induced-B cell activation is controlled by both T cell and MØ signals.

877 CELL-MEDIATED IMMUNITY TO HUMAN LUNG AND BREAST CANCER ASSOCIATED ANTIGENS, R.B. Herberman, J.L. McCoy, J.H. Dean, T.R. Jerrells and G.B. Cannon, National Cancer Institute, Bethesda, Md 20014 and Litton-Bionetics, Inc., Kensington, MD 20795

Although there have been many reports of cell-mediated immunity of cancer patients against tumor tissues, the specificity of the reactions usually has not been determined. We have studied lymphoproliferation (LP) and leukocyte migration inhibition (LMI) activity of lung and breast cancer patients in response to tumor extracts. In LP, 70% (28/40) of lung cancer patients reacted to autologous tumor extracts (median stimulation index, 9) and only one patient had marginal reactivity to normal lung. Antitumor reactivity could not be attributed to mitogens or microbial antigens in the extracts. In breast cancer, 41% (9/22) patients had LP responses to autologous tumor extracts and none reacted to normal breast tissues. The majority of patients also produced leukocyte inhibitory factor (LIF) in response to autologous tumor. Negative LP responses to tumor antigens of some patients were found to be due to adherent suppressors, with activity appearing after their removal or in the presence of indomethacin. Reactivity in LMI to allogeneic tumor extracts was also examined: 60% of lung cancer patients and 70% of breast cancer patients reacted to extracts of tumors or cell lines derived from tumors of the same organ. In contrast, less than 10% of normals reacted and about 15% of cancer patients reacted with extracts from unrelated cancers. Using a modified indirect microagarose LMI, we frequently have observed patients to produce LIF in titers of 10^{-2}-10^{-3}. This recent development should allow more detailed definition of the specificity of the antigens detected in LMI.

878 RECOGNITION OF AKR/GROSS VIRUS INDUCED TUMORS BY SYNGENEIC T CELLS, Christopher S. Henney, William R. Green, and Robert C. Nowinski, Fred Hutchinson Cancer Research Center, Seattle, WA, 98104.

Some mouse strains, e.g. AKR, have a high incidence of spontaneous leukemia, characterized by the display of AKR/Gross virus-encoded antigens in high density on the leukemic cell. Other strains e.g. C57BL/6 show little propensity to such murine leukemia virus (MuLV) associated leukemia. Since leukemic cells display increased quantities of AKR/Gross virus-encoded antigens, an attempt was made to determine if resistance to leukemogenesis correlated with the ability to generate cytotoxic lymphocytes specific for virally-encoded antigens expressed on MuLV induced tumors. C57BL/6 mice were immunized in vivo with an AKR leukemic cell (SL-3) and spleen cells from these animals then restimulated in vitro with virus positive cells of the same H-2 haplotype as the responder. Both EL4 of C57BL/6 origin and SL-1 (derived from an AKR H-2^b congenic) stimulated the production of cytotoxic T cells. The cytotoxic cells generated lysed (^{51}Cr release assay) EL4 and SL-1 cells, but not the SL-3 (H-2^k) tumor cells used for priming. Furthermore, cells of the H-2^b haplotype that serologically showed little or no AKR/Gross virus associated antigens (e.g. EL4 and C57L 691) were also insusceptible to lysis. This specificity was confirmed by cold target inhibition assays and is consistent with the conclusion that the effector cells generated are H-2 restricted and directed against AKR/Gross virus-associated antigens. (Supported by grants from NIAID and NCI.)

Learning of H-2 Restriction and Lymphocyte - Virus Interactions

879 LEUKEMIA CELL SURFACE SPATIAL RELATIONSHIPS BETWEEN H-2, MLV GP70, AND THEIR NEIGHBORING POLYPEPTIDES. David A. Zarling, Andrew Watson, Robert E. Duke, and Fritz H. Bach, Univ. of Wisconsin, Madison, 53706.

T-lymphocyte mediated lysis of Friend, Moloney, or Rauscher murine leukemia virus (FMR-MLV) transformed H-2^b leukemia cells is H-2^b and FMR-MLV restricted. The FMR virus antigen(s) contains a determinant of the MLV envelope glycoprotein, gp70. The hypothesis was tested whether there was a physical association between H-2^b and R-MLV gp70 antigens on the surface of R-MLV transformed H-2^b leukemia cells (RBL-5A). No covalent or non-covalent association existed between surface iodinated H-2^b and R-MLV gp70 antigens in NP-40 disrupted RBL-5A cell extracts after immunoprecipitation and analysis by SDS-PAGE. However, on intact RBL-5A cells a selective physical association between H-2^b and MLV gp70 antigens was measured in co-capping experiments (Zarling et al, Scand. J. Immunol., in press). A 13 A base-reversable cross-linking reagent not previously used in membrane protein studies was used to measure molecular associations between H-2^b, MLV gp70, and their neighboring polypeptides on the surface of RBL-5A cells. Cross-linked iodinated polypeptide products immunoprecipitated with αH-2^b or αR-MLV gp70 sera were analysed by two dimensional SDS-PAGE. The cross-linked proteins were separated in the first dimension and were cleaved before analysis in the second dimension SDS-PAGE. Analysis of the cleavage products of RBL-5A surface polypeptides cross-linked for various times showed both homomolecular and heteromolecular associations.