**800** TOPOGRAPHIES OF MULTIGENE FAMILIES, David S. Hogness, Department of Biochemistry, Stanford University School of Medicine, Stanford, CA 94305

Six multigene families in Drosophila melanogaster have been analyzed at the molecular level, mostly by the use of cloned DNA segments (Dm segments). These families are divisible into two classes: tandem and dispersed. Families in the first class consist of members that are contained in tandemly repeated units. By contrast, members of dispersed families occur singly at many different chromosomal sites that are widely distributed over the genome.

Molecular maps of four tandem families have been obtained in D. melanogaster: (a) the 18 and 28S genes (1-2), (b) the 5S RNA genes (3), (c) the histone genes (Karp, Lifton, Goldberg and Hogness - papers in preparation), and (d) a heat-shock gene family (Lis, Prestidge and Hogness - in preparation). Each has been mapped at a single site in the haploid genome - the first at the nucleolus organizer in the X or Y chromosomes, and the others at sites in the autosomes. A comparison of the structure of the tandem arrays that contain these four gene families will be made.

The discovery of dispersed families resulted from an examination of the genes that provide the abundant mRNAs found in D. melanogaster cell cultures. The members of the first dispersed family to be mapped occupy some 30 widely dispersed sites (4; Rubin, Finnegan and Hogness - in preparation). Cloned Dm segments that contain individual genes (plus flanking sequences) from six of these sites have been isolated and compared. While each contains a very similar, if not identical, mRNA region that is about 7 kb long, the flanking sequences appear to be site specific. Examination of restriction fragments of total D. melanogaster DNA indicate that this conclusion applies to virtually all of the sites in the family. A novel feature of these genes is that a 0.5 kb sequence at the 3'-end is repeated at the 5'-end in the same orientation (Finnegan, Rubin and Hogness - in preparation). A second dispersed family that provides another abundant mRNA has recently been examined and exhibits characteristics similar to those of the first (Young, Rubin, Finnegan and Hogness - unpublished results).

The functional advantages of the tandem and dispersed topographies will be considered at the end of this talk.

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**801** ORGANIZATION AND TRANSPOSITION OF IMMUNOGLOBULIN GENES, Susumu Tonegawa and Nobumichi Hozumi, Basel Institute for Immunology, Postfach 4005 Basel 5, Switzerland

Arrangement of mouse immunoglobulin V- and C-genes is being studied at the molecular level using bacterial restriction enzymes and nucleic acid hybridization (1-2). High molecular weight DNA of various sources were digested to completion with various restriction enzymes. The resulting DNA fragments were fractionated, by size, in preparative agarose gel electrophoresis. The DNA fragments carrying V- or C-genes were identified by hybridization with radioiodinated, purified, light chain mRNA's and their specific fragments. The results of this sort of experiment lead us to conclude that in early embryonic cells V- and C-genes are discontiguous and that gene transposition takes place during lymphocyte differentiation, in order to bring the two segments of DNA together to create a complete immunoglobulin gene. Such transposition events is restricted to lymphocytes and plasma cells among various adult tissues. In lymphocytes transposition takes place only in that pair of V and C genes which are used for expression in the particular clone. It is proposed that activation of a particular pair of V and C-genes to expression is intimately coupled with the transposition event.

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802 THE DIVERSITY OF IMMUNOGLOBULIN MOLECULES, L. Hood, J. Hubert, N. Johnson, E. Loh, J. Schilling, B. Black and V. Farnsworth, Division of Biology, California Institute of Technology, Pasadena, CA 91125

The diversity of immunoglobulin polypeptides from several different systems (NZB myeloma proteins of a closely related V region subgroup, phosphorylcholine-binding BALB/c myeloma proteins, etc.) will be presented (1). The diversity of these immunoglobulins will be discussed with regard to current theories concerning the organization and expression of antibody genes (2).

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**841** TRANSLATION AND IMMUNOPRECIPITATION OF THYMOCYTE KAPPA CHAIN MESSENGER RNA
David L. Putnam and Ursula Storb, Dept. of Microbiology and Immunology, University of Washington; Seattle, WA 98195

The identity of the T (thymus-derived) cell antigen receptor remains controversial in part due to the lack of convincing evidence whether or not T cells have the capacity to synthesize antibody-like molecules. We have approached this question by examining the T cell RNA pool for sequences that code for antibody Kappa light chain (K-chain). That such messages are present was first demonstrated by molecular hybridization with radiolabeled complementary DNA (cDNA) prepared by the reverse transcription of the 3' portion of purified K-chain mRNA from a mouse myeloma (Storb et al. (1976) Proc. Natl. Acad. Sci. USA, 73:2467).

Subsequently we have shown that these sequences are viable mRNA's which are translatable into complete Kappa chains in the following manner. Thymocyte RNA was enriched for the poly-adenylated mRNA species by passage over Oligo-dT. Then RNA's of 11-13S size were isolated by sucrose density gradient fractionation. This RNA stimulated the incorporation of $^{35}$S-methionine in cell-free protein synthesis. Synthesis of K-chains was established using a highly specific anti-Kappa direct immunoprecipitation method in the presence of detergent. Specific precipitates were recovered by centrifugation through sucrose and detergent gradients, and finally resolved by SDS polyacrylamide gel electrophoresis after reduction. Autoradiographic analysis of the gels verified that T cell mRNA translation products contain several K-chain precursors, similar to spleen translates. The possibility of B cell contamination in the initial thymocyte cell preparation is negligible as determined by immunofluorescence checks on each cell sample: 99.8% θ +, << 0.2% surface Ig +, virtually no detectable plasma cells. (Supported by grants 5T01 CA-05040, AI 10685, and

**842** REGULATION OF EXPRESSION OF ANTIBODY GENES. Malcolm Gefter, Gary Siebert, and John Harris. Massachusetts Institute of Technology, Cambridge, Massachusetts.

A recently developed method for readily obtaining cell hybrids with mouse myeloma cell lines has allowed for the construction of cells having novel properties. Hybrids have been constructed between MPC 11 ($\gamma_{2B}$-k) and MOPC 315 ($\alpha$-$\lambda_2$). In general agreement with previous results and the work of others (Cowan et al., 1976, Eur. J. Imm. 61, 355-368) these hybrids express all four parental immunoglobulin chains. By appropriate construction, cell lines have been obtained that express the $\alpha$ chain alone, or the $\alpha$ chain in combination with either its natural $\lambda_2$ chain or the MPC 11 k chain, or lines expressing the $\alpha$ chain and both $\lambda$ and k sumultaneously. Similarly, lines expressing the MPC 11 $\gamma$ chain and the $\lambda$ and k chains alone or in combination have been constructed. The results of the quantitation of the expression and covalent structure of each chain in each cell line will be described.

Several conclusions can be drawn from the study of these cell lines. There is preferential association of the "natural" heavy and light chain pairs compared to the constructed pairs. Failure of heavy chains to be secreted is due to protein turnover. Assembling of the H chain to an L chain prevents turnover and allows secretion.

These data suggest a plausible model for somatic evolution of H-L pairing that results in the formation of stable and functional pairs.

**843** SIZE OF THE TRANSCRIPTION UNITS FOR IMMUNOGLOBULIN GENES IN MOUSE MYELOMA CELLS,
Ralph Giorno and Walter Sauerbier, University of Colorado Med. Ctr., Denver, CO 80262

We have applied the technique of "u.v. mapping" to analyze the size of the transcription units for presumptive messenger RNAs for $\gamma$2b heavy and $\kappa$ light chains in MPC 11 mouse myeloma cells. Target sizes have been estimated for 19S presumptive $\gamma$2b heavy chain mRNA and 14S presumptive $\kappa$ light chain mRNAs, which have been fractionated by electrophoresis on polyacrylamide gels. Our analysis reveals that the target size, i.e. transcription unit, is twice as large as the 19S RNA species and 1.6 times as large as the 14S RNA species. Thus, we estimate the transcription unit to be some 2.5 to 3.0 times the size of the heavy and light chain polypeptides.

Studies are in progress to determine the purity of 19S and 14S RNAs by in vitro translation. Preparation of cDNA probes of these molecules has also been undertaken in order to isolate putative nuclear precursors by RNA·DNA hybridization.

844 A PUTATIVE NUCLEAR RNA PRECURSOR LARGER THAN IMMUNOGLOBULIN κ LIGHT CHAIN MESSENGER RNA. Maureen Gilmore-Hebert and Randolph Wall. Department of Microbiology and Immunology and the Molecular Biology Institute, University of California at Los Angeles. Los Angeles, California 90024

Using a procedure developed for the recombinant DNA cloning of poly(A)-containing mRNA (1), we have cloned complementary DNA (cDNA) synthesized from purified MOPC 21 immunoglobulin κ light chain mRNA. We have used such purified recombinant cDNA, covalently linked to sepharose, for the isolation of κ mRNA and for the detection of nuclear RNA containing κ mRNA sequences. Nuclear RNA from cultured mouse P3K cells labelled for 2 - 3 hours contained two discrete classes of immunoglobulin κ specific RNA sedimenting under stringently denaturing conditions at 25S and 14S. Polysomal RNA selected on recombinant cDNA sepharose yielded a single RNA species with an electrophoretic mobility identical to that of purified 14S immunoglobulin κ mRNA prepared by repeated poly(U) sepharose selections and sucrose gradient sedimentations. The 14S κ specific nuclear RNA was the predominant species in labelling periods of 2 - 3 hours. In contrast, nuclear RNA isolated after brief pulse labelling periods (10 - 20 min) contained the 25S κ specific nuclear RNA species but relatively little of the nuclear κ specific 14S species. These results suggest that the 25S κ specific nuclear RNA could be a precursor to immunoglobulin κ light chain mRNA.

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845 HEAVY CHAIN DISEASES IN MAN: ISOLATION AND TRANSLATION OF mRNA FOR A NATURALLY OCCURRING IMMUNOGLOBULIN VARIANT. A. Alexander and J. Buxbaum, Dept. of Medicine, N.Y.U. Medical Center and Manhattan Veterans Administration Hospital, New York, N.Y. 10010

Human heavy chain diseases (HCD) are lymphoid malignancies in which Ig forming cells synthesize and secrete aberrant heavy chain molecules and fail to produce light chains. We have established a cell line from the peripheral lymphocytes of one such patient whose isolated serum protein was a $\gamma_3$ fragment with a molecular weight of 39,500 and an N-terminal deletion (OMM) (Adlersberg et al. PNAS, 72:723, 1975). The cultured cells secreted a protein which was antigenically and electrophoretically (in SDS) identical to the HCD protein. Short pulse labeling of the cells (3.5') revealed no evidence for post-ribosomal degradation of a normal heavy chain. Extraction of RNA using hot phenol and SDS followed by poly-U sepharose chromatography yielded a population of poly-A containing molecules which, when added to a wheat germ cell free system, resulted in the production of a protein with the antigenic properties of the HCD protein and an estimated molecular weight of 38,000 daltons. Fractionation of the partially purified mRNA on sucrose gradients indicated that the protein was coded by an mRNA with a sedimentation constant of approximately 15.5 while γ-chain mRNA from a murine myeloma had an S rate of 17. There was no evidence for synthesis of light chain or a $V_H$ fragment either in the intact cell or the cell free system. These experiments indicate that the HCD protein is a synthetic product which is probably the result of a partial gene deletion resulting in a short transcription product. It is not yet clear if the absence of L-chain synthesis reflects gene deletion, defective transcription or defective translation. (Supported by NIH grants CA12152, CA09161, AM01431, and research funds from the Veterans Administration.)

846 HYBRIDIZATION PROPERTIES OF IMMUNOGLOBULIN mRNA. FAILURE TO DETECT COVALENTLY ASSOCIATED IgG-mRNA TRANSCRIPTS OF REITERATED AND UNIQUE MOUSE DNA, Edward P. Cohen and Mary K. Legler, La Rabida-Univ. of Chicago Inst., Chicago, IL 60649

mRNAs coding for complete immunoglobulin G were isolated from the cells of mouse myeloma RPC-5 using specifically purified antibodies to immunoprecipitate polyribosomes engaged in Ig gamma and kappa chain synthesis. More than 85% of the poly A-RNA obtained from the antibody-fractionated polyribosomes consisted of Ig-mRNAs as determined by translation in the wheat germ system. Approximately 95% of IgG-mRNAs, labeled with $^{125}I$, hybridized with mouse liver DNA sheared to about 300 base pairs ($S_{20,w}$=5.3) at a $C_0t_{1/2}$ of $4 \times 10^3$, indicating that complementary DNA sequences were present less than 5 times per haploid genome, (Biochem. 15: 4390, 1976). To determine if the IgG-mRNAs used contained covalently bound transcripts of unique and reiterated DNA, (homology between Igs of differing subclass specificities allows hybridization of mRNAs for one Ig and, if present, DNA for Igs of related but nonidentical types), hybrids formed with sheared DNA were isolated with or without RNase before fractionation and the apparent rates of hybridization compared. Even under less stringent hybridization conditions, a monophasic $C_0t$ curve ($C_0t_{1/2}$ of $4 \times 10^3$) resulted regardless of whether RNase was included in the isolation protocol. Under similar conditions, the apparent hybridization rates of hetergeneous nuclear RNAs from the same cell source containing covalently bound transcripts of repetitive and unique sequences were clearly different. (Supported by a Grant from the National Science Foundation)

847 BIOCHEMISTRY OF PRODUCTS OF THE MAJOR HISTOCOMPATIBILITY COMPLEX ON THE HUMAN LYMPHOCYTE SURFACE. Cox Terhorst, Richard Robb, and Jack L. Strominger. The Biological Laboratories, Harvard University, 16 Divinity Avenue, Cambridge, Mass. 02138.

Products of the HLA-A and HLA-B loci have been isolated from cultured human lymphocytes after solubilization with papain or with detergents. They are composed of two polypeptide chains, the heavier of which is a glycoprotein of 34,000 daltons (papain solubilized) or 44,000 daltons (detergent solubilized) and the lighter of which is a polypeptide of 12,000 daltons, identical to human urinary $\beta_2$-microglobulin. The amino acid composition and N-terminal sequence of p44, p39 (an intermediate in the papain digestion) and p34, indicate that a hydrophilic peptide of about 5,000 daltons is first removed from the C terminus followed by a hydrophobic peptide. The amino acid composition and N-terminal sequence of four p34 preparations have been examined (including products of both the HLA-A locus (HLA-2 from two different cell lines) and the HLA-B locus (HLA-7 and HLA-12)). The products of the two loci are strikingly similar to each other. Most HLA specificities have an acid labile peptide bond in between two intrachain S-S bridges. Additional cleavages with CNBr and NTCB have been carried out. The distance between the two half cystines is of the order of 60 amino acids. The Asn linked glycan is located on the N-terminal side of these cystines. Amino acid sequences around the half cystines show some homology with immunoglobulins. The gross structure of the HLA-A,B antigens will be outlined.

848 PARTIAL CHEMICAL CHARACTERIZATION OF Ia ANTIGENS DERIVED FROM MURINE THYMOCYTES. Benjamin D. Schwartz, Anne M. Kask, Susan O. Sharrow, Chella S. David and Ronald H. Schwartz. Departments of Medicine, Microbiology, and Genetics, Washington University School of Medicine, St. Louis, MO. 63110, the Laboratory of Immunology, NIAID and the Immunology Branch, NCI, Bethesda, MD. 20014.

The genetic and functional association between Ir genes and genes for Ia antigens have raised the question of the existence of Ia antigens on thymus-derived cells. Though serological and chemical studies have given contradictory results, functional studies seem to favor the presence of Ia antigens on T cells. We attempted to resolve this question by isolating C3H thymocytes free of other contaminating cells using the fluorescence activated cell sorter, and then chemically testing the purified populations for Ia antigens. The purified populations studied were Ig negative thymus cells, and thymus cells selected with an adsorbed rabbit anti-mouse brain antiserum. Proteins were radiolabeled in vitro, solubilized by the non-ionic detergent NP-40, reacted with anti-Ia antiserum, and analysed by SDS-polyacrylamide gel electrophoresis. Radiolabeled Ia antigens were obtained from both purified thymus cell populations. These antigens were synthesized by the thymocytes and were found on molecules composed of two chains of molecular weight 33,000 and 25,000 daltons respectively, similar to spleen cell Ia antigens. It was estimated that an average thymocyte has approximately 1/50 the amount of Ia antigens as does an average spleen cell.

849 CHEMICAL CHARACTERIZATION OF AN H-2D GENE MUTANT: H-$2^{da}$, J. L. Brown and S. G. Nathenson, Albert Einstein College of Medicine, New York, NY 10461

There are now available a number of mouse strains mutant in the major histocompatibility locus. Among these are two in which the genetic change has been localized to the D region. One of these is B10$D_2$(M504)(H-$2^{da}$). This mutant, found by Egorov, exhibits mutual CML, MLR, skin graft rejection and GVHR as well as slight serological differences with its parent, B10$D_2$. We have examined the primary sequence differences between the H-2D gene product of the mutant and its parent using radiolabeled peptide mapping. We have also determined the amount of M504 H-2D expressed on spleen cells, its molecular size, binding to lentil lectin, and carbohydrate side chain size as compared to those of the parent H-2D gene product. We find distinct differences in expression on spleen cells, binding to lentil lectin and molecular size as well as extensive differences in the comparative peptide maps. All results suggest this is not a point mutation.

850 EVIDENCE OF FUTHER SEROLOGICAL CROSS REACTIVITY BETWEEN PRODUCTS OF I-$C^d$ and I-$A^b$, Jeffrey A. Frelinger, Dept. of Microbiology, USC Medical School,Los Angeles,CA 90033

The origin of the H-2 K and D regions by gene duplication is widely accepted. Both serological and structural homology have been reported. Although reports of cross reactivity between I-A and I-C products have appeared, the origin of Ia antigens from different subregions is less clear. Using a new antiserum, B10.A(4R) anti B10.A(1R) we have been able to demonstrate cross reactivity of antigens coded by I-$C^d$ with those coded by I-$A^b$. This is the reciprocal of the cross reactivity reported by Murphy. This serum reacts with B10 lymph node cells in direct cytotoxic tests, killing 50% of the cells, with a titer of 1/80. This reaction cannot be caused by a shared I-C determinant between H-$2^{h1}$ and H-$2^b$ since 4R and B10 share the same H-$2^b$ haplotype in the I-C subregion. Therefore the reactivity with the H-$2^b$ haplotype must map to the H-2K side of the H-$2^{h4}$ recombinant, in the I-A region. This assignment is confirmed by testing D2.GD, and H-$2^{g2}$ strain. This haplotype is derived from two reactive haplotypes, H-$2^b$ and H-$2^d$, however D2.GD is unreactive, confirming the mapping of the positive reaction with B10 to the I-A subregion. We have noted that antisera which detect Ia.6 usually also detect this cross reacting specificity, and no antiserum which we have produced against I-$C^d$ which does not detect Ia.6 can detect this cross reactivity. This suggests that the cross reactive specificity may be Ia.6, although the absorbtion analysis to test this was inconclusive.

851 DIFFERENTIAL EXPRESSION OF Ia ANTIGENS ON MURINE MACROPHAGES, Carol Cowing, Benjamin Schwartz, and Howard Dickler, Immunology Branch, National Cancer Institute, NIH, Bethesda, MD 20014, and Department of Medicine, Washington University School of Medicine, St. Louis, MO 63110. There is accumulating evidence for a functional role of Ia antigens in macrophage-lymphocyte interactions, yet these antigens have only been found in relatively small amounts on peritoneal macrophages. In view of this apparent discrepancy, another population of mouse macrophages was examined. Splenic and peritoneal macrophages were evaluated for Ia antigens by indirect immunofluorescence. Greater than 50% of purified spleen macrophages had Ia on their surface, while less than 10% of peritoneal exudate macrophages had detectable Ia. When macrophages isolated from spleen were maintained in culture for seven days, the cells lost detectable Ia antigens and became indistinguishable from peritoneal macrophages. That the Ia antigens detected on freshly isolated spleen macrophages and on peritoneal macrophages were synthesized by these cells was confirmed by radioisotope labeling techniques. Sera and strain combinations within the B10 congenic series were used to identify subregions within the I region. With nine combinations it could be shown that antigens coded in the I-A subregion accounted for the majority of detectable Ia on splenic macrophages (57% $\pm$ 9); while in five instances, antigens coded by the rest of the I region were detected on only a minority of these cells (11% $\pm$2). These findings suggest the possibility that macrophages at different stages of differentiation may have different functional characteristics in the immune response.

852 IMMUNOLOGICAL STUDIES OF THE L2C LEUKEMIA - A B CELL LEUKEMIA OF INBRED STRAIN 2 GUINEA PIGS, Ira Green, Theodore Konen, Helen Hu, Benjamin D. Schwartz and Ethan M. Shevach, The NIH, NIAID, LI, Bethesda, Md. 20014.

The L2C leukemia cell bears IgM and receptors for C3b. Five closely related lines of the L2C leukemia have been described, all of which bear the same IgM idiotype. Four of the 5 lines also bear the Ia antigens of strain 2 guinea pigs. Previous studies using immunization protection tests have demonstrated that the L2C cells bear a TSTA. However the TSTA in the Ia negative line is not immunogenic. Hetero and allo antisera directed against the IgM idiotype were produced; these antisera were then used in conjunction with internal radioactive labelling followed by SDS polyacrylamide gel electrophoresis to study surface antigens on the $Ia^+$ and $Ia^-$ L2C cell. It was demonstrated that the IgM was made by the cell and that it was monomeric. Furthermore although the allo antisera potentially contained antibodies to an Ia idiotype, none were found. Furthermore the anti IgM idiotype antisera did not react with any Ia-like molecule; suggesting that there was no shared antigenic structure between the IgM idiotype and the Ia molecule of the L2C cell. In another phase of the studies, a KCl extract of the L2C cell was prepared that was able to successfully immunize normal strain 2 animals against a challenge with L2C cells. Immunochemical studies are currently in progress to determine the nature at the TSTA in the KCl extract.

853 STUDIES ON THE EXPRESSION OF MURINE IgD, IgM AND IgG, Samuel J. Black, Leonard A. Herzenberg, Michael R. Loken, Barbara A. Osborne, and Wessel van der Loo, Genetics Department, Stanford University School of Medicine, Stanford, CA, 94305.

Recent work from our laboratory has shown that most mouse B cells co-express IgM and IgD on their suface. Of these cells a small number also bear IgG. In order to investigate the co-expression of several Ig classes on a B cell membrane we are using allo and hetero-antisera specific for $\mu$, $\delta$ and $\gamma$ heavy chains in combination with fluorescence-activated cell separator analysis. At present we are actively pursuing the following questions: (1) On lymphocytes bearing more than one Ig class is there a quantitative relationship between the amounts of the classes expressed? (2) Do cells with surface IgM, IgD, IgG or any combination of these Ig classes represent functionally different groups?

To date (11/18/76) we have found that the relative amounts of different Ig classes expressed on the surface of a single lymphocyte vary with the age of the animal from which the cell was harvested along with the organ in which the cell was found. Thus, no general quantitative relationship has been observed. In addition, studies performed with antisera reacting with allotypic determinants on the $\delta$ heavy chain suggest that IgD is present on the membrane of unprimed and primed lymphocytes. There is no correlation between the class of antibody secreted by PFC differentiating from these cells on exposure to antigen and the amount of IgD on their surface. Similar studies on lymphocytes with membrane bound IgM and IgG are in progress.

854 FUNCTION OF LYMPHOCYTES BEARING DIFFERENT SURFACE RECEPTORS. David L. Brandon, Andrew Edwards, and R. M. E. Parkhouse. National Institute for Medical Research, London NW7 1AA, England.

We investigated whether the class of immunoglobulin on the surface of adult CBA mouse spleen cells is related to the presence of other surface receptors or to the ability of the cells to develop into antibody producing cells following stimulation by lipopolysaccharide (LPS). Cells which bear Fc receptor (FcR) and complement receptor (CR) were detected by rosette formation, using ox red blood cells, rabbit antibody, and AKR mouse serum as a source of complement. Surface IgM and IgD were detected by indirect immunofluorescence. There is a small subpopulation of B cells which lacks FcR but has Ig mostly of the IgD class. About 85% of CR-bearing lymphocytes have surface Ig of both the IgM and IgD classes, whereas 15% of the CR-bearing lymphocytes have no surface Ig detectable by immunofluorescent microscopy. Populations depleted of CR-bearing lymphocytes are able to respond to LPS by differentiating into cells which secrete IgM and IgG. Most of the B cells express both IgM and IgD on their membranes, but about 5% have only IgM and 5 to 10% have only IgD, as defined using the fluorescence-activated cell sorter (FACS). Spleen cells were fractionated using the FACS and cultured in the presence of LPS. Elimination of the most intensely stained IgM-bearing cells or IgD-bearing cells did not affect the response of the cells in culture. We conclude that precursors of IgM- and IgG-secreting cells are not limited to the CR-bearing cells or to those cells with a high density of surface IgM or IgD.

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855 LYMPHOCYTE SURFACE IgD IN RATS, MICE AND MEN. Marvin A. Cuchens and Gerrie A. Leslie, Dept. of Microbiology & Immunology, Univ. of Oregon Health Sciences Center, Portland, OR 97201.

Rabbit anti-rat IgD was prepared with rat spleen and lymph node lymphocyte membrane IgD isolated from a chicken anti-human $\delta$ immunoadsorbent (IA). The antiserum was made $\delta$ chain specific by passage through appropriate immunoadsorbents. It demonstrated reactivity with human IgD and not against rat serum components by immunodiffusion. Membrane FA of human PBL detected ~5% IgD positive cells and was specifically blocked by preincubation of the anti-rat IgD with human IgD. Similar studies with mouse splenic lymphocytes demonstrated very dull fluorescence. The questionable IgD positive cells represented <3% of the total number of Ig positive cells, in marked contrast to reports of >60%.

The tissue distribution of Ig bearing lymphocytes in the rat follows:

| | Blood | Spleen | Lymph Nodes | Thymus | Peyer's Patches |
|---|---|---|---|---|---|
| % IgM | 18.7 | 33 | 26 | <0.5 | 45 |
| % IgD | 6.4 | 15 | 17 | <0.5 | 50 |
| % Ig | 15.4 | 54 | 51 | <0.5 | 92 |

The presence of rat membrane IgM and IgD has been substantiated by SDS-polyacrylamide disc electrophoresis.

Cytoplasmic FA stains of white cell suspensions of various tissues detected 1-3% IgD positive cells. Both plasma cells and lymphocytes were found. Studies are in progress to determine if circulating IgD can be detected in the rat. These studies suggest that rat IgD may be antigenically more cross-reactive with human IgD than the putative mouse IgD.

856 IgD ALLOTYPE EXPRESSION IN THE MOUSE, James W. Goding, Noel L. Warner and Judith E. Layton, Walter and Eliza Hall Institute, Royal Melbourne Hospital, Parkville, 3050, Victoria, Australia.

Many mouse alloantisera prepared against lymphocytes contain antibodies to IgD, IgM or sometimes both. The presence of these antibodies has thus revealed genetic polymorphisms of both major B cell receptor classes, and allowed the identification of two new immunoglobulin heavy chain loci; Ig-5 (for delta chains) and Ig-6 ($\mu$ chains). Both are linked to the Ig-1 locus.

Immunofluorescence studies reveal that most B cells in spleen, lymph node, thoracic duct lymph and peripheral blood possess both $\mu$ and delta receptors. In bone marrow, about 60% of B cells bear IgM only, and the remainder are doubles. Even at one year, most B cells are doubles, and cells bearing only IgD are uncommon. Surface IgM and IgD have similar or identical variable regions. After activation with LPS, cells appear to lose surface IgD.

857 THE CAPACITY OF INDIVIDUAL IMMUNOCYTES TO RESPOND TO MORE THAN ONE ANTIGEN. P. Liacopoulos, J. Couderc and J. Panijel, Institut d'Immuno-Biologie, Hôpital Broussais 75674 Paris cedex 14 and Institut Pasteur 75724 Paris Cedex 15, France.

Stimulation of mice with two antigens results in the appearance of PFC specific for each of the antigens and of a low percentage (av. 2%) of PFC reacting to both. These double PFC produce two different types of antibody molecules since (a) in micromanipulated individual double PFC inhibition by soluble antigen of the one specific lysis do not interfere with the other lytic activity and (b) individual double PFC generate monospecific daughter PFC reacting to only the one or the other antigen. These results strongly suggest that PFC precursors are in fact pluripotential i.e. must bear more than one antigen specific receptors.

In other experiments the patterns of specific recovery of normal mouse spleen cell populations depleted of anti-Pigeon RBC cells were investigated. It was found that no anti-PRBC response occur when such populations transferred in irradiated recipients are stimulated with PRBC. However when recipients are also given a non-specific stimulation, an early recovery of the anti-PRBC reactivity is produced. This recovery cannot originate from a clonal expansion of specific cells possibly escaped the depletion, because when the adjuvant muramyldipeptide (MDP) is given the recovery occurs suddenly between the 4th 1/2 and 5th day (doubling time 4 hours) and when it is provoked by elicitation of an unrelated delayed type reaction (to picryl chloride or TNP) an important number of PFC reacting to both PRBC and TNP (5%) is detected. The early recovery could therefore be attributed to a recruitment into the specific response of pluripotential PFC precursors having at the time of depletion a too small number of PRBC receptors to permit their firm binding to PRBC immunoabsorbant column.

858 IN VITRO REGULATION OF SURFACE ALLOTYPE IN HETEROZYGOUS b4,6 RABBIT PERIPHERAL BLOOD LYMPHOCYTES, Linda M. Schoenberg and Benjamin Wolf, Univ. of Pa., Phila., Pa. 19174

It has been previously demonstrated that single and double expression in PBL from b4,6 rabbits is due to active synthesis. We have studied the effects of anti-allotype antibody on surface allotype expression in an unimmunized, restricted population of PBL after a short incubation too brief to stimulate blast transformation and cell replication. Neonatal and adult PBL (pronase-treated or untreated) were incubated overnight in the absence of anti-allotype antibody or in the presence of anti-b4, anti-b6 or anti-b4 + anti-b6. Assay by the mixed antiglobulin technique confirmed the decrease in surface allotype. Incubation in either anti-b4 or anti-b6 for as little as 4 hours led to the suppression of the homologous allotype and compensatory increase of the alternate allotype. This marks the first time that such a compensatory increase has been observed in vitro. Untreated PBL from neonates were then sequentially incubated, first with anti-b4 (4 hours) and followed by anti-b6 (14 hours). This resulted in a release from suppression of the b4 surface allotype. Reversing the procedure gave corresponding results for b6. This suggests a possible inter-allotype regulatory influence in these b4,6 cells. If these observations are not the result of altered allotype expression in the same b4,6 cell, one must suppose that previously non-expressing cells are either being stimulated or released from suppression by a regulatory cell.

This work was supported by NSF Grants GB-28694 and GB-44202 and the University of Pennsylvania Plan to Develop Scientists in Medical Research.

**859** GENETIC CONTROL OF THE IMMUNE RESPONSE TO H-2.32. Nobukata Shinohara & David H. Sachs, NCI, NIH, Bethesda, MD 20014

We have examined strains of B10 congenic mice of various H-2 haplotypes, and have found them all to produce a very weak humoral antibody response to H-2.32, the H-2D private specificity of $H\text{-}2^k$. The failure of these strains to mount an anti-32 response appears to be attributable to non-H-2 linked gene(s) present in the B10 background. Also, as has been previously reported, two strains of different background sharing the same $H\text{-}2^m$ haplotype show a significant difference in the magnitude of the anti-32 antibody response. Thus, B10.AKM mice are low responders to B10.BR whereas AKR.M mice are high responders to AKR. The (AKR.MxB10.AKM)$F_1$ mice are also high responders to B10.BR. In an analysis of backcross progeny of these two strains, (AKR.MxB10.AKM)$F_1$ x B10.AKM, the anti-32 antibody response was found to be predominantly controlled by a single locus. This locus was shown to be linked neither to the Ig heavy chain allotype locus nor to the Ly2 locus which is considered to be closely linked to the k light chain locus, indicating a probable regulatory rather than structural gene defect. There was no correlation between the magnitude of the antibody response to $H\text{-}2^d$ antigens and that of the anti-32 antibody response in individual backcross animals, suggesting the antigen specific nature of this Ir gene. Finally, despite the observed difference in antibody production, no significant difference between AKR.M and B10.AKM mice was observed in induction of $H\text{-}2D^k$-specific killer cells. Thus, this non-H-2 linked antigen-specific Ir gene apparently controls B cell but not T cell anti-H-2.32 reactivity.

**860** GENETIC CONTROL OF THE IMMUNE RESPONSE TO H-7 ALLOANTIGENS, Peter J. Wettstein, Dept. Microbiology, USC Medical School, Los Angeles, CA. 90033

H-2 linked genes are important in regulating presentation of H-7 alloantigenic determinants in H-7 -incompatible skin grafts and H-7 antigen-specific, recipient responsiveness. Tail skin was exchanged orthotopically between H-7 congenic partners sharing the $H\text{-}2^b$, $H\text{-}2^a$, or $H\text{-}2^d$ haplotypes. These congenic pairs carry the $H\text{-}7^a$ and $H\text{-}7^b$ alleles which code for the H-7.1 and H-7.2 antigens. Mice possessing the $H\text{-}2^b$ haplotype were fast responders to both H-7.1 (MST = 3.1 weeks) and H-7.2 (MST = 5.4 weeks) -incompatible grafts. $H\text{-}2^a$ and $H\text{-}2^d$ mice were slow responders to H-7.1 -incompatible skin which survived on both hosts with MST's of > 20 weeks. $H\text{-}2^a$ and $H\text{-}2^d$ mice were also slow responders to H-7.2 -incompatible grafts ($H\text{-}2^a$ : MST > 20 weeks; $H\text{-}2^d$; MST = 13.1 weeks). Fast responsiveness was inherited as a dominant trait. Grafting of $H\text{-}2^a/H\text{-}2^b$ heterozygotes with H-7 -incompatible skin demonstrated the influence of donor H-2 haplotype on relative "rejectability" of H-7 -incompatible grafts. $H\text{-}2^a/H\text{-}2^b$ mice rejected H-7.1 -incompatible skin from $H\text{-}2^b/H\text{-}2^b$ (MST = 3.7 weeks) and $H\text{-}2^a/H\text{-}2^b$ donors (MST = 3.5 weeks) more rapidly than skin from $H\text{-}2^a/H\text{-}2^a$ donors (MST = 8.0 weeks) ($p = .002$). H-7.1 -incompatible grafts obtained from $H\text{-}2^b/H\text{-}2^b$ donors were able to prime for the accelerated rejection of secondary H-7.1 -incompatible grafts from $H\text{-}2^a/H\text{-}2^a$ donors, and vice versa. Similar results were obtained with H-7.2 -incompatible grafts on $H\text{-}2^a/H\text{-}2^b$ and $H\text{-}2^d/H\text{-}2^b$ recipients. These results clearly demonstrate the importance of H-2-linked genes in regulating both the presentation of H-7 alloantigens in donor tissue and alloantigen-specific, recipient responsiveness.

**861** GENETICS AND REGULATION OF H-2-CONTROLLED GRAFT-VERSUS-HOST REACTIONS, Edward A. Clark, Yoko S. Mullen and William H. Hildemann, Dental Research Institute, UCLA, CA 90024

Genetic fine mapping studies identify at least four regions within the H-2 complex coding for products which induce GVH reactions: K, I-A, I-C, and D. Disparities at I-B, I-J, or S do not evoke GVH. To help clarify how H-2-controlled GVH reactions are regulated, we recently have examined the effect of preexposure of spleen cell donors to anti-recipient alloantibodies. Prior to encounter with B10.A host cellular alloantigens, B10 adult donors were injected intraperitoneally with either hyperimmune donor anti-recipient antiserum (B10 anti B10.A; hemagglutinin titer $2^8$-$2^{10}$) or normal serum (NMS). Two to seven days later spleen cell suspensions were prepared, washed twice, and counted. Ten to $20x10^6$ spleen cells were then injected into newborn B10.A or (B10.AxB10)F1 mice. Recipients injected with cells from alloantiserum-treated donors had reduced GVH splenomegaly (spleen index (S.I.) = 1.61±0.13 n=16) and much reduced mortality compared to littermate controls injected with spleen cells from NMS-treated donors (S.I. = 2.17±0.14 n=13). Additional experiments indicate: 1) specific inhibition disappears in donors by 8 to 14 days after pretreatment; 2) reciprocal B10.A recipient anti-B10 donor serum has no effect; 3) IgG enriched B10 anti B10.A antibodies purified by $NH_4SO_4$ precipitation and DE52 cellulose ion-exchange chromatography are inhibitory. Experiments are in progress to define the specificity of the inhibitory antibodies and the donor cell type affected.

862 RECOGNITION OF MINOR ANTIGENIC DETERMINANTS BY RAT CYTOTOXIC T CELLS, Ann Marshak and Darcy B. Wilson, Immunobiology Research Unit, Univ. of Pa. School of Medicine, Phil., Pa. 19174

In the rat, as in the mouse, recognition by cytotoxic T cells (CTL) of TNP-modified cells and minor histocompatibility antigens unlinked to the major histocompatibility complex (MHC) is restricted by gene products of the MHC (Ag-B in the rat). However, CTL raised within phenotypically identical Ag-B[1] strains cross-react extensively on third party target cells, regardless of the target Ag-B haplotype. Backross analysis suggests that these antigens, which operationally must be considered to be minor antigens by GVH and MLC criteria, map within or close to the Ag-B locus. Concanavalin A induced lymphoid blast cells, tumor cells, and normal T and B lymphocytes all express these "minor" major locus determinants. It is proposed that such antigens are either slight structural variations of the defined MHC determinants, or separate molecular structures exempt from MHC controlled restriction due to their association with the MHC complex.

Supported by grants AI-10961, CA-15822, and CA-09140.

863 H-2D-ASSOCIATED HYBRID RESISTANCE TO HISTOCOMPATIBLE TUMORS, Richard C. Harmon, Edward A. Clark, Linda S. Wicker, and William H. Hildemann, Dept. Microbiology and Immunology, UCLA, Los Angeles, CA 90024

Hybrid resistance to two methylcholanthrene-induced B10.A(4R) fibrosarcomas and to the C57BL lymphoma EL-4 was studied in H-2 heterozygous mice. Histocompatible tumor recipients were derived from mating C57BL/10, B10.A and $H\text{-}2^a/H\text{-}2^b$ recombinant B10.A(2R), 4R, 5R, and 18R mice in such a way as to provide heterozygosity at defined portions of the H-2 complex. Hybrid resistance following intraperitoneal injection of tumor cells was determined by comparing the mean survival times of H-2 heterozygous mice with those of homozygous 4R(sarcomas) or B10(EL-4) mice. Recipients which were heterozygous for the K, I, and S regions were not significantly resistant to tumor growth. However, mice heterozygous for the D region alone or for the D region in combination with other regions were tumor resistant. Hybrid resistance was not demonstrated if tumor cells were injected subcutaneously rather than by the intraperitoneal route. A positive correlation was demonstrated between tumor resistance of mice in vivo and expression of spleen cell-mediated, natural cytotoxicity against EL-4 in vitro. Additional tumor recipients have been derived from mating B10 or 4R mice with congenic $H\text{-}2^a/H\text{-}2^s$ recombinant mice. Preliminary results using these recipients suggest that the locus responsible for hybrid resistance lies between S and D. Further work using additional recombinants between S and D to map this locus is in progress.

864 SPECIFIC SUPPRESSION OF CYTOTOXIC RESPONSE TO SYNGENEIC TUMORS
Fumio Takei, Julia G. Levy and Douglas G. Kilburn, University of British Columbia, Vancouver, B.C., Canada V6T 1W5

P815 mastocytoma cells, when injected subcutaneously into syngeneic DBA/2 mice, induced T lymphocyte-mediated cytotoxicity, but it decreased as the tumor grew, and ultimately the tumor killed the mice. It was found that thymocytes and spleen cells from mice with progressively growing tumors inhibit the in vitro generation of anti-tumor cytotoxicity, indicating the presence of suppressor cells in these mice. The characterization of the suppressor cells suggested that they are T lymphocytes and their activity is specific to the tumor, i.e., they do not inhibit cytotoxic response to other tumor. Although both the suppressor T cells and the killer T cells seemed to be tumor-specific, they migrated in separated fractions by ficoll-hypaque density cell separation. Adult thymectomy resulted in a lower primary anti-tumor response. Normal thymocytes were found to enhance the response of lymph node cells, suggesting a cellular cooperation in the anti-tumor response.

865 GENETIC CONTROL OF RESISTANCE TO MPC-11 TUMOR CELLS. Mary Clare Walker and Julia M. Phillips-Quagliata, N.Y.U. Medical Center, New York, N.Y. 10016.

Genetic control of the resistance of $F_1$ hybrids between Balb/c and various mouse strains to the Balb/c transplantable, mineral oil-induced plasmacytoma, MPC-11, has been investigated. 3 x $10^5$ viable MPC-11 cells were injected subcutaneously into $F_1$ hybrids between Balb/c and C57Bl/10 (B10) or A congenic mice carrying the H-$2^b$, H-$2^a$, H-$2^s$ or H-$2^f$ haplotypes, between Balb/c and B10 congenic mice carrying the H-$2^m$, H-$2^k$, H-$2^{i5}$, H-$2^{h2}$ and H-$2^{y2}$ haplotypes and between Balb/c and C57Bl/6 (B6) (H-$2^b$), DBA/1 (H-$2^q$), D1.C (H-$2^d$), DBA/2 (H-$2^d$) and SJL (H-$2^s$). The incidence of tumor take, regression and survival for more than 80 days were scored. The results indicate that background genes present in B10, B6 and DBA/1 X Balb/c $F_1$ hybrids confer resistance to tumor take. Hybrids between Balb/c and A mice, carrying any of the 4 H-2 haplotypes tested, or SJL or DBA/2 are 100% susceptible to tumor take, as are homozygous Balb/c mice. Among B10 H-2 congenic X Balb/c $F_1$ hybrids there are differences in ability to cause regression of established tumors. Our results therefore suggest that genes mapping outside the H-2 complex play a crucial role in determining susceptibility to MPC-11 tumor take and that genes mapping within the H-2 complex may play a role in tumor regression. It is unlikely that the Hh-1 locus is involved in resistance as Balb/c X B10.D2 $F_1$ mice are resistant and are homozygous at both H-2D and Tla. Furthermore, cells susceptible to silica seem not to be involved in resistance. We are currently testing $F_1$ hybrids between Balb/c and Bailey recombinants to obtain a strain distribution pattern for resistance and are planning to type (Balb/c X B10) X Balb/c $F_1$ backcrossed mice for linkage of resistance to genes in various linkage groups. Supported by NIH grants CA 16247 and CA20045.

866 THE CYTOTOXIC RESPONSE OF SJL MICE TO SYNGENEIC IN VITRO RETICULUM CELL SARCOMA LINES. Janet M. Roman and Benjamin Bonavida. Dept. of Microbiol. & Immunol., UCLA, Los Angeles, Ca. 90024

We have utilized in vitro sensitizations to analyze the cytotoxic response of SJL mice to syngeneic reticulum cell sarcoma (RCS) tumors. Three RCS tumor lines passaged in vitro (RCS-LA1, RCS-LA6, and RCS-LA8) are capable of stimulating a syngeneic cytotoxic response in spleen and lymph node populations of SJL mice (ages 2-12 months); however, only RCS-LA6 and RCS-LA8 serve as good targets for this syngeneic killing. Curiously, SJL spleen or lymph node cells sensitized to EL-4, normal C57BL/6 spleen, or normal BALB/c spleen will lyse RCS tumor cells but not Con A stimulated SJL blast cells. This suggestion of antigens shared between RCS tumor cells and normal allogeneic cells is being further analyzed by cell-mediated killing and serological techniques. The presence of altered antigens or alloantigens on RCS cells would greatly facilitate analysis of this tumor system by providing (an) antigenic marker(s) enabling quantitation of the frequency of tumor cells in an in vivo RCS tumor. More significantly, presence of alloantigens on RCS cells would have important implications concerning strain distribution of genes coding for H-2 antigens, control of expression of these genes, and alteration of these control mechanisms associated with the neoplastic state. (Supported by USPHS CA19753 and CA9120.)

867 IMMUNOGENICITY OF MODIFIED LYMPHOMA CELLS, Morton D. Prager, F. Samuel Baechtel and William C. Gordon, U. of Texas Health Science Center, Dallas, Tx 75235

Following modification of mouse lymphoma cells by over 20 different chemicals, their immunogenicity in syngeneic hosts was determined in tumor rejection tests. Sulfhydryl blocking agents which are themselves poor haptens (e.g., iodoacetamide (IAd), iodoacetate, N-ethylmaleimide) have been favored because of their effectiveness and ease of use. IAd treatment of 6C3HED, P1798, L1210, and YAC lymphomas produced protective vaccines. Degree of substitution has been quantitated in kinetic studies with $^{14}$C-IAd; standard preparations were substituted with 2.5 μmoles/$10^9$ cells, but at 17 μmoles/$10^9$ cells protective capacity decreased. Specific antitumor antibodies are commonly elicited by vaccination with modified cell preparations, and peritoneal cells from immunized mice inhibit proliferation of tumor cells in vitro compared to those from non-immunized animals (IDS assay). However, BALB/c mice have evidenced resistance to P1798 without demonstrable antibody. When potent anti-P1798 antisera were obtained, reaction with host and other thymocytes was observed. Partially purified Ag from P1798 was effective immunoprophylactically and may be related to Thy-1. Complexing IAd-lymphoma cells with the lipophilic dimethyldioctadecylammonium (DDA) cation increased their protective capacity over cells treated with IAd alone which in turn were more immunogenic than X-irradiated(6C3HED)cells. In 2 of 3 lymphoma systems DDA increased antitumor antibody production, but because DDA interferes in the IDS assay, its effect on cellular immunity is unclear. These studies indicate that modification of malignant cells can increase their immunogenicity. (Supported by Grant CA 12089 from the National Cancer Institute, DHEW)

868 T-CELL PROLIFERATIVE AND ANTIBODY RESPONSE TO NUCLEASE IN MICE: H-2-LINKED CONTROL AT THE LEVEL OF INDIVIDUAL DETERMINANTS, Jay A. Berzofsky, Ronald H. Schwartz, Alan N. Schechter, and David H. Sachs, NCI, NIAID, and NIAMDD, NIH, Bethesda, MD 20014.

Antibody production to staphylococcal nuclease has previously been shown to be under H-2-linked Ir gene control in mice. When immunized with whole nuclease, the low responder B10 strain shows a selective defect in the ability to make antibodies to a determinant(s) in the region between residues 99 and 149 of nuclease, compared with the congenic high responder strain B10.A. In addition, the peptide fragment corresponding to this region of nuclease is immunogenic in the B10.A strain but not in the B10, whereas the fragment (1-126) is immunogenic in both strains. The same pattern of high or low responsiveness in a large number of strains has now been found to hold for in vitro T-cell proliferation using peritoneal exudate T-lymphocyte enriched cells (PETLES) from nuclease-primed mice. Moreover, when PETLES from nuclease-primed mice were tested for T-cell proliferation stimulated by different fragments, the fragment (99-149) was much less stimulatory in the B10 than in the B10.A compared to other fragments. These results suggest that both antibody production and T-cell proliferation to nuclease are under the same Ir gene control exerted at the level of individual antigenic determinants rather than the whole molecule. Previous mapping studies have assigned the Ir-nuclease gene to the I-B subregion of H-$2^b$. However, the T-cell proliferative response patterns of some intra-H-2 recombinants to a series of fragments show characteristics of both the B10 and B10.A patterns. This observation is consistent with the notion that genes mapping in different I subregions control responses to different individual determinants.

869 ROLES OF ADHERENT CELLS IN MURINE T CELL ANTIGEN RECOGNITION, Lanny J. Rosenwasser, and Alan S. Rosenthal, LCI, NIAID, NIH, Bethesda, MD 20014

We have developed a strict adherent cell dependent, in vitro proliferative assay for primed murine T cell function. In order to study the cellular requirements for murine T cell antigen recognition we have used specific alloantisera, complex multi-determinant soluble protein antigens as well as defined antigens under strict IR gene control to demonstrate at least two distinct functions for adherent cells in the T cell antigen recognition process. The first function is the production of an "immunostimulatory" factor which is not antigen specific nor H-2 related, in that adherent cells, regardless of haplotype, will produce factor. The immunostimulatory factor, however, is species and cell type specific in that guinea pig macrophages and murine fibroblasts will not produce it. A second function of adherent cells in our assay system appears to be antigen presentation. This function is H-2 related in that identity or partial identity at H-2 between macrophage and primed T cell is required. At present, studies are being undertaken to map the antigen presentation function further to a specific subregion of the H-2, and to assess IR gene function in the macrophage.

870 IN VITRO IMMUNE RESPONSE OF SPLEEN CELLS FROM BIOZZI MICE GENETICALLY SELECTED FOR HIGH AND LOW ANTIBODY PRODUCTION, Gino Doria and Giovanna Agarossi. CNEN-Euratom Immunogenetics Group, Laboratory of Radiopathology, C.S.N. Casaccia (Rome), Italy.

The aim of this study was the identification of the cell type in which genes selected for high (H) or low (L) response against SRBC express their functions. Spleen cells from H and L responder mice were immunized with SRBC, TNP-HRBC, or TNP-LPS in the Mishell and Dutton system. Each antigen elicited primary responses of different magnitude in cultures of H and L spleen cells, the differences being at least as great as those observed *in vivo*. These findings, under experimental conditions allowing the exclusion of any influence of the animal milieu during the immune response, suggest macrophages, B, and T lymphocytes as possible target cells of gene action. The results of *in vitro* cell separation and recombination experiments indicate that the genetic selection brought about differences between H and L responders much more pronounced in lymphocytes than in macrophages. Among lymphocytes, B cells but not helper T cells were found more responsive in cultures of spleen cells from H than from L mice.

871 GENETIC CONTROL OF IMMUNOLOGICAL UNRESPONSIVENESS TO AQUEOUS SOLUTIONS OF PROTEIN ANTIGENS. THE Ir-Z1 AND ir-Z2 LOCI IN SJL/J AND CE/J MICE, Boris Rotman, Brown University, Div.Biol.Med., Providence, RI 02912

Immunization with some heterologous proteins in aqueous solutions fails to elicit humoral antibody in several mammalian species. While we found this unresponsiveness to be prevalent in mice from 31 different inbred strains injected with bacterial β-D-galactosidase(Z), in two of the strains, SJL/J and CE/J, a high response was observed. Genetic analyses involving $F_2$ and backcrosses between responders and non-responders indicate that the responder trait segregates as a single dominant locus for SJL/J and as a single recessive for CE/J. These loci are termed Ir-Z1 and ir-Z2, respectively.

There is no correlation between H-2 type and response to aqueous Z at the strain level. No linkage between Ig-1 and Ir-Z1 or ir-Z2 was detected.

Responder mice produced mainly IgG which was qualitatively undistinguishable from IgG produced in non-responder mice immunized with enzyme-adjuvant mixtures. Both precipitating and activating(i.e., capable of activating a defective β-D-galactosidase) antibody were found in responder animals.

The immunogenicity of Z in responder mice is specific and is not associated with a functional catalytic site since mutant enzymes elicit antibody. Neither bovine serum albumin, human serum albumin, nor bacterial galactose-binding protein elicited specific antibody under our conditions.

Non-responders acquired specific immunological memory after injection of Z.

872 MULTIGENIC CONTROL OF MURINE RESPONSE TO OVALBUMIN Martha Turner Lubet and John R. Kettman, University of Texas Health Science Center, Dallas, Texas 75235

The ability of different doses of ovalbumin (OVA) to generate in mice a primary antibody and to sensitize the mice to transfer delayed type hypersensitivity (DTH) was examined. In 7 of the 10 strains examined, there was good correlation between minimum doses of OVA necessary to give a primary anti OVA response and to sensitize to transfer DTH. As others have reported, the minimum dose of antigen necessary to generate a primary anti OVA response varies with strains. Strains bearing the H-$2^a$ or H-$2^k$ haplotype, in general, require higher doses of OVA. In congenic strains, which differ only at the MHC, B10.Br require 10 ug while B10 and B10.D2 require 1 ug to show a primary antibody response. But strains of mice that bear the same H-2 but different backgroups show different dose response profiles. C3H/HeJ (H-$2^k$) respond to OVA only at 100 ug but B10.Br respond to 10 ug OVA; B10.D2 (H-$2^d$) respond to 1 ug while DBA/2 require 10 ug of OVA. These results suggest that the minimum dose of OVA necessary to elicit a primary antibody response depends on H-2 haplotype as well as other genes. The dose profile for the DTH responses is similar. The C3H/HeJ respond to 100 ug but B10.Br respond to 10 ug OVA. B10 and DBA/2 require 10 ug of OVA to sensitize for DTH, but B6D2 $F_1$ and B10.D2 requires only 1 ug of OVA. Thus there is complementation seen in the control of DTH, but it is not known if this complementation is related to that controlling the primary antibody response.

873 GENETIC CONTROL OF THE ANTIBODY RESPONSE TO TNP-MSA, Walter J. Urba and William H. Hildemann, Dept. Microbiology and Immunology, UCLA, Los Angeles, CA 90024

The antibody response of inbred mice to 2,4,6-trinitrophenol (TNP) hapten conjugated to autogenous mouse serum albumin (MSA) is regulated by gene products of the H-2 complex (Rathbun and Hildemann, J. Immunol., 105:98, 1970). Low antibody responsiveness to $TNP_{15}$MSA is determined by the H-$2^a$ haplotype (B10.A and A/J) as a dominant trait, whereas homozygosity for the H-$2^b$ haplotype (A.BY and B10) leads to high responsiveness. Unlike most other H-2-linked immune response genes, the $F_1$ hybrids, (B10.A x B10)$F_1$ and (A.BY x A/J)$F_1$ mice, are low responders.

In recent fine-mapping studies, H-2 recombinant mice were immunized ip with 50μg $TNP_{15}$MSA in CFA and bled 7, 14 and 21 days later. We used a modified Farr assay to determine the percent antigen bound by 25μl of a 1:5 dilution of antiserum incubated with 25μl of 2 x $10^{-8}$M $^{125}$I-$TNP_{10}$BSA. Using this assay, we confirmed that B10 and A.BY (bbbbbb) were high responders while A/WySn and B10.A (kkkddd) and (B10.A x B10)$F_1$ and (A.BY x A/WySn)$F_1$ mice were low responders. 2R (kkkddb), 3R (bbbddd), 5R (bbbddd) and 4R (kkbbbb) strains were all low responders after primary immunization.

These results suggest that complementary genes within the H-2 complex may regulate the immune response to $TNP_{15}$MSA. Experiments in progress should a) map the TNP-Ir gene(s) within the H-2 complex and b) identify the immunoglobulin classes affected by high versus low responsiveness.

874 ANTIBODY RESPONSE TO DEXTRAN OR DNP-DEXTRAN IN MICE IS H-2 - LINKED, Bonnie Blomberg and Melvin Cohn, The Salk Institute, San Diego, CA 92112

Our previous genetic studies on the immune response to dextran B-1355S have shown that a specific structural $V_H$ (heavy chain variable region) gene, $V_H$-DEX, is required for a good primary antibody response in mice. H-2 - linked genes are also involved in the ability to respond to dextran and can only be revealed when the $V_H$-DEX gene contribution is removed; this is accomplished either by measuring the anti-dextran response in animals which genetically lack $V_H$-DEX (i.e. in general do not carry the BALB/c allotype) or by measuring the anti-DNP response to DNP-dextran in all animals (and where the $V_H$-DEX gene will not contribute to B cell response differences). In both cases the $H\text{-}2^b$ haplotype is associated with responsiveness: this is manifested as a higher hemagglutinating response to dextran itself and the ability to generate an IgG response to DNP on DNP-dextran. The H-2 - linked control of the IgG anti-DNP-dextran is "thymus-independent" as measured in nude(athymic) mice carrying either $H\text{-}2^d$ or $H\text{-}2^b$.

875 GENETIC CONTROL OF ANTIBODY RESPONSE TO THYMUS-INDEPENDENT ANTIGEN LINKED TO THE MAJOR HISTOCOMPATIBILITY COMPLEX, Judith Lovchik and Erwin Diener. Department of Immunology, The University of Alberta, Edmonton, Alberta, Canada.

Genetically-controlled antibody responses toward only thymus-dependent antigens have been found to be linked to the major histocompatibility complex. To date, genetic control of antibody responses to thymus-independent antigens have not been observed which are linked to the major histocompatibility complex. We have found, however, that the antibody response to the thymus-independent antigen, polymerized flagellin (POL 1338), is very low in mice of the $H\text{-}2^d$ haplotype both <u>in vivo</u> and <u>in vitro</u>. $F_1$ hybrids between high responder and low responder strains behave as responders, indicating dominance of the gene(s) coding for responsiveness. $H\text{-}2^s$ and $H\text{-}2^p$ mice are also low responders, while $H\text{-}2^b$ mice respond well to POL 1338. Congenic strains possessing the $H\text{-}2^d$ (low responder) haplotype on genetic backgrounds from high responder mice behave as low responders, suggesting H-2 linkage. Additional studies with recombinant congenic mice suggest that responsiveness to POL is not controlled by Ir-region genes but will probably map in the D-end of the H-2 complex.

Low responder mice are capable of recognizing POL 1338, as their anti-TNP response is as great as that of high responder mice when TNP-POL is used as antigen.

876 IMMUNE RESPONSE TO α-1,3 DEXTRAN IN MICE SHARING THE $Ig\text{-}1^b$ ALLOGROUP AND THE $H\text{-}2^b$ HAPLOTYPE, Claude de Preval and Melvin Cohn; The Salk Institute for Biological Studies.

It is well documented that certain inbred strains of mice, of which BALB/c is a prototype, respond rapidly by producing a pauciclonal germ line encoded response to α-1,3 dextran. The absence of the BALB/c $V_H^{dex}$ germ line gene allows us to observe the effect of the other genes governing this immune response. Two phenotypes are found in mice sharing the $Ig\text{-}1^b$ allogroup and the $H\text{-}2^b$ haplotype: the strain C57BL/6, the congenic strain CB20 B, the recombinant inbred strains (from BALB/c and C57BL/6 mice) CXBE and CXBI are "Low Responders", whereas the recombinant inbred strain CXBK is almost "Non Responder". This indicates that one, or several, genes contained in either the BALB/c or C57BL/6 background contribute to this phenotypes. The $V_K$ genes are good candidates to account for this difference, and these experiments could allow us to detect a $V_K$ marker. The antibody specificity (α-1,3/α-1,6) and their chain isotypes were analyzed by radioimmune assay and the light chain clonotypes were analyzed by isoelectric focussing.

877 COMPLEMENTATION GROUPS FOR THE CONTROL OF IMMUNE RESPONSES. Martin Dorf and Baruj Benacerraf. Harvard Medical School, Boston, Massachusetts 02115.

In mice the immune responses to the family of L-glutamic acid, L-lysine (GL)-containing terpolymers, including GL phenylalanine (GLØ) and GL-leucine (GLleu) are controlled by two complementing Ir genes localized within the I region of the H-2 complex. This was shown by the demonstration of specific immune responses in selected F1 hybrids and intra-I region recombinants between appropriate non-responder strains. However, the specific Ir alleles can no longer be considered as simply positive or negative since we have demonstrated distinct complementation patterns involving certain H-2 haplotypes. These complementation patterns or complementation groups indicate that considerable polymorphism exists at the Ir loci. In addition, the data are consistent with a genetic concept of coupled complementation similar to that described for the H-2-linked immune suppressor (Is) genes. Finally, in the GL-leucine system we have also identified non-H-2 influences on the magnitude of the immune response.

878 HETEROGENEITY OF THE HLA-B5 COMPLEX AS INDICATED BY CML,MLC AND THE RESPONSE TO ANTIGEN. L.J.Greenberg, L.T.Furcht, M.A.Long and E.J.Yunis. Univ. of Minnesota, Mpls MN 55455 and Harvard Univ.,Boston,MA 02115.

In a Chippewa Indian family we have identified two siblings that are homozygous at HLA-B5, mutually non-stimulatory in MLC and who fail to stimulate either parent in MLC.These siblings also appear to be homozygous at HLA-D, however, when their cells were used to stimulate a panel of Indian and Caucasian individuals of predominantly the HLA-B5 phenotype, no typing responses were observed. The data suggest that these stimulator cells are either too restrictive to be useful as typing cells or that there is greater polymorphism at the D locus associated with HLA-B5 than has been observed for other D locus specificities. Employing the CML assay, thought to discriminate at the B locus of HLA, individuals of the HLA-B5 phenotype could be separated into two groups, designated 5.1 and 5.2, again suggesting polymorphism at HLA-B5. Although all the cells tested responded normally to PHA stimulation, they showed a heterogenous response to a purified streptococcal antigen.The response to this antigen has previously been shown to be linked to a hypothetical determinant associated with HLA-B5, however there was no correlation between the response to this antigen and the dissociation of HLA-B5 into 5.1 and 5.2 by the CML assay.

879 RhLA AND IMMUNE RESPONSE TO HIGHLY PURIFIED ALLERGENS IN MONKEY FAMILIES, R. Hussain, D.G. Marsh, and H. Balner, Johns Hopkins Univ. and Primate Centre, The Netherlands

43 members of two large Rhesus harems (family groups with one father) were immunized with low doses of 4 highly purified pollen antigens (Ragweed AgE, Ra3 and Ra5 and Rye grass group I). Monkeys were immunized with alum precipitated antigens at day 0 and 21 and aqueous antigens at day 35. Specific IgG antibody responses at day 0, 35 and 42 were studied quantitatively by double antibody radioimmunoassay.

Individual monkeys showed selectivity of response towards the 4 antigens. Results with AgE (1 µg doses) were largely uninformative for linkage. Some Ra3 (10 µg) Rye I (1 µg) and most Ra5 (10 µg) responses showed segregation in families but association was not consistently found with a specific familial haplotype. An example is shown in Table I.

We found no clear evidence for association between specific RhLA haplotype and any immune response in agreement with our previous data in man (Black et al., Immunogenetics 3:349, 1976). The genetic complexity of immune response may require several generations of selective inbreeding to demonstrate RhLA-linked Ir loci, assuming they exist.

(Supported by NIH Grant #AI11370, Howard Hughes Medical Institute, and Contract #E.E.G.6243-22/6/001.)

| # Monkey | Haplotype | ∝RyeI ng Abn/ml. | # Monkey | Haplotype | ∝Ra5 ng Abn/ml. |
|---|---|---|---|---|---|
| 599 ♂ | AB | 67 | 600 ♂ | AB | 23 |
| CV ♀ | CD | 160 | 597 ♀ | CD | 3 |
| CV | BD | 6.5 | AB | BC | 4548 |
| EI | AD | <1 | BF | BC | 1388 |
| GD | BD | <1 | DW | AC | 530 |
| | | | HO | AD | <2 |
| 581 ♀ | CD | 130 | IW | BD | <2 |
| G | BC | 124 | | | |
| GG | BC | 94 | 669 ♀ | CD | <2 |
| DV | BC | 3 | AC | AD | 30 |
| AG | AC | 4 | BI | AD | <2 |
| CF | AC | 20 | CR | AD | 72 |
| | | | EL | BC | <2 |
| 498 ♀ | CD | 42 | FS | BC | <2 |
| NN | AC | 41 | | | |
| AQ | AC | 2800 | 603 ♀ | CD | 8 |
| ED | AD | 3 | T | BD | 21 |
| GU | BD | 532 | DF | BD | 5 |
| | | | FO | BD | 11 |
| | | | GY | BC | 2 |

880 GENETIC CONTROL OF THE IMMUNE RESPONSE TO CYTOCHROME C. Beverly D. Deak and Hugh O. McDevitt. Division of Immunology, Department of Medicine, Stanford University School of Medicine, Stanford, CA 94305.

MHC linked genetic control of the immune response to specific determinants in structurally well-defined protein molecules such as lysozyme, insulin, and staphylococcal nuclease has been described in the mouse and the guinea pig. Cytochrome c, a molecule highly conserved in evolution, has been structurally characterized in more than forty eukaryotic species.

We have studied the immune response of mice to cytochrome c from the yeast Candida krusei, which differs from mouse cytochrome c by 45 amino acid residues. The response to C. krusei cytochrome c is clearly linked to the MHC. High response associates with the H-$2^f$ haplotype, intermediate response with the H-$2^s$ haplotype, and low response with the H-$2^{b,d,k,j,q}$ haplotypes. Studies are now in progress to map the MHC gene(s) controlling this response. The response to horse cytochrome c is linked to the MHC but requires an additional gene(s) present in the C3H background, and absent in the B10 background. High response associates with the H-$2^q$ haplotype, intermediate response with the H-$2^b$ haplotype, and low response with the H-$2^q$ haplotype. We have been able to demonstrate very little cross reactivity at the antibody level with cytochrome c from several distinct species.

803 GENETIC CONTROL OF Ia ANTIGEN EXPRESSION, D. C. Shreffler, Department of Genetics, Washington University, School of Medicine, St. Louis, MO 63110

The mouse major histocompatibility complex (H-2) controls three broad classes of immunological functions, controlled by the four major regions of the complex, K, I, S, D (1). The K and D regions determine cell-membrane structures expressed on almost all body tissues, which appear to function as self-markers for immune surveillance mechanisms. The S region controls components of the complement system. Greatest interest currently centers on the I region, which determines cell-membrane molecules that are expressed principally on lymphocytes and macrophages. These molecules are involved in the cell interactions that mediate lymphocyte activation and regulation. The serological detection and definition of an extensive set of I region gene products (the Ia antigens) has opened a very important new avenue for the genetic, chemical and functional definition of the molecules that mediate these mechanisms. At least four discrete Ia gene products have been defined and mapped to four discrete I subregions (1,2,3). The products of the I-A, I-E and I-C subregions are found on B lymphocytes and some subpopulations of T lymphocytes (1,2). I-J subregion products appear to be restricted to T lymphocytes, apparently to suppressor T lymphocytes (3). Ia antigens are also expressed on macrophages, although the specific molecules expressed have not yet been clearly identified. Ia antigenic determinants have also been found on a number of soluble mediators produced by T cells and macrophages.

The Ia antigens found on B cells are glycoproteins comprised of two polypeptides of molecular weights 25,000 and 33,000 daltons (4). Discrete molecular products of the I-A, I-E and I-C subregions have been defined (2,4). The anti-Ia sera which detect and define these Ia antigens have been extensively tested for their capacity to inhibit immune functions, to absorb soluble mediators, or to eliminate functional subpopulations of lymphocytes. Such studies have implicated the Ia antigens in MLR stimulation, response to LPS and Con A mitogens, T cell helper and suppressor functions, T cell proliferative responses to antigen, macrophage interactions with T cells, and a number of other phenomena. The general features of the Ia antigens will be reviewed and the possible I region functional mechanisms that are suggested by the current evidence will be discussed.

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804 THE ROLE OF MHC GENES IN T-CELL MEDIATED RESPONSES TO SYNGENEIC MODIFIED CELLS, Gene M. Shearer, Anne-Marie Schmitt-Verhulst, Terry G. Rehn, Pierre A. Henkart, and Howard B. Dickler, Immunology Branch, National Cancer Institute, Bethesda MD 20014

Cell-mediated lympholysis (CML) has been generated in vitro against trinitrophenyl- (TNP-) modified syngeneic murine spleen cells (1). The specificity of the effectors generated is such that the stimulator and target cells must both be modified by the same agent (1) and must also express the same H-2K and/or H-2D haplotype (1). Further analysis of the specificity indicated that the effector cells generated against TNP-modified autologous cells could not lyse: (a) H-2-matched targets modified with TNP separated from the cell surface proteins by a tripeptide spacer (2); nor (b) H-2-matched targets in which TNP was presented on the cell surface by the insertion of a TNP-fatty acid-dextran conjugate into the lipid bilayer of the cell membrane. Furthermore, cells presenting TNP either by (a) or (b) were ineffective inhibitors of the lysis of H-2-matched TNP-modified targets by effector cells. These findings indicate that the immunogenic unit recognized by these cytotoxic effectors includes more than the modifying agent and involves cell surface proteins. Evidence suggesting that the additional contribution to the immunogenic complex involves H-2K and H-2D products is provided by the observations that: (a) lysis of TNP-modified targets by syngeneic effectors can be blocked only by TNP-modified cells sharing the K and/or D haplotypes with the modified stimulating and target cells; and (b) that treatment of stimulating cells with anti-H-2 sera at the time of TNP-modification reduces their immunogenicity. These results are compatible with a model involving recognition of the modifying agent in association with H-2-coded structures by a unique receptor on the T-cytotoxic lymphocyte.

Cultures primed in vitro with TNP-modified autologous cells can generate secondary effectors only if restimulated with TNP-modified stimulators sharing H-2K and/or H-2D with the primary stimulators. Secondary proliferative responses can be induced only with TNP-modified cells sharing either K, I-A, or I, or D with the primary stimulating cells. These results extend the requirement for K and/or D homology in CML to include a role for I region products when proliferative responses are studied in the modified cell system.

In addition to the role of H-2 products in the specificity of the TNP-modified self system, the MHC also influences the ability to generate cytotoxic effectors against TNP-$H\text{-}2D^{d}$ (1). The phenomenon appears to be controlled by multiple Ir genes, one of which maps to the left of I-A, and the other between the I-A and I-J subregions of the H-2 complex (3).

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805 SELECTIVE EXPRESSION OF GENE PRODUCTS ON LYMPHOCYTE SUBCLASSES AND THEIR RELEVANCE TO CELL-CELL INTERACTIONS, H. Cantor, Department of Medicine, Harvard Medical School and the Farber Cancer Center, Boston, MA 02115

The surface components of lymphocytes have been more extensively characterized than those of any other mammalian cell type. Many of these surface components have been serologically defined and the genes coding for them precisely mapped. Expression of these different gene products can therefore serve as identification markers of lymphocytes at different stages of differentiation. In addition, many assays of immune function in vitro are now available and can be used to determine the possession or lack of different immune functions within a given population of lymphocytes. Thus the 'surface-antigen profile' of a subpopulation of lymphocytes can be readily correlated with its functional properties, in order to delineate each step or branch in the cell's differentiative path.

The results of this experimental approach have indicated that unique subclasses of lymphocytes mediate distinct immune responses. Separation of these subclasses of lymphocytes have allowed new insights into the mechanism of lymphocyte interactions resulting in the production of cell-mediated and humoral immunity.

This information will be summarized with particular emphasis on the special lymphocyte interactions required for the production of antibodies possessing $V_H$ components encoded by germ line genes.

881 EXPRESSION OF Ly 1, Ly 2, Thy 1 AND TL DIFFERENTIATION ANTIGENS ON MOUSE T-CELL TUMORS, Bonnie J. Mathieson, Pamela S. Campbell, Michael Potter and Richard Asofsky, Nat. Inst. of Allergy and Infectious Diseases and Nat. Cancer Inst., National Institutes of Health, Bethesda, MD 20014.

Transplanted lymphomas induced in BALB/c mice with 1-ethyl-1-nitrosourea (ENU) and transplanted spontaneously occurring lymphomas of AKR mice were examined for the expression of the $T^+$ cell antigens Ly, TL and Thy 1 using three serological methods. Most of the Thy $1^+$ and/or $TL^+$ tumors, i.e., T cell tumors, expressed either Ly 1 or Ly 2 antigen, but not both. Thus these tumors expressed restricted Ly phenotypes comparable to phenotypes previously described for functional peripheral T cells. Because tumor phenotypes were stable over a number of transplant generations, they appeared to be an intrinsic property of the specific tumors. The majority of the BALB/c lymphomas were Ly $1^- 2^+$ and also positive with anti-TL antiserum. This predominant phenotype on the BALB/c tumors may be related to either the mode of tumor induction or to the mouse strain, but since the restricted Ly pattern was observed both in BALB/c and AKR tumors, the phenotypic restriction itself is not a consequence of either of these factors. Tumor induction by ENU per se is not responsible for Ly or TL antigen expression since several non-T cell BALB/c tumors, also induced by ENU, did not express either Ly or TL antigens.

Data presented here suggest that the target cell for leukemogenesis may be a partially differentiated thymus cell. The restricted expression of Ly antigens on differentiating thymus cells to either the Ly $1^+$ or Ly $2^+$ phenotype may occur prior to the loss of TL antigen.

882 ALA-1: A MURINE DIFFERENTIATION ANTIGEN OF ACTIVATED T AND B CELLS. Ann J. Feeney, Dept. of Biology, Univ. of California, San Diego, La Jolla, CA 92093.

Ala-1 (activated lymphocyte antigen-1) is a murine alloantigen expressed only on peripheral T and B cells after activation by antigen or mitogens. Anti Ala-1 does not lyse normal thymus, lymph node or spleen cells nor Con A-stimulated thymocytes, but it does kill >90% of Con A-stimulated T cells and of LPS-stimulated B cells in cytotoxicity assays.

Four functional subsets of lymphocytes activated by antigen in vivo were found to express Ala-1: killer T cells, IgM PFC, IgG PFC, and primed helper cells. Killer T cells were produced by immunizing mice with histoincompatible tumor cells, and removing and purifying the immune peritoneal lymphocytes 12 days later. Pretreatment of the killer cell population with anti Ala-1 and C reduced lymphocytotoxicity by >95%. 92% of IgM plaque-forming cells found in spleen populations 4 to 6 days after immunization with SRBC were eliminated by pretreatment of the spleen cells with anti Ala-1 and C. Ala-1 pretreatment also eliminated 74% of IgG plaque-forming cells present in spleens of mice hyper-immunized with SRBC. Pretreatment of carrier-primed spleen cells with anti Ala-1 and C reduced the helper cell activity of that population by 80%. Helper cells were assayed by monitoring the ability of small numbers of carrier (SRBC)-primed cells to augment the in vitro anti-hapten (TNP) response of normal spleen cells cultured with hapten-carrier conjugate (SRBC-TNP). Precursors of helper cells and of IgM-PFC were found to be Ala-$1^-$, since pretreatment of normal spleen cells with anti Ala-1 and C followed by a 4-day culture with SRBC did not diminish the in vitro primary response compared to untreated cultures. Thus, Ala-1 expression appears to be restricted to the late stages of differentiation of T and B cells, after antigen or mitogen activation.

883 SEPARATION OF SUBSETS OF MOUSE SPLEEN CELLS WITH SEPHADEX G-10 COLUMNS. Michael K. Hoffmann, Karin Pickel, Herbert F. Oettgen and Ulrich Hammerling. Memorial Sloan-Kettering Cancer Center, New York, N. Y. 10021

Ly and Mishell reported that passage through a Sephadex G-10 column depletes mouse spleen cells of macrophages. We report that a Sephadex G-10 column also retains suppressor T cells and, after coating with antigen-antibody complexes and complement, certain subsets of B cells. Suppressor cells were induced by injecting (C57BL/6 x DBA2)F1 mice with C57BL/6 spleen cells. Spleens of the recipient mice were rich in suppressor T cells, of C57BL/6 origin, and directed against components of the H-2 region of the opposite parental strain in F1 hybrids. They differed from helper T cells in their Ly phenotype (suppressor T cells Ly $1^+2^+$; helper T cells Ly $1^+2^-$), and were retained by Sephadex G-10 columns which did not retain helper T cells. Suppressor T cells induced by injecting mice with sheep erythrocytes or BCG were similarly retained. Coating of Sephadex G-10 columns with antibody-antigen complexes and complement permitted removal of cells which carried complement receptors ($CR^+$) from spleen cell suspensions. The effluent contained T cells, and B cells which carried Ig, Ia and Fc receptor on their surface. Spleen cells lacking $CR^+$ cells cooperated with primed but not unprimed T cells after macrophages were added. Agents which induce CR could replace macrophages in this context, 2-mercaptoethanol could not.

884 SEPARATION OF T CELL SUBSETS WITH A FICOLL VELOCITY SEDIMENTATION GRADIENT, Harley Y.-S. Tse and Richard W. Dutton, Dept. of Biology, UCSD, La Jolla, CA 92093.

We have previously demonstrated that Con-A activated helper and suppressor T cells can be physically separated by a 5-20% ficoll velocity sedimentation gradient when assayed on primary anti SRBC response. Helper activity is associated with a pool (Pool I) of small, slowly sedimenting cells which have incorporated little thymidine in the pregradient culture period. Suppressor activity is associated with a pool (Pool IV) of more rapidly sedimenting cells that contain blast cells and the bulk of the incorporated thymidine (J.E.M. 143:1199, (1976)). In the present studies, we further show that the isolated Con A helper cells are Ly-$1^+$ and suppressor cells are Ly-$23^+$ and that both cell types do not require DNA synthesis in the induction phase. Ablation of thymidine incorporation response by irradiation, mitomycin treatment or inclusion of colcimid in the pregradient cultures does not inhibit the manifestation of helper activity in Pool I or suppressor activity in Pool IV cells although the unfractionated culture is not suppressive. We have also applied the gradient technique to demonstrate separation of positive and negative allogeneic effects from the same initial in vitro C57BL/6 anti $BDF_1$ cultures. In this case, although the unfractionated culture is usually stimulatory, enrichment after gradient separation of enhancing cells in Pools I and II and detection of suppressor cells in Pool IV are again observed. Cytotoxicity, too, is mainly confined to Pool IV cells. There is, however, one difference. While C57BL/6 anti $BDF_1$ cells suppress both normal C57BL/6 and $BDF_1$ responses to SRBC, they are only cytotoxic to $BDF_1$ and DBA/2 LPS blasts but not to C57BL/6. We suggest that suppressor cells could be different from cytotoxic cells. (Supported by USPHS AI 08795 and ACS IM-1J.)

885 SEPARATION OF MURINE THYMOCYTE SUBPOPULATIONS USING A LOBSTER AGGLUTININ (LAgl) A.L.Hartman, P.A.Campbell, B.M.Rickard and C.A.Abel, Dept. of Path., Univ. Colo. Med. Ctr. & Natl. Jewish Hosp. and Res. Ctr, Denver, Colorado 80206

Two distinct populations of cells can be identified in the murine thymus on the basis of their sensitivity to cortisone. Cortisone-resistant thymocytes (CRT) and T cells have a high sialic acid content while thymocytes have a low sialic acid content. Incubation of spleen T cells and CRT for brief periods in the presence of LAgl, a lectin with sialic acid specificity isolated from the hemolymph of the lobster Homarus americanus, results in agglutination of most cells, while only a small percentage of normal thymocytes are agglutinated upon incubation with LAgl. Treatment of cells with LAgl does not appear to affect their viability; agglutination can be reversed by the addition of EDTA. When single cell suspensions are added to LAgl covalently linked to cyanogen bromide-activated Sepharose 6MB beads the same pattern of differential agglutination of cells is seen. Cells bound to these beads can also be released by the addition of EDTA. The mitogenic properties of this lectin were examined. LAgl is not mitogenic for murine thymocytes or spleen T cells, while it is mitogenic for murine B cells at doses that agglutinate both spleen T and B cells. These results suggest that LAgl covalently linked to Sepharose 6MB beads can be used for the separation and isolation of cortisone sensitive thymocyte populations. (Supported by NIH Grants AI-11742 and CA-92703)

886 SELECTIVE EXPRESSION OF ALLOANTIGENS ASSOCIATED WITH X CHROMOSOMES LINKED IR GENES ON T LYMPHOCYTES SUBPOPULATIONS, Marc Zeicher, Edna Mozes, and Peter Lonai, Department Chemical Immunology, The Weizmann Institute, Rehovot, Israel.

A new polymorphic alloantigen system controlled by loci on the X chromosome has been identified using antisera raised in F1 hybrid mice differing in their X chromosome. These alloantigens are associated with the X-linked immune response genes controlling the immune response to the so called "thymus independent antigens" type III pneumococcal polysaccharide, Poly (I). Poly (C) and denatured DNA. They also show association with the histocompatibility locus present on the X chromosome. They were mainly detected on a T lymphocyte subpopulation present in Thymus, spleen and lymph nodes but absent from peripheral blood. That subpopulation was compared with T lymphocyte subpopulations defined by Ly I and Ly 2.3 alloantigens.

887 ANTIGEN- AND TIME-DEPENDENT MATURATION OF T CELLS, George Berry, Barbara Araneo, Philippa Marrack and John Kappler, University of Rochester, Rochester, NY 14642.

Previous studies have shown that mouse peripheral T cells can be assigned to one of three classes, T1, T2 and effector. T1 cells are short-lived, sessile and virgin, T2 cells are long-lived, recirculating and memory, and effector cells are short-lived and sessile.

Alloantigen-reactive cells, as measured by cell division in mixed lymphocyte cultures against H-2 incompatible target cells, are found almost entirely in the T2 population of cells. These cells cannot be generated from their T1 precursors by priming mice containing T cells of only the T1 type with spleen cells of the appropriate H-2 incompatible strain. This result contrasts with that obtained when the T1 precursors of sheep red blood cell-specific T2 cells were studied. In that case antigen was very efficient at driving the T1 precursors into the T2 pool.

In another series of experiments we showed that T2 cells specific for alloantigens and for sheep red blood cells were generated from their T1 precursors in the absence of deliberate antigen priming at about the same rate.

These results will be discussed in terms of the fact that T2 cells specific for alloantigens are very common by comparison with those with other specificities.

888 T-T CELL SYNERGISM BETWEEN IMMUNOGLOBULIN POSITIVE AND IMMUNOGLOBULIN NEGATIVE SUBPOPULATIONS OF PRIMED THYMOCYTES, F. Paraskevas and S.T. Lee, Dept. of Medicine University of Manitoba, Winnipeg, Manitoba.

The helper cell function of thymocytes collected 6 hours after priming with sheep erythrocytes (SRBC) has been examined. Such thymocytes contain a cell population which shows easily demonstrable surface immunoglobulin. Six hour primed thymocytes combined with bone marrow cells were injected in sublethally irradiated mice which were challenged one hour later with SRBC. The 6 hour primed thymocytes exerted a highly specific helper cell function as they induced a two to threefold increase of 19S PFC and a six to sevenfold increase of the 7S PFC as compared to unprimed cells. Six hour primed thymocytes, treated with a rabbit anti-mouse Ig and complement, had no effect on the helper cell function of unprimed cells but it abolished the augmented helper cell function of primed cells. Anti-θ treatment of unprimed cells completely abolished their helper cell function. In contrast the anti-θ treated 6 hour primed cells having lost their ability to give an augmented helper cell function were still able to induce a significant number of plaque forming cells. This residual helper cell function is contributed by a subpopulation of cells which is resistant to lysis by anti-θ serum. The augmented helper cell function of 6 hour primed T cells was also abolished after treatment with an anti-SRBC serum.

Since the sum of PFC given by anti-Ig and anti-θ primed cells is much smaller than the number given by untreated cells, a T-T cell synergism between these two subpopulations is implicated.

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

889 TERMINAL DEOXYNUCLEOTIDYL TRANSFERASE (TDT) IN MARROW OF ATHYMIC NUDE MICE. J. J. Hutton and F. J. Bollum, VA Hospital and Depts. of Medicine and Biochemistry, University of Kentucky, Lexington, Kentucky 40506

TDT is an unusual DNA polymerase normally found only in thymus and bone marrow. It is thought to play a role in the generation of immunological diversity in T and B lymphocytes. TDT activity is much higher in thymus than marrow and it is possible that the small numbers of TDT containing cells in marrow originate in the thymus and migrate to marrow rather than originating from stem cells of marrow during lymphoid differentiation. If the cells migrate from thymus then TDT should be absent from the marrow of congenitally athymic or "nude" mice. We have found identical TDT activities per cell or per mg DNA in marrow from athymic and normal mice of the BALB/c strain. TDT was absent from lymph nodes of each. Criteria for the presence of TDT included inhibition of transferase activity by antiserum to calf thymus TDT and stimulation of activity by the initiator, oligo $DA_{50}$. The presence of TDT in marrow of congenitally athymic mice is consistent with the presence of the enzyme in lymphoid progenitor cells that can mature in marrow independent of thymic influence.

890 SPECIFIC ENRICHMENT OF SUPPRESSOR T CELLS BEARING THE PRODUCTS OF I-J SUBREGION, Ko Okumura, Toshitada Takemori and Tomio Tada, Laboratories for Immunology, School of Medicine, Chiba University, Chiba, Japan

Carrier-specific and allotype specific suppressor T cells belong to the Ly 2.3 subclass and express Ia antigen(s) determined by a gene or genes mapped in I-J subregion. However, no direct evidence is available to indicate that the suppressor T cell is in fact killed by anti-I-J antisera. We have successfully enriched the carrier-specific suppressor T cell by specific means, and studied its function and genetic expressions. B cell-depleted spleen cells of KLH-immunized mice were slowly passed through a KLH-coated Sephadex G-200 column at 37°C, and the retained cells were eluted by flushing the column with cold (0°C) medium. The recovery of cells by this method was less than 1% of original spleen cells. About 20% of such cells were directly killed by anti-I-J and complement (C). Treatment of the cells with anti-Ly 2.3 and C, but not with anti-Ly 1 and C, removed the cells which were killed by anti-I-J and C. The cells expressing the product of I-J subregion induced a striking suppression of an adoptive secondary antibody response in vivo, and the extract of such cells suppressed the in vitro antibody response of primed spleen cells at a dose equivalent to as low as $10^4$ cells. Such a suppressive activity was not affected by removal of Fc receptor-bearing cells. The method provides a new tool to study directly the genetic expressions on the suppressor T cell and its product (Supported by a Grant from the Ministry of Education, Culture and Science of Japan).

891 SUPPRESSOR AND CYTOTOXIC CELLS IN LYMPH NODES AND SPLEENS OF ALLOSENSITIZED MICE. G.A.Truitt, R.R.Rich, and L.Chu. Baylor College of Medicine, Houston, TX 77030.

Irradiated allosensitized lymphocytes from tissues of BALB/c mice (4 days after footpad injection of allogeneic spleen cells) have been tested for ability to suppress mixed lymphocyte reactions (MLR) and generation of cytotoxic lymphocytes (CL), in vitro. We have established that regional lymph node cells (LNC) contain many more cytotoxic cells than spleen. However, in contrast to spleen cells, LNC were relatively ineffective suppressors of MLR. Both populations of activated cells suppressed the generation of CL. We studied the influence of antigen concentration on generation and suppression of cytotoxic responses. While generation of CL was constant over the dose range of stimulatory cells used, suppression of the CL response by allosensitized spleen cells increased as antigen increased; contrary to results expected with antigen removal by cytotoxic cells. Further, we studied the specificity of suppression by spleen cells and found that while suppression is only observed when the appropriate sensitizing alloantigen was included in the cultures, suppressor cells activated by either $H\text{-}2^b$ or $H\text{-}2^k$ alloantigens suppressed cytotoxic responses generated simultaneously to both antigens. We conclude that pursuant to secondary stimulation alloantigen-activated suppressor cells suppress cell-mediated responses against different alloantigens (i.e.nonspecifically). Finally, the data suggest the possibility of functional discrimination between suppressor and cytotoxic lymphocytes. (Supported by USPHS NO1-AI-42529 and HL-17269, NASA NAS9-14368 and a grant from the Kelsey-Leary Foundation).

892 T CELL SUBPOPULATIONS SUPPRESSING HUMORAL AND DELAYED-TYPE HYPERSENSITIVITY (DTH) RESPONSES, R. L. Whisler and J. D. Stobo, Mayo Medical School, Rochester, MN 55901

Although T cells are capable of suppressing both humoral and DTH responses, it is not clear whether this represents the activities of a single or distinct T cell subpopulations. The following suggests the existence of suppressor T cell subpopulations.

Nylon wool column effluent populations from the spleens of C3H/HeJ mice immunized two weeks previously with $4x10^9$ sheep red blood cells (SRBC) specifically suppressed the direct, indirect plaque forming cell (PFC) and DTH response of syngeneic recipients to SRBC. Sedimentation of these splenic T cell populations on five step discontinuous bovine serum albumin gradients demonstrated a rapidly sedimenting fraction suppressing both PFC and DTH responses. In addition, T cells in a slowly sedimenting fraction suppressed DTH responses 3 fold. The slowly sedimenting populations resulted in amplification of recipients direct and indirect PFC responses. Pretreatment of donors 2 days prior to transfer with 200 mg/kg of cyclophosphamide (CY) eliminated both rapidly and slowly sedimenting T cells suppressing DTH reactivity. Rapidly sedimenting T cells from animals pretreated with 20 mg/kg failed to suppress either DTH or PFC responses. However, slowly sedimenting T cells from the same animals continued to suppress DTH reactivity while amplifying the direct and indirect PFC responses. In vitro irradiation of rapidly and slowly sedimenting T cells with 650 R ablated the ability to suppress both PFC and DTH responses. However, the ability of slowly sedimenting T cells to amplify recipients PFC responses was not diminished. These studies suggest:
1) There exist two distinct T cell subpopulations capable of suppressing DTH responses;
2) One of the subpopulations can be distinguished from T cells suppressing PFC.

893 MODE OF ACTION OF A TYPE OF SUPPRESSOR T CELL, Lee Harwell, Philippa Marrack, and John Kappler, Department of Microbiology, University of Rochester, Rochester, NY 14642.
Non-specific suppressor T cells are generated by incubation of spleen cells for 48 hours in 4 ug/ml Concanavalin A (ConA). Less than 1000 of these cells will suppress the response of $2x10^5$ B cells to sheep red blood cells incubated with a non-specific, T cell-derived helper mediator (NSM). Many more of the ConA-activated cells are required, however, to inhibit the response of the same number of B cells to the thymus independent antigen, trinitrophenylated lipopolysaccharide. Inhibition by the suppressor cells is eliminated by treatment of the ConA-activated cell preparations with anti-T and complement. If B cells are incubated for 24 hours with suppressor cells and antigen before addition of NSM, no inhibition is observed, suggesting that the suppressor cells do not act directly on B cells. If NSM is incubated with suppressor cells for 24 hours before the cells are removed and the NSM added to cultures of B cells and antigen we observe that all the helper activity of the mediator is gone. Thus ConA-activated suppressor cells act by inhibiting NSM rather than B cells.

894 MODE OF ACTION AND PROPERTIES OF SUPPRESSOR T CELLS OPERATING IN LOW ZONE TOLERANCE, Eckehart Kölsch, Joachim Heuer and Rudolf Stumpf, Heinrich-Pette-Institut für experimentelle Virologie und Immunologie an der Universität Hamburg, Martinistrasse 52, 2000 Hamburg 20, West Germany.
Low zone tolerance (LZT) to phage fd seems to be a type of tolerance which is primarily caused by suppressor T cells. We have attempted to analyze their mode of action. For the induction of antigen-specific suppressor cells in CBA mice, we use tolerogenic and immunogenic doses of antigen together with a pretreatment with hydrocortisone. Suppressor activity can be demonstrated upon transfer of spleen cells into normal syngeneic mice. After immunization these animals are unable to produce IgG antibody against fd, whereas the IgM response is not suppressed. The half-life of transferred suppressor cells in nonimmunized animals is 5-6 weeks. The target of suppression are unprimed T helper cells, whereas primed helper cells cannot be blocked. T helper cells become "resistant" to suppression 18-36 h after contact with antigen. Differentiation from unprimed B into B memory cells is unaffected, yet under suppression conditions persisting B memory cells are blocked in IgG production. We will also discuss experiments aimed at enriching suppressor cells according to various separation methods. The experimental data are incorporated into a model of LZT.

895 CHARACTERIZATION OF SUPPRESSOR T CELLS IN TOLERANT MICE, Israel Zan-Bar and Samuel Strober, Division of Immunology, Stanford Medical Center, Stanford University, Stanford, California 94035
We recently characterized the size and cell surface markers on suppressor T cells in mice tolerized with deaggregated bovine serum albumin. Lethally irradiated (BALB/C x C57BL/Ka)$F_1$ mice were injected with T cells primed to BSA in complete Freund's adjuvant (CFA) and B cells primed to DNP-BSA in saline and the antibody response to BSA was measured by a tanned red blood cell hemagglutination assay. The response to DNP was measured by a modified Farr Assay. We were able to suppress the adoptive response by injecting graded numbers of spleen cells from tolerant mice into the adoptive hosts. The suppressor cells could be eliminated by *in vitro* treatment with antisera directed against the θ, Ia, and Ly-2.3 cell surface antigens in the presence of complement. Antisera directed only against the J subregion of the Ia region also eliminated suppressor cells. Separation of tolerized spleen cells according to size by 1 x g velocity sedimentation showed that the suppressor activity is confined to the large cell fraction ($S > 4.5$ mm/hr).

896 SUPPRESSION OF IgE PRODUCTION IN MICE. Z. Ovary and N. Watanabe, NYU Medical School, New York, N.Y. 10016.

To have high titer anti-DNP IgE antibody mice were injected with 1μg DNP13KLH (13 Groups/$10^5$ Daltons) in 1 mg $Al(OH)_3$ i.p., infected 21 days later with 750 3rd stage larvae of Nippostrongylus brasiliensis and challenged with N.brasiliensis protein coupled to DNP(DNP17Nb) in 1 mg $Al(OH)_3$, 14 days after infection. Whereas other strains of mice(BALB/c,CBA,ASW etc) had persistent, high titer anti-DNP IgE, in SJL IgE was transient. However, when SJL mice were irradiated (540 R) one day after challenge the higher titer anti-DNP IgE antibody persisted. If these irradiated mice received $5x10^7$ normal SJL spleen or mesentric lymph node cells or thymocytes, anti-DNP IgE antibody was suppressed. The suppressor cells were destroyed by α-Thy1.2 and complement (C), but not by α-Ly2.2 and (C). By the same immunization schedule(SJLxBALB/c)F1 produced persistent α-DNP IgE antibody. Therefore, the suppressor activity was also eliminated by α-Ly1.2 and (C). The suppressor trait is recessive. Using backcross F1 to SJL it has been shown that the suppressor trait was not linked to H-2 and the percent of segregation was consistent with a trait determined by a single autosomal gene. The suppressor substance could be extracted from normal spleen cells, it is a heat labile, high molecular weight protein.

897 SUPPRESSOR CELLS IN EXPERIMENTAL TRYPANOSOMIASIS. Anil N. Jayawardena, Diane D. Eardley, and Byron H. Waksman. Dept. of Pathology, Yale University School of Medicine, New Haven, Conn. 06510.

African trypanosomiasis is a fatal disease characterized by a profound suppression of immunological responsiveness. An experimental model system - T brucei (S42 strain) in the CBA mouse, has been used to analyze the mechanism underlying this state of immunological hyporesponsiveness. Spleen cells from infected mice show markedly suppressed DNA synthetic responses to Con A, PHA, LPS, and PFC responses to SRBC and DNP-Ficoll; the degree of suppression increases as the infection progresses. When these cells are co-cultured with normal cells they suppress the ability of the normal cells to elicit DNA synthetic responses to mitogens and allogeneic cells, and PFC responses to thymus dependent and independent antigens. Spleen cells from infected nude mice fail to suppress responses to mitogens. The suppressed proliferative responses of spleen cells from infected mice are markedly improved by depletion of glass wool adherent cells and the separated adherent cells are more suppressive than the unfractionated population. Treatment of the suppressor population with anti-Thy I or anti-brain serum specific for T cells reduces its ability to inhibit the PFC response of normal spleen cells to SRBC. These results suggest that T. brucei infections result in the activation of a nonspecific suppressor T cell population which may play a role in the failure of resistance in this disease. The T cell subsets involved in these interactions and the possibility that specific suppressor T cells are also generated are being investigated.

898 AGE DEPENDENT DEVELOPMENT OF HELPER AND SUPPRESSOR T CELLS IN ANTI-μ BURSECTOMIZED CHICKENS. Vali Kermani-Arab and Gerrie A. Leslie, Dept. of Microbiology and Immunology, Univ. Oregon Health Sci. Ctr., Portland, OR 97201.

Chickens provide a unique system for studying T-B cell interactions in humoral immune development. Line $6_3$ inbred chickens when injected IV with 4 mg of specifically purified goat anti chicken IgM (μ) antibody on day 14 of embryonation were agammaglobulinemic and lacked immunoglobulin bearing B cells as determined by (1) immunofluorescence, (2) lactoperoxidase catalyzed $^{125}I$ cell surface labeling, and (3) capacity to restore immunoglobulin synthesis in cyclophosphamide induced agammaglobulinemic birds at 4 and 12 weeks of age. Lymphocytes from these chickens exhibited a normal response to T cell mitogens i.e., PHA and Con A. Of special significance was the observation that the IV injection of $75 \times 10^6$ splenic, thymic and peripheral blood lymphocytes from these birds, at 4 weeks of age, into normal syngeneic 15 day old embryos or newly hatched chicks enhanced early immunoglobulin synthesis, whereas, administration of the same number of splenic, thymic, and bone marrow cells from similar donors 12 weeks of age into 1-day-old recipients rendered these hosts severely hypogammaglobulinemic or agammaglobulinemic. The effector cells from the anti-μ Bx donors were inactivated by rabbit anti chicken T cell antiserum plus complement. These data suggest that help and suppression of B cell differentiation are functions of T cells and are age dependent phenomena. (Supported by NIH AI 12273 and NSF 1176.)

899 MULTIPLE IF-PHENOTYPES OF MOUSE LIGHT CHAINS. David M. Gibson, University of Sherbrooke, Sherbrooke, P.Q. Canada. Light chains isolated from normal immunoglobulin can be resolved into a finite series of discrete "focusing groups" by isoelectric focusing in polyacrylamide gels. Initial studies showed the existence of two isoelectric focusing phenotypes in a limited survey of the light chains of 9 inbred mouse strains (Gibson, D., J. Exp. Med. 144, 298 (1975) ). I wish to report here that at least 4 different isoelectric focusing phenotypes can be distinguished amongst the light chains of inbred mouse strains. Strains sharing these different phenotypes are (A) C57Bl/6J, C57Bl/10J, C57L/J, DBA/2J, St/bJ, C3HeB/FeJ, C57Bl/10Sn, CBA/J, A/J, SWR/J, DBA/1J; (B) AKR/J, RF/J and PL/J; (C) C58/J and (D) NZB/BlnJ and BDP/J. In each case the differences between alternate phenotypes appears as the presence or absence of a distinctive focusing band or bands, suggesting that each band in question may represent the product of a single (v-) gene. Genetic studies have shown that the band IF-1, shared by the strains AKR, RF, PL and C58 is inherited in a simple fashion (see reference above) and that this marker is closely linked to the Ly-2,3 locus on chromosome 6 of the mouse (Gibson, D. Taylor, B.A. and Cherry, M. in preparation).
Analysis of the isoelectric focusing phenotypes of a series of mice obtained in the backcross (C58/J x SWR/J)$F_1$ x SWR/J however suggests that other loci may also affect the isoelectric focusing pattern, since several phenotypes differing from both of the parental types were observed. The results of further studies on the nature of this phenotypic variation will be reported.
Supported by the Medical Research Council of Canada Grant No. MT-4317.

900 ON THE REGULATION OF EXPRESSION OF THE MURINE $\lambda_1$ LIGHT CHAIN, William R. Geckeler and Melvin Cohn, Developmental Biology Laboratory, Salk Institute for Biological Studies, San Diego, CA 92112.

The known genetically determined, antigen-specific response differences map either to H-2 or to the heavy chain allogroup locus. We are analyzing a single genetic locus which controls the expression of the $\lambda_1$ light chain and which is unlinked to either the H-2 or heavy chain allogroup loci.

Two alleles at this locus, $\lambda_1^+$ and $\lambda_1^{lo}$, produce effects involving $\lambda_1$ light chain expression. $\lambda_1^+$ homozygotes have normal levels of $\lambda_1$ associated with serum immunoglobulin. With the appropriate heavy chain complement, they are uniformly high responders to $\alpha$-1,3 dextran with an antibody whose light chain is entirely $\lambda_1$. $\lambda_1^{lo}$ homozygotes, on the other hand, have at least thirty-fold less $\lambda_1$ associated with serum immunoglobulin. While their response to $\alpha$-1,3 dextran is in the $\lambda_1$ light chain class, the $\lambda_1^{lo}$ gene produces large fluctuations in the magnitude of the response by individual $\lambda_1^{lo}$ homozygotes.

An analysis of heterozygote ($\lambda_1^+/\lambda_1^{lo}$) mice suggests that the phenotypic effects are produced by alleles of the $\lambda_1$ structural gene itself or by alleles for a regulatory element linked to the $\lambda_1$ structural gene. Experiments in progress will decide between structural and regulatory models, and will determine the stage of B-cell differentiation at which the $\lambda_1^{lo}$ gene exerts its effect.

901 GENETIC RELATIONSHIP BETWEEN THE PRECURSOR REGION AND THE VARIABLE REGION OF K LI( CHAINS. Stephen Rose and W. M. Kuehl. University of Virginia, Charlottesville, Va. 22901

Structural studies have indicated that mouse K light (L) chains can be organized int a large number of variable (V) region subgroups. Sequence studies of mature KL chains de rived from MPC-11 and MOPC-21 mouse myeloma tumors suggest that these chains are derived from the same or related subgroups, even though the MPC-11 L chain contains a partially duplicated $NH_2$ terminal segment of 12 amino acids (Smith, 1973). These extra $NH_2$ termina residues in the MPC-11 L chain may be part of the structural gene for the V region or cou be due to incomplete processing of a precursor light (PL) chain. Partial $NH_2$ terminal se quence studies on the PL chains synthesized in vitro from purified MPC-11 and MOPC-21 mRN have yielded the following: 1) both precursors contain an additional 29 $NH_2$ terminal res dues compared to the mature L chain and 2) both precursor regions contain methionine resi dues at positions 1, 6 and 10. These preliminary studies indicate that the additional NH terminal sequence in the mature MPC-11 L chain is not a consequence of incomplete process of PL chain. In addition, these results extend Schecter's (1976) studies which indicate t for each subgroup (or for each V region) the $NH_2$ terminal precursor gene is adjacent to t V region structural gene.

**902** COMPARATIVE PEPTIDE MAPPING OF RADIOIODINATED RABBIT LIGHT CHAINS, Robert L. Raison and John J. Marchalonis, Dept. of Microbiol., Monash University Medical School, Prahran, Victoria, Australia and Walter and Eliza Hall Institute, Parkville, Victoria, Australia.

Peptide maps of rabbit L chains derived from immunoadsorbent purified antibodies to group B and group C streptococcal carbohydrates were prepared from tryptic digests of material eluted from polyacrylamide gels and labelled with $^{125}I$. Comparison of the tryptic fingerprints of the tyrosine containing peptides of L chains from 5 anti-group B and 2 anti-group C antisera showed (i) 4 peptides common to all L chains examined; (ii) a unique peptide ("C") present only in L chains from anti-group C antibodies; (iii) no allotype-related differences; (iv) a unique peptide present in the L chains from antibody produced in an Australian wild rabbit and (v) 2 L chains with identical peptide maps.

A total of 14 different radioiodinated peptides were observed, each L chain containing from 9 to 12 of these in its individual fingerprint. This restriction of iodinated tryptic peptides may be indicative of restriction of amino acid residues in both framework and hypervariable regions in the L chains of anti-streptococcal antibodies produced in outbred rabbits.

**903** AMINO ACID SEQUENCE OF LIGHT CHAINS FROM PEDIGREED RABBITS. Dietmar G. Braun and Hans Huser, Basel Institute for Immunology, Basel, Switzerland.

Amino acid sequence analyses of light chains isolated from rabbits which have been pedigreed over the last 10 years indicate that $V_L$ regions of antibodies to the streptococcal group A-variant polysaccharide are under a germ line encoded control. This is indicated by recurrent framework sequences of the $V_L$ regions and by identical or very similar amino acid sequences in the complementarity-determining regions. For example, the light chains of two littermates isolated from anti-group A-variant antibodies had an identical sequence throughout the $V_L$ region.

Furthermore, evidence is available that recombination may be a mechanism by which additional variability is achieved. It is not clear at this point whether $V_L$ regions that appear to be the product of recombination have been arrived by a genetic or by a somatic event. For example, one light chain was found that shares part of the framework with a light chain belonging to one subgroup and the subsequent part of its framework, including the first hypervariable region, with another light chain.

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**904** THE STRUCTURAL BASIS OF A MAJOR DETERMINANT OF THE GROUP a ALLOTYPE IN RABBIT Ig HEAVY CHAINS, Michael N. Margolies, L. Edward Cannon, Edgar Haber, Thomas J. Kindt and Blair Fraser, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114 and the Rockefeller University, New York, NY 10021.

The genetic implications of an inherited marker, the group a allotypes, in the rabbit heavy chain variable region ($V_H$) require the identification of those amino acid residues involved in the allotypic determinants. The amino acid sequence of the $V_H$ from three homogeneous antibodies elicited by type III pneumococcal vaccine was determined. Each antibody displayed the $V_H$ allotype a1. Two of these heavy (H) chains (3374, 3381) had identical amino acid sequences at all $V_H$ framework positions which are correlates of the group a allotype. The third H chain (3T72) demonstrated a variant sequence at positions 15 and 16 but was otherwise identical to the other two H chains at all positions which are correlates of group a allotype. The a1 allotypic specificities of all three homogeneous antibodies were compared by quantitative radiobinding and inhibition of radiobinding assays using insolubilized anti-a1 antisera as well as allotypic antiserum fractions made specific for the homogeneous antibody 3374. Antibodies 3374 and 3381 were allotypically identical and share an a1 allotypic specificity which also predominates in pooled a1 IgG. However, antibody 3T72 lacked this major a1 specificity. The correlation of this serologic difference with the amino acid sequence variation at position 15 and 16 of the H chain indicates that these residues constitute a major a1 allotypic determinant.

905 LINKAGE AND STRAIN DISTRIBUTION OF ANTI NUCLEASE ANTIBODIES, C. Garrison Fathman, David S. Pisetsky and David H. Sachs.

Rat antibodies raised against anti-nuclease antibodies, from mouse strains A/J and SJL, detect strain specific idiotypic determinants related to the antigen combining site. Using these rat anti-idiotypic antisera it has been possible to examine the genetic linkage and strain distribution of these anti-nuclease idiotypes. Although the immune response to staphylococcal nuclease is controlled by H-2 linked Ir genes, the expression of the anti-nuclease idiotypes is independent of genes linked to the H-2 region: A/J anti-nuclease idiotype was present in immune sera from strains A/J ($\underline{H\text{-}2}^a$) and A.BY ($\underline{H\text{-}2}^b$) but absent in sera from strains B10 ($\underline{H\text{-}2}^b$) and B10.A ($\underline{H\text{-}2}^a$). Analysis of A/J anti-nuclease idiotype expression in a backcross of (A/JxB10.A)xB10.A demonstrated linkages of the A/J anti-nuclease marker to the $\underline{Ig\text{-}1}^e$ heavy chain allotype marker. The expression of both A/J and SJL anti-nuclease idiotype markers in antibodies raised in strain BALB/c but only the A/J idiotype marker in strain BAB.14 antibodies suggest that it might be possible to use these idiotypic markers to construct a map of distinct variable region genes.

906 IMMUNOGLOBULIN GENETICS: STUDIES WITH RECOMBINANT INBRED STRAINS OF MICE, Benjamin A. Taylor, The Jackson Laboratory, Bar Harbor, ME 04609

Recombinant inbred (RI) strains have proved useful in studies of immunoglobulin genetics. RI strains are derived by brother-sister inbreeding beginning with the $F_2$ generation of a strain cross. Such strains are used to discover or demonstrate genetic segregation, linkage and pleiotropism. The heavy chain allotype locus $\underline{Ig\text{-}1}$ was shown to be linked to serum prealbumin ($\underline{Pre\text{-}1}$) with about 12 percent crossing over. Several $V_H$ markers have been shown to be closely linked to $\underline{Ig\text{-}1}$. Several presumptive $V_H$ region recombinants have been identified among the 24 RI strains derived from C57BL/6J and DBA/2J, permitting preliminary mapping of five $V_H$ markers. The RI strains derived from AKR/J and C57L/J have been used to demonstrate close linkage of isoelectric focusing variants of kappa chains to the $\underline{Ly\text{-}2,3}$ lymphocyte cell surface antigen determining locus on chromosome 6.

907 SHARED, ALLOTYPE-LINKED ISOELECTRIC FOCUSING PATTERNS OF RECOMBINANT INBRED MICE. Dietmar G. Braun, Basel Institute for Immunology, Basel, Switzerland.

The immune response of (C57BL/6xBALB/c)F1 hybrids to the streptococcal group A polysaccharide is characterized by two clonotypes of the low responder $Ig\text{-}1^b$ allotype. These clonotypes A and B were now also identified in C57BL/6 mice, and in all of the Bailey recombinant inbred mice with this allotype. $Ig\text{-}1^b$ recombinant mice share additional 3-4 clonotypes which were also traced in C57BL/6 mice. (C57BL/6xBALB/c)F1 mice also express antibodies to the group A polysaccharide that are products of the $Ig\text{-}1^a$ locus; one of these clonotypes was shared with those of two CxBG mice and some BALB/c mice. It was remarkable that these two CxBG mice shared with the exception of two bands on isoelectric focusing a complete set of clonotypes consisting of 6-7 monoclonal antibodies. Since both the products of the $Ig\text{-}1^b$ and the $Ig\text{-}1^a$ locus were completely adsorbed to N-acetyl-glucosamine sepharose columns it is concluded that each of the two parent strains (C57BL/6 and BALB/c) contain in their germ line the information of at least 6-7 monoclonal antibodies that can bind the streptococcal group A polysaccharide.

908 IDENTIFICATION OF A B-CELL SURFACE STRUCTURE INVOLVED IN ANTIGEN-DEPENDENT TRIGGERING, Brigitte T. Huber and Harvey Cantor, Harvard Medical School, Boston, MA

CBA/N mice have an X-linked B cell maturation defect which is reflected in part in an absence or dysfunction of a subclass of mature B cells. We have immunized the defective ♂ offspring of the mating (CBA/N ♀ x BALB/c ♂) with BALB/c spleen cells. The resulting antiserum (αLyb3) selectively reacts with a component on the surface of a portion of B cells from a panel of H-2 different mouse strains. Binding of αLyb3 serum to this B cell subclass results in substantial (10-20 fold) enhancement of the antibody response to low doses of SRBC. Both binding and enhancing activity are removed by absorption with B cells from B6, BALB/c, but not CBA/N mice. Absorption of the serum with BM cells, T cells or thymocytes from $Lyb3^+$ strains does not remove activity.

Since the enhanced PFC responses are specific for the immunizing antigen, and since no PFC response is produced by injection of the antiserum alone, this enhancement probably reflects a second signal produced by specific interaction between antibody and the surface Lyb3 component. Moreover, this signal can partially replace the requirement for T cells in the production of antibody to a 'thymus-dependent' antigen. These findings (taken in conjuction with the previously described immune defects in CBA/N mice and other studies of B cell maturation) suggest to us that Lyb3 is a cell surface component expressed selectively on a mature B cell subclass. This component is important in B cell triggering by antigen and fails to develop in CBA/N mice, due to a dysfunction of a regulatory gene on the CBA/N X-chromosome.

909 FUNCTIONAL RESTRICTION OF COLONY FORMING B LYMPHOCYTES, Paul W. Kincade, Grace Lee, and Christopher J. Paige, Sloan-Kettering Institute, Rye, New York 10580

CBA/N mice are deficient in B lymphocytes and unable to respond normally to thymus-independent antigens. B cells from these mice do not proliferate in response to mitogens in semisolid gel culture. Under conditions which allow focal proliferation of normal cells as a linear function of the number of cells cultured; CBA/H mice have a normal incidence of colony forming cells, (CBA/N♀ X CBA/H)$F_1$ ♂ mice have none, and $F_1$ ♀ mice are intermediate. The B cells which CBA/N mice have and presumably the cells which mediate T-dependent responses (B2 cells) can be activated to division and differentiation by T-independent mitogens in liquid cultures prepared at high cell density but not when cell contact is minimized by dilution or physical separation. B cells which CBA/N mice lack and which form colonies *in vitro* and respond to T-independent antigens *in vivo* (B1 cells) are present as early as 17 days' gestation in fetal liver, are in some respects heterogeneous, and comprise an equivalent percentage of the B cells in newborn spleen, adult spleen, and lymph nodes. In the absence of other detectable hemopoietic abnormalities in CBA/N mice, and since colony forming cells can be restored by lethal irradiation and grafting with CBA/H fetal liver or bone marrow cells, the following tentative conclusions are possible: 1) the sex-linked immune deficiency of CBA/N mice results from the complete absence of a functional subpopulation of B cells; 2) the semisolid cloning technique quantitatively and selectively detects these cells, and 3) T-independent B1 cells and T-dependent B2 cells may represent divergent pathways of differentiation.
Supported by NIH grant AI-12741 and a Senior Investigatorship from the Arthritis Foundation.

910 REGENERATION OF SURFACE Ig AS A MEASURE OF IMMUNOLOGICAL MATURATION OF CBA/N MICE. I.M. Zitron, I. Scher, and W.E. Paul, NIAID and NMRI, Bethesda, Md. 20014.

The CBA/N mouse strain carries an X-linked defect, manifested in its B cells, which gives rise to an inability to respond to certain thymus-independent antigens. On the basis of analysis of amount and class of membrane Ig, expression of complement receptors and Mls stimulatory determinants, and pattern of immunologic responsiveness, the defects in these mice are believed to arise from an arrest in the maturation of B cells or of a B cell sub-line. Recently, it has been reported that Ig-bearing cells of young mice of normal strains fail to re-express membrane Ig after overnight incubation with anti-Ig. This has been suggested as a mechanism by which immunologic tolerance is achieved in neonatal mice and might account for the failure of CBA/N mice to make certain immune responses. We have confirmed the finding that splenic B cells of neonatal, nondefective animals fail to re-express sIg after overnight incubation with anti-Ig reagents. Taking advantage of the X-linked nature of the CBA/N defect, we have compared adult (CBA/NxDBA/2)$F_1$ male [defective] mice with their female [non-defective] littermates, with respect to this function. Both the male and female animals are capable of the re-expression of membrane Ig. This has been shown to hold true when initial incubation involved either a polyvalent anti-Ig or an anti-μ chain reagent. Thus the maturational arrest in the defective animals, if it occurs as a single discrete defect, must occur at a stage such that their B cell population is more mature than that of the neonatal mouse. The lack of certain responses in CBA/N mice is, then, not simply ascribable to their being at a stage of maturation such that Ig receptors are irreversibly modulated due to encounter with a polyvalent ligand.

911 THE GENERATION OF MATURE ANTI-SRBC SPECIFIC B CELLS IS ANTIGEN DEPENDENT. Yvonne J. Rosenberg and Alastair J. Cunningham, Australian National University, Canberra, A.C.T. Australia 2601

The time required for B cells from various tissues to generate antibody-forming progeny after antigenic stimulation within irradiated recipients can be used to assess their functional maturity. In such experiments cells from adult bone marrow or foetal liver required a longer period to give an adoptive anti-SRBC plaque-forming cell response than those in spleen. This delay is not overcome by allowing the cells a 7-day 'sojourn' in the irradiated recipient before antigen challenge. The requirements for the generation of mature splenic type B cells have been studied using two-stage experiments in which the generation of mature cells in vitro could be measured by their ability to yield anti-SRBC plaque-forming cells after stimulation with mitogen in vitro. The results show that a critical factor which influences the final differentiation of bone marrow or foetal liver cells into fully mature B cells is exposure to antigen. This is consistent with the idea that immunocompetent cells in an adult animal are generated after antigenic stimulation of more primitive cells.

912 ONTOGENETIC PARAMETERS OF IMMUNE ACTIVITY. H.M. Etlinger and J.M. Chiller, National Jewish Hospital and Research Center, Denver, Colorado 80206

Antigen binding studies have indicated previously that lymphocytes specific for a variety of antigens are present in neonatal mice. Whether the presence of specific receptors is translatable to positive or negative consequences of antigen-lymphocyte interaction is unclear. We have examined the ontogenetic capacity of mice to respond to antigens which are either relatively T-independent (DNP-Ficoll), T-dependent (aggregated human gamma globulin (AHGG)) or somewhere in between (sheep RBC). Four day old mice were found to respond at near adult levels to DNP-Ficoll (direct PFC). In contrast, adult level responses to AHGG were not reached until some 40 days of age (indirect PFC). The ontogenetic response profile to SRBC demonstrated that, although 4 day old mice were capable of responding to SRBC (direct PFC), no indirect PFC were observed until the animals were some three weeks of age. These ontogenetic patterns may reflect absence of functional T help, the dominance of T suppression and/or the response of subpopulations of B cells. The question of whether the inability of young mice to respond positively to AHGG reflected a negative interaction was examined by injecting animals with AHGG 30-40 days after the initial neonatal treatment. Such animals were indeed specifically unresponsive to further challenges of AHGG, a state which appeared to be restricted to B cells since 1) there was a normal response to DNP in animals challenged with DNP-HGG, 2) the kinetics of unresponsiveness were similar to those of B cells described in the deaggregated HGG system and 3) a T cell activity marked by lymph node cell proliferation induced in vitro with HGG was normal. (Supported by USPHS Grants AI-05343, 13131 and 00133.)

913 DIFFERENTIATION OF MYELOMA CELL IMMUNOGLOBULIN EXPRESSION. J.W. Rohrer and R.G. Lynch, Washington Univ. Med. Sch., St. Louis, MO 63110

The BALB/c myeloma MOPC-315 produces a TNP-specific IgA, but neither increased serum IgA nor a-TNP hemagglutinin are detected for 7 days after 315 injection. To determine if qualitative changes occur in the 315 cells diffusion chamber 315 cells were implanted i.p. and harvested later. Surface expression and secretion of 315 Ig were detected by monitoring TNP rosette-forming cells(RFC) and plaque-forming cells(PFC),respectively. 315 stem cells were monitored using a spleen colony assay(PCFU). Only 50% of the 315 cells were recoverable 1 day later but began to increase exponentially by day 4. RFC concentration was halved by day 1, began to increase on day 3, and attained original levels by day 7. As RFC increased, so did the number of red cells bound per RFC. PFC concentration decreased 90% by day 2, remained low thru day 5,and then increased rapidly. PCFU concentration doubled by day 1, continued to increase thru day 7, and then declined. A morphological shift from plasmacytoid to lymphocytoid cells occurs by day 1 followed by a gradual reappearance of plasmacytes during the next week. Hence, Ig expression by 315 cells in DC mimics antigen-driven normal B cell differentiation. Preliminary studies show promotion of 315 cell growth and differentiation by TNP-carrier in the presence of soluble, carrier-specific helper T cell factors. We believe MOPC-315 may provide a monoclonal model for studies of B cell regulation. (Sup NIH grants CA15306, ca17114, CA09118, and GM00897).

914 NON-SPECIFIC EFFECTS OF ANTIGEN ON B-CELL DIFFERENTIATION, Ken Shortman, Maureen Howard and John Fidler, The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia.

B-cell subsets representing stages in differentiation have been isolated and characterised by sedimentation, density, electrophoretic, and adherence column cell separation procedures. Their functional status has been assessed by adoptive and cell culture assays for AFC-progenitor activity. Memory B cells have been separated and distinguished from virgin B cells. Virgin B cells may be found in a number of developmental states. Two distinct types of virgin AFC-progenitor activity can be distinguished kinetically and by physical criteria. The earlier form ("pre-progenitors") are activated non-specifically by environmental or experimentally injected antigens, into larger, atypical forms (physical characterisation) and finally into division and proliferation ($^{3}$H-thymidine suicide experiments). The later virgin B cells ("direct AFC-progenitors") arising from this proliferative phase only respond specifically to antigen, producing AFC. Memory B cells likewise only respond specifically to antigen. Three stages of B-cell development are thus proposed: (i) An antigen independent phase; (ii) A phase dependent on the non-specific effects of environmental stimuli; (iii) A specific antigen dependent phase.

915 APPROACHES TO THE STUDY OF THE DIFFERENTIATION OF B AND T LYMPHOCYTES, Robert A. Phillips, Richard G. Miller and Sydney L. Abramson, The Ontario Cancer Institute, Toronto, Canada M4X 1K9.

The major problems in the study of the early events in the differentiation of lymphocytes are the lack of suitable markers to identify lymphocyte precursors and the inability of these precursors to differentiate in culture. We have approached these problems in two ways. In the first approach we have used radiation-induced chromosome markers to study the interrelationships between the stem cells for the myeloid and lymphoid systems. The distribution of marked cells in these mice suggested the existence of at least three different classes of stem cells: a pluripotent and two restricted stem cells, one for myeloid cells and one for T cells. There was no evidence for a lymphoid restricted stem cell. In the second approach we have used an improved culture system to study the TNP-LPS response of various subpopulations of cells in adult bone marrow. The small lymphocytes give a peak PFC response on day 4. In contrast, the large, rapidly sedimenting cells give little or no response on day 4, but give a large PFC response between days 6 and 9. Our data indicate that the large, rapidly sedimenting cells are the precursors of the small B lymphocytes. This in vitro assay for B cell precursors is now being used to investigate the stem cell heterogeneity described above. (Supported by the MRC and the NCI of Canada).

916 B CELL HETEROGENEITY AS DISTINGUISHED BY RESPONSES TO TNP-POLYACRYLAMIDE BEADS. James J. Mond, Donald E. Mosier and William E. Paul, NIAID, NIH, Bethesda, Md. 20014.

Developmental heterogeneity has been previously demonstrated by the analysis of membrane markers and certain functional properties of thymus-independent (B) lymphocytes. In order to study the requirements of B lymphocytes at different developmental stages for activation by thymus-independent antigens, we have studied the capacity of a series of TNP-polyacrylamide beads ($TNP_x$-PAB) differing only in epitope density to elicit primary in vitro anti-TNP responses in dissociated spleen cell cultures. Initial experiments were carried out using immunologically defective (CBA/N x DBA/2N)$F_1$ male mice and their normal $F_1$ female littermates. Both the high and low epitope $TNP_x$-PABs elicited primary anti-TNP responses in normal $F_1$ female spleen cells, whereas only the more heavily substituted beads were found to trigger the defective $F_1$ male cells. In addition, greater numbers of beads were required for optimal responses by the $F_1$ male spleen cells. Since $F_1$ males appear to have a defect in B cell maturation, these experiments suggest that the activation of relatively immature B cells requires a greater hapten concentration than does the activation of more mature B cells. To further explore this idea, we studied the ontogeny of this response in neonatal and adult $BDF_1$ mice. It was found that spleen cells from 2-3 week old mice were able to mount a response only to the more heavily substituted $TNP_x$-PAB whereas adult spleen cells responded to both low and high density $TNP_x$-PABs. Since $TNP_x$-PABs function as T-independent antigens in this system, these experiments indicate that immature B cells may have a higher threshold for activation by antigen than do more mature B cells.

917 SUBPOPULATIONS OF CHICKEN B LYMPHOCYTES, Yong Sung Choi and John Lifter, Sloan-Kettering Institute for Cancer Research, Rye, New York 10580.

Immunoglobulin synthesizing cells from the spleen and bursa were fractionated by the 1 x g sedimentation velocity technique and characterized by their ability to synthesize immunoglobulin and by staining with fluorescent antilight chain. Four subpopulations of immunoglobulin synthesizing cells were identified. In the bursa, slowly sedimenting (S 2.3 mm/hr) and rapidly sedimenting (S greater than 3.5 mm/hr) subpopulations with surface immunoglobulin were present; in the spleen, a slowly sedimenting (S 2.3 mm/hr) subpopulation with surface immunoglobulin and plasma cells (S greater than 3.5 mm/hr) with large concentrations of intracellular immunoglobulin existed. The subpopulations differed most markedly in their ability to synthesize immunoglobulin (cpm Ig synthesized/$10^6$ Ig positive cells); the rates of immunoglobulin synthesis were in the ratio of 1:2:1:900. The slowly sedimenting B cells from the spleen and both subpopulations of B cells from the bursa released small amounts of immunoglobulin into the culture media, whereas, the plasma cells released immunoglobulin at a rate as much as 3700 times greater. Bursal B cells could be further distinguished from splenic B cells by a greater amount of DNA synthesis.

918 HUMAN PERIPHERAL B LYMPHOCYTES DO NOT HAVE HIGH AFFINITY Fc RECEPTORS, Elaine L. Alexander and Pierre A. Henkart, NIH, Bethesda, Maryland 20014

The binding and interaction of FcR positive human peripheral lymphocytes (HPBL's) with antigen-antibody (Ag-Ab) complexes immobilized on plastic surfaces has been developed as a means of preparing purified populations of FcR positive and FcR negative cells. During these studies, we noted that the FcR negative nonadherent population was not depleted of sIg nor C3R bearing cells. The FcR positive adherent cells failed to stain in situ for C3R or sIg, and did not develop these surface markers after removal from substrate and culture in vitro. Conversely, we have studied the adherence of lymphocytes to substrate coated with goat αHIg Fab. The nonadherent population was depleted of sIg and C3R bearing cells, but not of FcR bearing cells. The FcR negative adherent cells were sIg and C3R positive. These observations suggested that sIg and FcR's were segregating on independent populations of lymphocytes. We designed double labeling experiments to examine directly the distribution of sIg, C3R, and FcR on HPBL's. sIg was detected by goat αHIg, which unlike rabbit αHIg, does not bind to lymphocyte or monocyte FcR's. C3R's were detected by the binding of fluoresceinated bacteria coated with complement. FcR's were assessed by an indirect immunofluorescence assay for the binding of soluble Ag-Ab complexes used at a concentration of approximately 1 mg/ml. The results demonstrated that the majority of human peripheral B lymphocytes bearing sIg also bear C3R, but not FcR. Similar results were obtained with human tonsillar B lymphocytes. Thus, these experiments demonstrate that human B cells do not have high affinity Fc receptors.

806 THE I REGION GENES IN GENETIC REGULATION. Baruj Benacerraf, Martin Dorf, Carl Waltenbaugh, Jacques Thèze and Judith Kapp. Department of Pathology, Harvard Medical School, Boston, Massachusetts 02115.

The I region of the major histocompatibility complex of mammals is concerned with the regulation of immune responses to thymus-dependent antigens. For many antigens, the control of immune responses results from the interaction of Ir genes in the I-A (β genes) and in the I-C region (α genes). These genes complement in both the cis and trans position. Moreover, evidence of coupled complementation has been obtained, indicating that every β gene does not complement successfully every α gene. Evidence has also been obtained in several systems and different laboratories that the I region gene products play a critical role in the specificity of antigen presentation by macrophages to specific T cells. These systems will be discussed and their relevance to Ir gene defects evaluated. Many, but not all, genetic nonresponder strains develop specific suppressor T cells following injection with the relevant antigen. The genetic control of specific T cell suppression has also been ascribed to the I region of the murine H-2 complex and the same phenomena of coupled complementation has been observed for specific immune suppression genes as for Ir genes. Treatment with cyclophosphamide inhibits the stimulation of specific suppressor T cells and restores responsiveness to strains bearing appropriate H-2 haplotypes. A specific suppressor factor can be obtained from thymocytes or peripheral T cells of specifically suppressed mice. Such a factor has been obtained from DBA/1 or A.SW strains for the terpolymer GAT and from BALB/c and B10.BR mice for the copolymer GT. They suppress specific immune responses of selected allogeneic as well as syngeneic strains. The most informative example of suppression across H-2 concerns the behavior of BALB/c GT factor in A/J mice. This factor suppresses the response of A/J mice to GT-MBSA although this and other strains bearing the H-$2^a$ haplotype are not suppressed by GT alone, indicating two steps in the generation of GT suppressor T cells. Moreover, evidence has been obtained in both the GAT and GT system that specific suppressor extracts are able to stimulate the stimulation of specific suppressor cells. The immunochemical properties of specific suppressor factors are being investigated. The GAT factors can be specifically retained by an antigen-Sepharose column, and by anti-I immunoabsorbent but not by an anti-mouse Ig column. The factor can be eluted from the antigen column. The data to be reported illustrate, therefore, that the I region regulate immune responses by two correlated processes: 1) the stimulation of specific helper or suppressor T cells by controlling the manner in which antigen is presented to these cells, and 2) the production of antigen specific T cell factors endowed with helper or suppressor properties on the response of other immunocompetent cells.

807 REGULATION OF THE ANTIBODY RESPONSE BY T CELL PRODUCTS DETERMINED BY DIFFERENT I SUBREGIONS, Tomio Tada, Laboratories for Immunology, School of Medicine, Chiba University, Chiba, Japan 280

Studies on the genetic control of immune responses have been achieved mainly through two different approaches which are now closely inter-related; 1) the genetic analyses of the ability of animals to mount an immune response to given antigen; and 2) the demonstration and characterization of the products of I region genes which mediate the regulatory cell-interactions. The first approach has led to the discovery of specific Ir and Is genes, and gene complementation controlling the immune reactivity of animals to antigen. I shall mainly discuss the second approach which is concerned with cell surface material and 'factors' bearing I region gene products.

Recent studies from various laboratories on antigen-specific T cell factors, both helpful and suppressive, made it clear that these factors contain products of genes which are mapped in I region of MHC. They are produced by functionally different subsets of T cells which are distinguishable by cell surface markers determined by non-H-2 genes. In our own experiments two functionally distinct antigen-specific factors, one suppressive and the other enhancing, have been shown to exist, and they are encoded by genes mapped in different I subregions, i.e., I-J and I-A. Taussig and Munro reported an antigen-specific helper factor which is a product of I-A subregion, and Erb and Feldmann demonstrated a macrophage-derived factor which is also an I-A subregion gene product. Some of the antigen-nonspecific factors are also shown to be the products of MHC genes. Furthermore, the receptor sites (acceptors) for these factors on target cells have been shown to be the I region product, and thus the I region products appear to play an integral role in the cell to cell communication in the regulation of immune response.

In view of the multiplicity and complexity of these T cell factors, questions now to be asked are 1) What is the inter-relationship between these factors, and more specifically whether these factors and their acceptor sites are coded for by the same single locus or multiple loci ? 2) Whether or not these T cell factors belong to a new class of molecule which is distinct from conventional Ia antigens ? 3) How the different subregion loci are selectively expressed on functionally different subsets of lymphoid cells ? 4) What regulatory mechanism governs the generation and maintenance of such a complicated network of cells having different I subregion products ? and 5) What does the coexistence of antigen- and I region specificities on the single molecule imply ? These problems will be discussed referring to the recently described third T cell type which has Ly $1^+$ $2^+$ $3^+$ and acceptor sites for T cell factors, and which is perhaps involved in the feed-back generation of actual helper or suppressor T cells.

808 GENETIC REGULATION OF MACROPHAGE-T LYMPHOCYTE INTERACTION. Ethan M. Shevach and David W. Thomas, LI, NIAID, NIH, Bethesda, Md. 20014

The products of the major histocompatibility complex (MHC) play a critical role in the regulation of the interactions of immunocompetent cells. In the guinea pig effective interaction between antigen pulsed macrophages and immune T cells requires that the macrophage and T cell be syngeneic (1). Genetic studies have demonstrated that this interaction is mediated by the Ia antigens of the guinea pig MHC and not by the products of the guinea pig equivalent of the murine K or D region genes (2). It was hypothesized that the I-region genes code for specific cellular interaction structures and that homology between these structures was necessary for effective cellular interactions (3). However, as these studies were performed with T cells from animals primed in vivo, the results are also compatible with the view that T cells do not recognize antigen per se, but can only be sensitized to antigen-modified membrane components or to complexes of antigen with certain membrane molecules. Thus, allogeneic macrophage-associated antigen failed to activate immune T cells in vitro because the T cell had been primed in vivo only to antigen associated with syngeneic macrophages.

We have recently developed an in vitro assay for the generation of a primary response to soluble protein antigens in which non-immune T cells can be sensitized and subsequently challenged in tissue culture with antigen pulsed macrophages (4). This technique provides a method by which the mode of antigen presentation can be easily manipulated and experiments can be performed in which any combination of allogeneic or syngeneic lymphocytes and macrophages can be tested. T cells primed with syngeneic antigen-pulsed macrophages were only restimulated by syngeneic and not allogeneic antigen-pulsed macrophages. When $F_1$ T cells were primed with parental macrophages they were only restimulated by the parental macrophage used for initial sensitization and not with those of the other parent. When allo-reactive guinea pig T cells were rendered unresponsive to allogeneic macrophages by treatment with bromodeoxyuridine and light, the remaining T cells could be subsequently primed and rechallenged in culture with antigen-treated syngeneic or allogeneic macrophages. T cells primed with antigen-treated allogeneic macrophages could be restimulated only with antigen treated allogeneic, but not syngeneic macrophages. The results of this experiment strongly support the concept that Ia homology is not required for efficient T cell-macrophage collaboration in response to antigen and that the genetic restriction of this interaction is imposed only by the histocompatibility type of the macrophage used for initial sensitization.

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919 LACK OF GENETIC RESTRICTIONS IN THE SECONDARY IMMUNE RESPONSE IN VITRO.
Susan L. Swain and Richard W. Dutton, Department of Biology, University of California, San Diego, La Jolla, California 92093

The 2° in vitro PFC response to hapten-carrier is very sensitive to negative allogeneic effects. These suppressive effects are mediated by Ly2+ (Ly1-) T cells which recognize antigens on the responding cells coded for by the K or D or sometimes I subregions on the responding cells coded for by the K or D or sometimes I subregions of the MHC. It seems probable that the B cell is the target of suppression and that suppression and cytotoxicity are effected by distinct mechanisms. It thus seemed possible that this allosuppression might be responsible for the failure of carrier primed T cells to collaborate with allogeneic B cells. Procedures were devised to minimize allosuppression and the question of T-B collaboration was reexamined. The degree of allosuppression can be greatly reduced by pretreating T cell populations with anti-Ly2 serum plus C'. When this was done, we found that in vivo primed T and B cells differing from one another at K and I regions, at the whole H-2 region, or at the I region alone could collaborate. The response was carrier dependent (arguing against positive allogeneic effects), and at limiting amounts of T cell help there appeared to be no preference for syngeneic rather than allogeneic B cells. In separate experiments we found that syngeneic and allogeneic macrophages were equally effective in antigen presentation under conditions where the antigen was limiting. This lack of genetic restriction in the secondary in vitro response suggests that T cells can recognize and respond to antigen in solution or on cells with MHC determinants not present during in vivo priming.

920 ANTIGEN PRESENTATION IN THE MURINE T LYMPHOCYTE PROLIFERATIVE RESPONSE, Akihiko Yano, Ronald H. Schwartz and William E. Paul, LI, NIAID, NIH, Bethesda, Md. 20014.

The recent introduction of a reliable T lymphocyte proliferation assay, which ultilizes thioglycollate-induced, nylon column-passed, peritoneal exudate lymphocytes from immune mice (PETLES) has allowed us to investigate the genetic control of murine immune responses at the T cell level. Studies in guinea pigs using a similar T lymphocyte proliferation assay have demonstrated the requirement for antigen presentation by syngeneic macrophages in order to achieve stimulation of primed T lymphocytes. In the present studies, we have utilized the PETLES assay to demonstrate a similar restriction in the mouse and to analyze the genetic basis for this phenomenon. Spleen cells and non-immune PETLES were the best types of antigen presenting cells, while unfractionated thioglycollate-induced peritoneal exudate cells and bone marrow cells were less efficient. Antigen-presenting cells obtained from syngeneic mice gave greater net stimulation (Δcpm) than allogeneic presenting cells. $F_1$ cells gave intermediate levels of stimulation although they stimulated mixed lymphocyte reactions as great as those obtained with allogeneic cells. Antigen-pulsed spleen cells from strains differing at the Mls locus but similar at H-2 also generated large mixed lymphocyte reactions, yet these cells presented antigen as well as syngeneic cells. Finally, through the use of congenic resistant lines and their recombinant strains, one of the genes controlling the allogeneic restriction of antigen presentation to primed cells was mapped to the I-A subregion of the murine MHC. These results indicate that mixed lymphocyte reactions by themselves do not prevent antigen presentation and that genetic similarity in the region which codes for the major MHC-MLR stimulating antigens (I-A) is critical for effective presentation.

921 GENETIC CONTROL OF IMMUNE RESPONSES IN VITRO: MULTIPLE Ir GENES INVOLVED IN T MACROPHAGE, T-B, AND T-T INTERACTIONS, Marc Feldmann, Peter Erb* and Sarah Howie, ICRF Tumour Immunology Unit, Department of Zoology, University College London, and *Institute of Medical Microbiology, University of Basel, Switzerland.

The induction of helper cells in vitro (and presumably in vivo) involves the interaction of macrophages (M) with two T cells, a short-lived Ly-1,2,3 cell, and a long-lived Ly-1 cell. Macrophages after exposure to antigen release an immunogenic Ia-antigen fragment termed GRF, which activates T cells to become T helper cells. The genes controlling this interaction were mapped in three ways (all in I-A), first by genetic restrictions in T-M interaction, secondly by the serology of GRF, and thirdly by genetics of binding of the factor to T cells, indicating that there is a pair of genes, both present in the I-A subregion, involved in this interaction. Serological studies suggest that the receptors for GRF are not classical Ia antigens, since binding was not blocked by anti-Ia antisera. While the current experimental protocols detect genetic restrictions on T-M cooperation, others have reported results at variance with this concept. Experiments were thus performed to investigate these differences.

A simple way of studying the Ir genes involved in the response to synthetic polypeptide antigens is to ascertain the strains which will or will not make helper or suppressor factors, together with which combinations of mouse strains will respond to these factors. Such techniques have been used, in vitro, with (T,G)-A--L and related peptides, and GAT and related peptides, to reveal a heterogeneity of sites of Ir gene defects.

922 RESTRICTION IN THE COOPERATIVE FUNCTION OF SINGLE HELPER T CELLS, Herman Waldmann and Jenny Phillips, Dept. of Pathology, University of Cambridge, Tennis Court Rd., Cambridge CB2 1QP, England.

The In-Vitro antihapten response to TNP-KLH was assessed in the Lefkovits microculture system in such a way that helper T cells were titrated to limiting dilution in the presence of excess B, and similarly B cells titrated in the presence of excess T helper cells. The average number of PFC generated per T cell was of the same order as that calculated per responding B cell. In conditions of excess B cells, cultures showed marked restriction in the number of B cell clones responding when T cell help was limiting. This data suggests that even though one T cell may be confronted with many potential hapten reactive B cells, probably only one B cell is activated. Two interpretations of the data, namely, direct T-B cell contact, or particular suitability of a given B cell to the T helper cell will be discussed.

923 ALTERNATIVE PATHWAYS OF COOPERATION BETWEEN T CELLS AND SUBPOPULATIONS OF B CELLS, George K. Lewis, Raymond Ranken and Joel W. Goodman, The University of California, San Francisco, CA 94143

Murine T cells cooperate with B cells that carry a receptor for C3 (CR+) and with at least some B cells which lack the C3 receptor (CR-) in a primary in vitro antibody response. Depletion of serum C3 in vivo by cobra venom factor reduced the primary IgM anti-SRBC response by about 75%, suggesting a partial dependence of the response on C3. In vitro culture experiments using populations of B cells fractionated on the basis of the C3 receptor showed that CR+ cells were unable to make T-dependent antibody responses in the presence of anti-C3 antibody, whereas the response of CR- B cells was unaffected. Using irradiated, carrier-primed spleen cells from B10.A mice as a source of helper cells for B cells derived from various congenic strains in an in vitro primary IgM response to TNP-KLH, CR+ B cells cooperated across haplotype differences in the I region of the MHC, whereas CR- B cells did not. The genetic restriction for cooperation between CR- B cells and T cells was tentatively mapped to the IJ-IC region of the MHC. The findings suggest the existence of alternative cooperative pathways between T cells and B cell subpopulations which can be differentiated on the basis of the surface receptor for C3.

924 GENERATION AND EXHAUSTION OF SRBC-SPECIFIC T MEMORY HELPER CELLS, B.A. Araneo, E.E. Sercarz, P.C. Marrack, and J.W. Kappler, Department Bacteriology, University of California, Los Angeles, Ca. 90024

The maturation of peripheral T cells in mice involves at least two precursor stages, T1 and T2, which are distinguishable by adult thymectomy (ATx) and small doses of anti-thymocyte serum (ATS) in vivo. T1, recently derived from the thymus, are defined by their relatively short life time after ATx and relative insensitivity to ATS. T2, having the longest residence in the periphery, are long-lived and recirculating as determined by their relative sensitivity to ATS and insensitivity to ATx. Functionally, T1 and T2 do not represent sublines restricted to particular immunologic functions when stimulated by antigen in vivo, as precursors of several effector types are equally distributed between both populations. However, T1 and T2 can be distinguished on the basis of their kinetics of response to antigen. T2 proliferate and provide effective help sooner than T1. T memory cells reside in the T2 pool, according to their kinetics and sensitivity to ATS. To provide evidence for either parallel or sequential mode of development, the generation of T2 precursors from T1 precursors has been examined. The results indicate that T1 cells differentiate into T2, memory, cells in an antigen driven step. While the effect of antigen on this memory population is to generate active effector cells, there is also expansion of the precursor pool. The possibility of exhausting this expansion step is being studied.

925 THE ROLE OF INHIBITOR T CELLS IN PREVENTING SUCCESSFUL COOPERATION BETWEEN T & B CELLS HISTOINCOMPATIBLE FOR PORTIONS OF THE MHC COMPLEX OTHER THAN I-A
Herman Waldmann, Dept. of Pathology, University of Cambridge, Tennis Court Rd, Cambridge CB2 1QP, England.

Studies with parental T helper cells on F1 hybrid B cells, or of T & B cells within B10 congenic strains differing in parts of the MHC other than I-A show that at high T cell input their cooperation was extremely inefficient. This was found to be the consequence of the generation of inhibitor T cells. Combinations of T & B cells with differences across K or D ends of H-2 seem to provoke the activity of these cells much more than combinations possessing I region differences only. Irradiation abolished the activity of this inhibitor. Single (P->F1) or double (TBMC) chimaeras lacked cells capable of inhibiting secondary responses of the partner strains but not of third party cells. The properties of the inhibitor T cell and its relevance to the problems underlying the regulation of physiological T-B collaborator will be discussed.

926 SPECIFICITY OF ADHERENT CELL REGULATION OF HUMAN ALLOGENEIC REACTIONS (MLC'S),
Norman T. Berlinger, Carlos Lopez, and Robert A. Good, Sloan-Kettering Institute, 1275 York Avenue, New York, NY 10021.

Mixed leukocyte cultures (MLC's) of normal cells can be weakly or only moderately reactive due to suppression by adherent cells isogeneic to the responder. Sephadex G-10 column removal of the responder's adherent cells can sometimes allow significantly increased MLC responses. This procedure depletes responding cells of phagocytic, alpha-naphthyl esterase positive cells while not disturbing the relative proportions of T- and B-lymphocytes and Ripley positive lymphocytes. Replacing adherent cells reconstitutes the suppressed response. These effects do not appear attributable to artifacts related to cell density or to proliferative kinetics. Mononuclear cells of individuals with a congenital absence of B-cells also demonstrate the capacity for suppressed MLC responses to certain allogeneic stimuli, suggesting little participation of responder B-lymphocytes in the induction of this type of suppression. When a suppressed response to allogeneic stimulating cells of a certain individual occurs, a suppressed response will also occur with allogeneic stimulating cells of a different individual who is an HLA, MLC identical twin or sibling, suggesting a likely genetic component in the induction of suppression in mixed cell cultures. This type of allogeneic regulation could result from the interactions of MHC gene products and adherent cells which may qualify as mononuclear phagocytes.

927 GENETIC CONTROL OF T CELL PROLIFERATIVE RESPONSE TO INSULIN IN THE MOUSE,
Lanny J. Rosenwasser, Marcello A. Barcinski and Alan S. Rosenthal, LCI, NIAID, NIH, Bethesda, MD 20014

The genetic control of the immune response to insulin has been described in the mouse and guinea pig. We have employed an 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 insulin. We find that 1) H-$2^a$ and H-$2^k$ mice are non-responders to either pork or beef insulin, 2) H-$2^d$ mice will respond to either pork or beef insulin, 3) H-$2^b$ mice will respond to beef insulin but not to pork. In addition, using recombinant strains of mice, we have mapped the genetic control of the immune response of H-$2^b$ mice to beef insulin to the left of IB, probably in the IA subregion of the H-2 complex. Utilizing naturally occurring insulin "mutants" we have mapped the intramolecular regions of the insulin molecule which are recognized as distinct antigenic determinants in the appropriate responder mice.

928 AN ABSOLUTE REQUIREMENT FOR ANTIGEN-SPECIFIC HELPER T CELLS IN THE GENERATION OF CYTOTOXIC T CELLS FROM THYMOCYTE PRECURSORS. L.M. Pilarski, Department of Immunology, University of Alberta, Edmonton, Alberta, Canada.

A new, highly efficient system for generation of alloantigen-specific cytotoxic T cells has been developed which utilizes Marbrook acrylamide tissue culture rafts. This system has been used to generate cytotoxic T cells from precursors resident in the thymus. CBA thymocytes ($10^6$ cells per culture) cocultured with irradiated Balb/c stimulator cells yielded no anti-Balb/c cytotoxic activity. However, when $10^6$ CBA thymus cell responders were cocultured with both irradiated Balb/c stimulators and irradiated CBA spleen cells, substantial T cell-dependent anti-Balb cytotoxicity was generated (20% specific $^{51}Cr$ release at a lymphocyte to target ratio of 1:1). The activity present in the irradiated CBA spleen population had all the characteristics of a helper T cell. It was sensitive to treatment with anti-theta serum plus complement. It was antigen-specific as measured by a lack of anti-Balb/c helpers (but not of anti-C57BL/6 helpers) in CBA spleen cells which were tolerant of Balb/c antigens. The helper activity was not strain-specific in that CBA ($H\text{-}2^k$) thymus cells were helped by C57BL ($H\text{-}2^b$), C3H-NB ($H\text{-}2^p$) and DBA/1J ($H\text{-}2^q$) irradiated spleen cells. Control experiments indicated that all of the cytotoxicity generated was derived from thymocyte precursors, not from the irradiated helper population. These results indicate that cytotoxic T cells require helper T cells for either, or both, of the following events: (a) initial triggering events in a cytotoxic T cell response and/or (b) for clonal expansion. These results further suggest that killer precursors and helper T cells recognize the hapten and the carrier determinants, respectively, on the irradiated stimulator cell. Thus, T-T collaboration may be analagous to T-B collaboration.

929 CROSS PRIMING AND CROSS BOOSTING IN VIVO FOR A CYTOTOXIC RESPONSE TO MINOR HISTOCOMPATIBILITY ANTIGENS. Polly Matzinger and Michael J. Bevan, Dept. of Biology, Univ. of California, San Diego, La Jolla, CA 92093, and The Salk Institute for Biological Studies, P. O. Box 1809, San Diego, CA 92112.

CTL immunized against H-2 similar cells that differ at minor H loci lyse only targets that have both the same H-2 alle and the same minor H allele as the immunizing cell. An example of such H-2 restriction is the response of BALB/c (C; $H\text{-}2^d$) to B10.D2 ($H\text{-}2^d$ but differing at many minor H loci). CTL from C mice primed and boosted with B10.D2 will lyse B10.D2 but not C57Bl/10 (B10, $H\text{-}2^b$). It has been shown, however, that injecting a (BALB/c x BALB.B)$F_1$, [(C x C.B)$F_1$; $H\text{-}2^{d/b}$] with B10 lymphoid cells primes the $F_1$ for a secondary in vitro response to either B10 or B10.D2. In contrast to the priming step, boosting these primed spleen cells in vitro with B10 generates CTL directed only against B10 while boosting in vitro with B10.D2 generates CTL against B10.D2 and not against B10. In other words, the priming step does not seem to be H-2 restricted but the boosting step is restricted. We show here that (1) boosting a primed (C x C.B)$F_1$ in vivo with either B10 or B10.D2 generates CTL against both targets, (2) a GVH reaction is neither necessary nor sufficient for boosting, (3) the effector CTL are restricted and those directed against B10 are a different population from those directed against B10.D2.

930 REGULATION OF THE GENERATION OF CONTACT SENSITIVITY. Mark I. Greene, Ann Pierres, and Baruj Benaceraf. Harvard Medical School, Boston, Massachusetts 02115.

The ability of picryl sulfonic acid (PSA)-induced regulatory T cells to modulate the generation of contact sensitivity (CS) to picryl chloride has been studied. In this regard, suppressor cells obtained from the lymph nodes and spleen, and to some extent the thymus of PSA injected mice depress the generation of CS to PCl. Furthermore, soluble suppressor factor(s) (SF) with similar biological activity have been obtained. This soluble material was shown to stimulate the development of suppressor cells in the spleen and lymph nodes in normal recipients capable of suppressing CS and PCl. The precise contribution of membrane products to the activity of SF is currently being clarified, since we have verified that picrylated red cell membrane may also be used to induce tolerance to PCl CS when such products are administered intravenously. The possibility exists, therefore, that membrane interactions with PSA, may be able to induce suppressor cells through the conjugation of certain relevant membrane proteins as H-2 with the hapten, and that this material may, thereafter, present a tolerogenic signal to other lymphoid cells.

931 CYTOTOXIC T LYMPHOCYTE RECOGNITION OF CELL SURFACE H-2/VIRUS MOLECULAR COMPLEXES, K.J. Blank, J.E. Bubbers and F. Lilly, Albert Einstein College of Medicine, Bronx, NY 10461

Cytotoxic T lymphocytes from BALB.B ($H\text{-}2^b$) mice immunized against syngeneic Friend virus (FV) induced tumor cells (HFL/b) display H-2 restriction, i.e., these immune T cells kill in vitro only FV antigen positive tumor cells which also express $H\text{-}2^b$ antigens. This H-2 compatibility is necessary at least in the H-2D region since unlabelled HFL/g [derived from FV-infected BALB.G ($H\text{-}2^g$) mice] blocked the killing of radiolabelled HFL/b target cells by BALB.B anti-HFL/b killer cells. Killing in this system was also blocked by adding either anti-$H\text{-}2D^b$ or anti-gp69/71 antisera to the reaction medium. This antiserum-mediated blocking suggested that T cell killing required the recognition of specificities associated with both $H\text{-}2D^b$ and gp69/71 molecules which, as previously hypothesized, may interact on the tumor cell surface. Indeed, we have been able to demonstrate interaction between $H\text{-}2D^b$ and virus molecules by the lysostrip method in which the capping of virus molecules by treatment of infected spleen cells with anti-FV antiserum reduced the susceptibility of these cells to lysis by antiserum directed against $H\text{-}2D^b$ but not $H\text{-}2K^b$ molecules. In addition, NP-40 solubilized FV virions produced in BALB.B mice contain $H\text{-}2D^b$ but not $H\text{-}2K^b$ molecules as shown by the ability of these solubilized virions to inhibit the activity of anti-$H\text{-}2D^b$ but not anti-$H\text{-}2K^b$ antisera. It thus seemed likely that a specific interaction between $H\text{-}2D^b$ and virus molecules led to the incorporation of this H-2 molecule into the virion.

These studies lead us to believe that cytotoxic T cell recognition of syngeneic FV-induced tumor cells is directed against an H-2/virus molecular complex on the target cell surface.

932 RAPID IN VIVO DESTRUCTION OF SEMI-SYNGENEIC AND ALLOGENEIC CELLS BY NON-IMMUNIZED MICE AS A CONSEQUENCE OF NON-IDENTITY AT H-2, George A. Carlson and Thomas G. Wegmann, Dept. of Immunology, University of Alberta, Edmonton, Alta. T6G 2E1, Canada.

By monitoring the death and metastatic distribution of $^{131}$I-iododeoxyuridine labelled $H\text{-}2^d$ leukemia cells, hybrid and allogeneic resistance were studied in vivo. All mice tested were able to recognize and kill leukemia cells which were not H-2 identical with self. Resistance was found not only against completely allogeneic cells, but also against semi-syngeneic parental strain cells in $F_1$ hybrid mice. This resistance against semi-syngeneic and allogeneic cells was manifest in two ways: Firstly, the total survival of incompatible cells as determined by whole-body $\gamma$ counting was less than the survival in H-2 identical hosts, with killing of the grafted cells apparent within 24 hours of injection; secondly, colonization of the spleen by the H-2 homozygous leukemia cells was inhibited in H-2 heterozygous and H-2 incompatible mice when measured as early as three days after injection. This splenic rejection of grafted leukemia cells was organ specific as the sharp reduction of radioactivity seen in the spleen was not paralleled by a concomitant loss in other organs such as the liver. Differences at either the K through I-B regions of the H-2 gene complex or at the D region alone were sufficient to initiate rejection.

Although all non-immunized, non-irradiated mice showed early recognition and rejection of cells which were not H-2 identical with self, irradiation abolished rejection in some strains of mice while in others the rejection process was unaffected. While differences at H-2 provided the targets for recognition, non-H-2 genes determined the susceptibility of the rejection process to irradiation.

933 T-CELL MEDIATED CYTOTOXICITY DIRECTED AGAINST TUMOR-ASSOCIATED ANTIGENS IS NOT H-2 RESTRICTED. William E. Biddison and Jon C. Palmer. The Wistar Institute, Philadelphia, Pa. 19104

Cytotoxic T lymphocytes (CTL) were generated in DBA/2 mice against the syngeneic mastocytoma P815Y by intraperitoneal injection of $10^3$ viable P815Y cells. Peritoneal cells were harvested, fractionated by velocity sedimentation at unit gravity, and assayed for cytotoxicity against a panel of target cells in a 4 hour $^{51}$Cr-release assay. Cytotoxicity was observed against P815Y, the DBA/2 lymphoma L5178Y, and the DBA/2 Friend virus-induced leukemia GM 86, but not against the DBA/2 leukemia L1210 or DBA/2 Con A-induced lymphoblasts. DBA/2 anti-P815Y CTL could also lyse the C57BL/6 carcinogen-induced tumors EL4 (lymphoma), MC57G (sarcoma), G26-23 (glioma), and a C57BL/6 SV40-transformed fibroblast, C57SV. However, DBA/2 anti-P815Y CTL did not lyse LPS- or Con A-induced C57BL/6 lymphoblasts, or the strain A spontaneous tumors A10 (mammary adenocarcinoma) and C-1300 NA (neuroblastoma). Since P815Y expresses the cell surface determinants gp69/71 and p30 of Rauscher MuLV, the expression of these determinants was compared to the susceptibility of the aforementioned tumor cells to lysis by DBA/2 anti-P815Y CTL, but no correlation was found. We conclude that the T-cell killing to tumor-associated antigens on P815Y is not restricted to tumor targets which share private H-2 specificities with the stimulator P815Y cells. In addition, the gp69/71 and p30 determinants of Rauscher MuLV are not the target specificities recognized by DBA/2 anti-P815Y CTL.

934 DIFFERENTIAL H-2 RESTRICTION ON THE INDUCTION AND EXPRESSION OF SUPPRESSOR T CELLS IN TOLERANCE TO DNFB CONTACT SENSITIVITY, Stephen D. Miller, John W. Moorhead, Man-Sun Sy, and Henry N. Claman, Univ. of Colo. Med. Ctr., Denver, Colo. 80262.

Hapten-specific tolerance to DNFB contact sensitivity can be induced in mice by the injection of either the reactive hapten $DNBSO_3$ or in vitro hapten-modified lymphoid cells (DNP-LC). Such specifically unresponsive mice are termed "phenotypically tolerant." Tolerance can be transferred from these animals to normal mice with T cells, so at least part of the phenotypic tolerance is mediated via suppressor T cells (Ts). We examined possible genetic restrictions for both the induction and expression of Ts. Balb/c mice were tolerized on day -7 with either syngeneic or allogeneic DNP-LC. On day 0 these mice were tested for tolerance to DNFB (phenotypic tolerance) as well as for their ability to transfer tolerance to normal recipients (induction of Ts). It was found that both syngeneic and allogeneic DNP-LC induced phenotypic tolerance. However, induction of Ts required H-2 compatibility between the DNP-LC and the donor mice. A similar H-2 restriction was shown in CBA mice in that Ts were induced with CBA DNP-LC but not with Balb/c DNP-LC. In contrast to Ts induction, the expression of Ts activity was not H-2 restricted. It was found that Ts induced by the hapten $DNBSO_3$ in one strain of mouse could transfer tolerance to other strains which were H-2 incompatible. The data suggest that the induction of Ts requires recognition of the hapten on H-2 compatible membranes; on the contrary, the function of these cells after induction does not appear to be genetically restricted.

935 STUDIES ON THE SPECIFICITY OF CYTOLYTIC T LYMPHOCYTE CYTOLYSIS OF TNP-DERIVATIZED SYNGENEIC AND ALLOGENEIC TARGETS. Steven Burakoff, Paul Billings, Francois Lemonnier, Ronald Germain and Baruj Benacerraf. Department of Pathology, Harvard Medical School, Boston, Massachusetts 02115.

1) Normal spleen cells, when cultured with irradiated trinitrophenyl (TNP)-derivatized syngeneic spleen cells, develop cytolytic T lymphocytes (CTL) that lyse most effectively a TNP-derivatized target that is H-2 compatible with the effector. However, these CTL also lyse to a lesser extent TNP tumor and TNP spleen targets that are H-2 incompatible. This cross-reactive lysis (CRL) can be inhibited by antisera to the K and/or D loci of the target cell. Non-radiolabeled TNP-derivatized lymphoid cells syngeneic to either the stimulator or the target are able competitively to inhibit CRL. CTL from mice bearing the $H\text{-}2^b$, $H\text{-}2^d$, $H\text{-}2^s$ and $H\text{-}2^r$ haplotypes have been shown to demonstrate CRL of TNP-derivatized allogeneic targets, including TNP derivatized $H\text{-}2^k$ targets. However, CTL from $H\text{-}2^k$ strains demonstrate minimal, if any CRL in this system. Preliminary data, with the use of recombinants, indicate that strains bearing the k alleles in the K, IA, IB region do not demonstrate CRL of TNP derivatized allogeneic targets.

2) We have confirmed and extended the observation that C57BL/6 ($H\text{-}2^b$) CTL stimulated by DBA/2 ($H\text{-}2^d$) allogeneic spleen cells are able to lyse TNP derivatized tumor (EL4) or spleen cells syngeneic to the effector. Inhibition of cytolysis with non-radiolabeled targets indicates that the cytolysis of TNP-syngeneic targets by B6 anti-DBA/2 CTL is specific and suggests that TNP derivatization of the gene products of the $H\text{-}2^b$ haplotype may create new antigenic determinants which are similar to native determinants coded for by the $H\text{-}2^d$ complex.

936 SPECIFICITY OF CML AND MLR CLONES RESPONDING TO CHEMICALLY MODIFIED SYNGENEIC AND ALLOGENEIC CELLS, Anne-Marie Schmitt-Verhulst and Gene M. Shearer, Immunology Branch, National Cancer Institute, Bethesda, MD 20014

Secondary cell-mediated lympholysis (CML) and mixed lymphocyte reactions (MLR) were generated in vitro against trinitrophenyl (TNP)-modified murine syngeneic spleen cells. H-2 homology at H-2K and/or H-2D was required between the primary and secondary TNP-modified stimulating cells in order to restimulate in the secondary CML. Strong proliferative responses were detected only in the secondary cultures, and when there was H-2 homology at I or D, or K and/or I-A between the primary and secondary immunogens. Results of experiments designed to test the specificity of the T-effector cells generated against TNP-modified autologous cells are compatible with a model involving recognition of the modifying agent in association with H-2 coded structures by a single receptor on the cytotoxic lymphocyte (see Shearer, et al., symposium of this conference). An independent approach for analysis of the requirement for H-2-associated recognition consisted of eliminating clones of lymphocytes reactive against allogeneic cells by treatment with bromodeoxyuridine and light prior to their restimulation. It was found that a population of lymphocytes depleted of precursors of CML-reactive cells against alloantigens was still able to generate CML-reactive cells against TNP-modified cells syngeneic to the responding cells, but not against TNP-modified cells expressing the haplotype of the initial allogeneic cells. This result could be explained (a) if clones specific for altered alloantigens do not exist; or (b) by the elimination of those clones by depletion of the allogeneic reactive cells.

937 CLONOTYPE EXPRESSION VERSUS ANTIGENIC FORM IN THE IMMUNE RESPONSE TO PHOSPHORYLCHOLINE, Katie Williams and Latham Claflin, Dept. Microbiology, University of Michigan, Ann Arbor, MI 48109.

Previous studies on the immune response to phosphorylcholine (PC) have shown that; 1) Clones selected during the response to PC comprise a limited set, ie. each strain produces anti-PC antibodies having idiotypic and structural features equivalent to those of the BALB/c PC-binding myeloma proteins T15, M511 and 'M603', but not to M167 or W3207. 2) The immune response to PC is conserved; a similar set of clones exists in each strain. This analysis was conducted on IgM antibody using the "T-independent", PC-containing bacteria S. pneumoniae str. R36A. Recently we have observed similar phenomena with a T-dependent antigen PC-KLH and with the bacteria Proteus morganii (mouse isolate) which stimulate IgG in addition to IgM anti-PC antibodies. R36A and PC-KLH, whose PC-antigens are different, bind to each myeloma protein about equally well, whereas P. morganii reacts preferentially with M603. By quantitative idiotypic measurements and analytical light chain isoelectric focusing analysis BALB/c and A/J produce anti-PC antibodies to PC-KLH and P. morganii which are similar to T15, M511, and 'M603'. No antibody with features of M167 and W3207 are detected. The T15-clonotype is preferentially expressed after initial stimulation with PC-KLH, but M511 and 'M603' clonotypes appear after a second injection. The 'M603' clonotype predominates in the response to P. morganii. With all 3 immunogens, especially P. morganii, no antibody idiotypically identical with M603 is detected. An antibody, 'M603', appears whose light chain only is similar to M603. Thus with 3 different PC-containing antigens a similar set of clonotypes is expressed suggesting genetic conservation of information coding for these antibodies.

938 THE IMPACT OF ANTIGENIC CROSS-REACTIVITY ON CLONOTYPE EXPRESSION. Dietmar G. Braun, Basel Institute for Immunology, Basel, Switzerland.

In the course of studying the immune response patterns of BALB/c mice to the streptococcal group A polysaccharide it was found very difficult to predict the kind of isoelectric focusing patterns single mice would express. This difficulty is not similarly prevalent in A/J, C57BL/6 or (C57BL/6xBALB/c)F1 mice if immunized with group A vaccines. Here predictions of the clonotype patterns that will be expressed can be made. Furthermore, immunization of Bailey recombinant inbred mice enabled to trace clonotypes which are products of the Ig-$1^a$ locus. This data suggests that BALB/c mice also possess these clonotypes but are somehow blocked to express them at similar frequencies. In this context reference will be given to the finding that antigenic cross-stimulation by related but distinct antigens has been shown in several systems to deeply influence any subsequent clonotype expression in anamnestic responses by a previously established clonal hierarchy. In this system the affinity of the antibody expressed appears to be only of marginal importance. What is more important is the number of precursor cells available of the cross-reactive clonotypes.

939 CHANGES IN THE IDIOTYPIC PATTERN OF AN IMMUNE RESPONSE, FOLLOWING SYNGENEIC HAEMOPOIETIC RECONSTITUTION OF LETHALLY IRRADIATED MICE, A.A. Augustin, M.H. Julius and H. Cosenza, Basel Institute for Immunology, Postfach 4005 Basel 5, Switzerland.

The level of antibody obtained to a given epitope in lethally irradiated reconstituted mice is similar to that of normal mice. However, the size of an immune response does not reflect which individual clones are expressed following antigenic stimulation. In order to analyze selective clonal expression, we followed the "idiotypic pattern" of an antibody response in reconstituted mice. Upon immunization of normal Balb/c mice with phosphorylcholine (PC), 95% of the anti-PC antibodies produced share an idiotypic marker ($T15^+$) with TEPC15 (T15) myeloma protein of Balb/c origin. When irradiated Balb/c mice recontituted with either foetal liver or bone marrow (BM) regain responsiveness to PC (at 12-15 weeks after reconstitution), the anti-PC response predominantly consists of antibodies lacking the T15 idiotype ($T15^-$). However, reconstitution of lethally irradiated mice with syngeneic spleen cells results in an idiotypic pattern similar to that of normal Balb/c mice. The same pattern is also obtained in mice reconstituted with a mixture of BM and normal spleen cells. This change of idiotypic pattern was further investigated in experiments testing: (a) possible regulatory interactions between idiotypes and anti-idiotypic antibodies and (b) differential distribution of PC-specific clones in lymphopoietic and mature compartments of the immune system.

940 CLONAL DOMINANCE IS INDEPENDENT OF ANTIBODY AFFINITY

W.K. Schalch and D.G. Braun, Basel Institute for Immunology.

It has been established in the past that the average affinity of antibodies increases with time after administration of antigen. Contrary to this is the finding that maturation of the immune response to the streptococcal group A polysaccharide in mice is neither observed at the IgM nor at the IgG level. Rabbit antibodies to the streptococcal group A-variant polysaccharide (Av-CHO) often belong to the low affinity class of antibodies. This fact even pertains to antibodies that dominate the response. We therefore reinvestigated this problem. Rabbits were hyperimmunized i.v. with streptococcal group A-variant vaccines, and only those antisera were taken for analysis which contained at least one dominant clonotype. Purification of the clonotypes was achieved by preparative agarose block electrophoresis and/or chromatography on affinity columns with covalently linked Av-CHO. For example, antiserum K 116-700 contained among 8-10 expressed Av-CHO specific spectrotypes one dominating clonotype. Even though this clonotype was of low affinity its concentration in the antiserum was about ten times that of the high affinity spectrotypes. Therefore, contrary to systems where average affinities were measured, individual clonotypes do not necessarily follow the presumed rule of expansion determined by antigen affinity. Independent data have focused attention to the role which the number of antigen-sensitive precursor cells play in clonal dominance. Taken together it appears that antigen affinity of receptors on precursor cells is predetermined and not regulated by antigen.

941 PERSISTENCE OF IDIOTYPES RELATED TO PARTICULAR CROSS-REACTIVE ANTI-PNEUMOCOCCAL ANTIBODIES IN RABBITS DURING IMMUNIZATION

Yeh-Zen Huang, Donald Kennerly and A. M. Pappenheimer Jr., Biological Laboratories, Harvard University, Cambridge, MA 02138

Two rabbits were immunized over a period of 6-10 months with Type III and Type VIII pneumococcal vaccines and produced maxima of 70 mg/ml and 45 mg/ml, respectively, of precipitable antibodies against the homologous polysaccharide. Both antisera showed unusual cross-reactivities with heterologous polysaccharides; the anti-S3 serum with S8 and the anti-S8 serum with Klebsiella K44 capsular polysaccharide. In each case the cross-reaction was related to a particular idiotype and persisted throughout almost the entire course of immunization. The cross-reacting antibodies were isolated by ion-exchange chromatography and used to elicit anti-idiotypic antibodies in other rabbits of the same allotype. Radioimmunoassay for the anti-S3,8 and anti-S8,K44 idiotypes showed that the idiotype, like the cross-reaction, persisted during the course of immunization despite changes in charge and repeated replacement of new components with increasing affinity. However, the anti-idiotypic antibodies had the greatest affinity for Fab fragments prepared from the cross-reactive antibody used as antigen and were lower for Fab fragments prepared from earlier as well as later bleedings from the same rabbit. After absorption of the anti-S8 serum with K44 polysaccharide, more than 80% of the idiotype was removed. Most of the S8-precipitable anti-S8 still remained in the supernate after K44 absorption, but was of a different idiotype. Both cases suggest that idiotypes are closely related to the particular cross-reaction of anti-pneumococcal antibodies.

942 REGULATION OF IgE ANTIBODY SYNTHESIS BY AUTO-ANTIIDIOTYPE IMMUNIZATION IN GUINEA PIGS

Alain L. de Weck, Andrew F. Geczy and Olga Toffler, Inst. Clin. Immunology, Univ. Bern, Switzerland.

We have formerly demonstrated (J.Exp.Med. 144, 226, 1976) that syngeneic immunization in strain 2 and 13 guinea pigs using purified antibodies against chemically defined antigens (e.g. immunization of strain 13 animals with immunosorbent purified anti-penicilloyl-bovine gamma globulin (BPO-BGG) antibody from strain 13) produces anti-idiotype antibodies specifically preventing antigen-induced proliferation of immune T lymphocytes in vitro. In further experiments, it has now been found that passive and active immunization in vivo with anti-idiotype antibodies specifically blocks IgE synthesis of guinea pigs immunized in such a way (low doses of antigen in $Al(OH)_3$) as to produce IgE antibodies against BPO-BGG. Various ways to achieve long lasting suppression of IgE biosynthesis by immunization with idiotypes are currently investigated. Parallel studies are performed in outbred animals passively or actively immunized with their own antibodies. Aside from the demonstration that IgE shares idiotypes with other Igs and with T cell receptors, these studies suggest new approaches in the management of allergic diseases.

943 INTERACTION OF *H-2* AND NON *H-2* GENES IN PRODUCTION OF MIXED LYMPHOCYTE REACTION SUPPRESSOR FACTOR. S.Solliday Rich, F.M.Orson, and R.R.Rich,Baylor College of Medicine, and The Methodist Hospital, Houston, TX 77030.

MLR suppressor factor, derived from alloantigen-primed splenic T cells and the suppressor factor receptor are gene products of the murine major histocompatibility *(H-2)* complex. Suppression requires *I-C* subregion identity between suppressor and MLR responder cells. Non-*H-2* background genes previously appeared irrelevant. However, as an exception we have observed that factors derived from alloantigen-activated C57BL/6(B6)mice failed to suppress B6, C57BL/10 (B10)$(H\text{-}2^b)$ or (BALB/c x C57BL/6)$F_1$ responses. Other $H\text{-}2^b$ strains(B10 and A.BY)both produced suppressor T cell factors and were suppressed by active $H\text{-}2^b$ factors. While B6 could not produce suppressor factor, B6 MLR responses were suppressed by B10 and A.BY factors. Thus, the defect does not appear to be *H-2* linked and does not affect expression of factor receptor on responding cells. The factor obtained from a [suppressor(BALB/c) x nonsuppressor(B6)]$F_1$ hybrid suppressed responses of $F_1$ cells as well as those of both parental strains. Therefore production of suppressor factor is genetically dominant; it may be postulated that gene(s) provided by BALB/c non *H-2* background allow codominant expression of B6 and BALB/c reactive molecules in $F_1$ suppressor cells. To confirm the role of B6 non *H-2* gene background in faulty suppressor factor production the strain B6.C-$H\text{-}2^d$, congeneic to B6 but with $H\text{-}2^d$ haplotype, was tested. B6.$H\text{-}2^d$ factor failed to suppress responses of syngeneic and $H\text{-}2^d$ compatible strains, as well as responses of B6 cells. The data indicate that expression of MLR suppressor genes is controlled by separate regulatory genes not linked to the *H-2* gene complex. (Supported by USPHS N01-AI-42529, HL-17269 and NASA NAS9-14368).

944 IMMUNOCHEMICAL PROPERTIES OF THE GAT IMMUNOSUPPRESSIVE FACTOR(S) EXTRACTED FROM LYMPHOID CELLS OF NONRESPONDER MICE PRIMED WITH L-GLUTAMIC ACID$^{60}$-L-ALANINE$^{30}$-L-TYROSINE$^{10}$. Jacques Theze, Judith A. Kapp and Baruj Benacerraf, Harvard Medical School, Boston, MA 02115.

The GAT-specific suppressor T cell factor (GAT-$T_S$F) extracted from lymphoid cells from GAT-primed, nonresponder DBA/1 mice has been partially characterized. It is a protein that has affinity for GAT and determinants encoded by the I-region of the H-2 complex.
On the basis of specificity and avidity. GAT-$T_S$F resembles anti-GAT-MBSA antibodies produced by DBA/1 mice in spite of the fact that it is too small to be classical antibody and has no constant-region determinants of heavy or light chain. Further, GAT or a fragment of GAT is associated with the GAT-$T_S$F.
GAT-$T_S$F has been partially purified from the crude extract by absorption to GAT-Sepharose and elution with 0.4 to 0.6 KCl. GAT-$T_S$F purified on the basis of its affinity for GAT bears I-region determinants but not detectable GAT or GAT-fragment.

945 CHARACTERIZATION OF AN Ia-POSITIVE ALLOGENEIC EFFECT FACTOR, Terry L. Delovitch, Banting and Best Department of Medical Research, University of Toronto, Toronto, Canada M5G 1L6.

An allogeneic effect factor (AEF) was produced from a mixed lymphocyte culture of thymocyte derived, Ia "negative", activated responder cells and H-2 congenic, T cell depleted, irradiated stimulator cells. It reconstituted the helper cell function of hapten (DNP)- primed T cell depleted B cells of stimulator but not responder origin. Ia antigens derived only from the stimulator haplotype and not from the responder haplotype are active components of this genetically "restricted" AEF. "Restricted" AEF does not contain H-2 and immunoglobulin determinants; it's activity is not removed on an antigen ($DNP_6$ - KLH) - coated column. While the source of activity of "restricted" AEF has not been precisely mapped in the H-2 complex, it has been demonstrated that genes in the K ------ I-J segment may be involved. Since "restricted" AEF may be a product of the stimulator B cell (and/or macrophage), the data obtained suggest that the recognition by allogeneic T cells of Ia antigens on B cells activates the B cell to IgG antibody production.

946 MECHANISM OF ACTION OF A SOLUBLE IMMUNE RESPONSE SUPPRESSOR (SIRS) PRODUCED BY CONCANAVALIN A-ACTIVATED T CELLS. Carl W. Pierce and Takushi Tadakuma, Department of Pathology, The Jewish Hospital of St. Louis, Washington University School of Medicine, St. Louis, MO. 63110.

Concanavalin A-activated murine Ly $2^+$, $3^+$ T cells release a factor(s) which non-specifically suppresses plaque-forming cell(PFC) responses by murine spleen cells in vitro. SIRS has been characterized as a glycoprotein with a molecular weight in the range between 48,000 and 67,000 whose suppressive activity has not been dissociated from MIF activity also present in the culture fluids. SIRS lacks strain and antigen specificity and Ig and H-2 gene product determinants. A 2 hr exposure of spleen cells to SIRS is sufficient to suppress PFC responses and SIRS acts on macrophages (Mϕ), but not responding T or B cells. SIRS does not interfere with Mϕ functions essential for initiation of PFC responses since responses develop normally for the first 72 hrs and then prematurely decrease. SIRS-treated Mϕ suppress responses by an active process involving, in part, release of a soluble factor(s) which is effective across a millipore filter. Cell proliferation in cultures with SIRS-treated Mϕ decreases 24 hrs before the decrease in the PFC responses at 96 hrs. These data suggest that SIRS activates Mϕ to release a soluble inhibitor of cell proliferation which acts on B cells to produce the observed delayed suppression of PFC responses. (Supported by USPHS Grant AI-13915 and Grant 1040 from the Council for the Tobacco Research, U.S.A., Inc.)

947 IMMUNOSUPPRESSIVE FACTORS IN THE SERUM OF TUMOR BEARING MICE. Julia G. Levy, R.B. Whitney, and R. McMaster, Department of Microbiology, University of British Columbia, Vancouver, B.C. Canada. Mice with progressively growing tumors have in their serum immunosuppressive factor(s) which inhibit the immunological function of normal syngeneic or allogeneic lymphoid cells in vitro. The suppressive effect appears to be directed toward T-cell function in either the generation of helper cells in vitro in the development of anti-SRBC plaques, or in the generation of cytotoxic T cells in vitro to either allogeneic or syngeneic tumor cells. The inhibitory substance is effective only during the first 48 hours of in vitro incubation of lymphoid cells implying that it functions by blocking the differentiation or proliferation events involved in the generation of either killer or helper cells. Studies on the characterization of this factor indicate that it has a molecular weight of approximately 150,000, that it can be removed by passage through immunoadsorbent columns specific for mouse γ, μ or K chains, and that it is stable in terms of suppressive activity and molecular weight after gel filtration on Sephadex G-150 at pH 2.5. Attempts to determine whether the suppressive molecule is a low molecular weight component associated with immunoglobulin molecules have yielded equivocal results. We have shown that a comparable immunosuppressive component is generated in the serum of mice undergoing chronic graft vs host reactions, and argue that the factor may be present in normal animals at all times but that in animals with prolonged chronic antigenic stimulus, quantitative differences in the levels of the component may appear in the serum.

948 THE NATURE OF IMMUNOSUPPRESSOR T CELLS AND THEIR FACTORS IN THE TUMOR-BEARING HOST (TBH). Mark I. Greene, S. Fujimoto, A. H. Sehon, Harvard Medical School and the Department of Immunology, University of Manitoba, Canada.

Immunosuppressor T cells (IST) and their factor(s) capable of modulating the immune response to a methylchloranthrene-induced fibrosarcoma (S1509a) in A/J ($H\text{-}2^a$) mice have been studied. Suppressor T cells have been isolated from various lymphoid cells of A/J mice bearing the S1509a. These IST are able, when adoptively transferred to A/J mice which have been rendered immune to the S1509a tumor, to cause significant suppression of that tumor immune rejection. These IST carry or produce antigen-specific immunosuppressive factors (ISF) with similar biological activity. ISF have been chemically defined as non-immunoglobulin, antigen-specific factors, which carry antigenic determinants of the K-I end of the major histocompatibility comples (MHC) of the $H\text{-}2^a$ haplotype. Further, these factors can be absorbed out with other lymphoid cells. The IST and their specific factors (ISF) may be important in the suppression of the immune cytolytic effector response of TBH.

949 SOLUBLE ANTIGEN SPECIFIC SUPPRESSIVE FACTOR IN TOLERANCE TO HGG, Gerard Chaquat, Inserm, Paris, France

Spleen cells from high and low zone tolerant mice cultured 8 to 16 hours in presence of tolerogenic HGG release a factor which can replace suppressors cells in vivo. This product is antigen specific, and can be absorbed by anti H2 or anti I sera, but not by various anti immunoglobulins. Furthermore, its molecular weight estimated by both amicon ultrafiltration and sephadex gel chromatography ranges between 55000 45000 daltons.
Target cell of the factor was determined by two step cell transfer into irradiated recipients and found to be a T cell itself.

950 CROSS-IDIOTYPIC SPECIFICITY AMONG IMMUNOGLOBULINS IN SUB-ACUTE SCLEROSING PANENCEPHALITIS AND MULTIPLE SCLEROSIS; A.D.Strosberg, B.Marescau, K.Thielemans, D.Kunkel and A.Lowenthal. Free University of Brussels Medical School and Born-Bunge Institute Antwerp, Belgium.

The existence of cross-idiotypic determinants among human oligoclonal Subacute Sclerosing Panencephalitis (SSPE) immunoglobulins from several patients was shown by the use of rabbit anti-idiotypic antisera. Identity between SSPE cerebrospinal fluid immunoglobulins and SSPE serum proteins was also demonstrated. Sharing of idiotypy was also observed in sera from patients with Multiple Sclerosis (MS), another disease of the central nervous system. No reaction was observed with sera from other neurological diseases. Appropriate controls excluded artefacts due to a prevalent subolass of immunoglobulins in SSPE or MS.

Our results confirm previous reported patterns of cross-specificity observed for other groups of human monoclonal proteins such as the IgM cold agglutinins or anti-$\gamma$-globulin antibodies (Kunkel et al. 1973. J. Exp. Med. 137: 331), and support the hypothesis of a close involvement of the antigen combining site in the idiotypic specificity.

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951 GENETIC LINKAGE OF THE A/J ANTI-NUCLEASE IDIOTYPE. David S. Pisetsky and David H. Sachs. Immunology Branch, National Cancer Institute, NIH, Bethesda, MD 20014

Rat antisera detecting site-specific idiotypic determinants on anti-nuclease antibodies from A/J mice have been used to define a variable region marker (A/J anti-nuclease idiotype) and to investigate its linkage relationships. Although nuclease is an antigen under H-2-linked Ir gene control, no influence of the H-2 locus on A/J idiotype expression was found. The A/J idiotype was present in immune sera from strains A/J ($H\text{-}2^a$) and A·BY ($H\text{-}2^b$) but absent from sera from strains B10·A ($H\text{-}2^a$) and B10 ($H\text{-}2^b$). Analysis of the segregation of the A/J idiotype in progeny of the backcross (B10·A x A/J) x B10·A showed linkage of the idiotype to the $Ig\text{-}1^e$ heavy chain allotype locus. There was, however, a very high apparent frequency of recombination of approximately 7%. In addition, all recombinants were accounted for by $Ig\text{-}1^b/Ig\text{-}1^b$ homozygotes which bore the A/J idiotype, and none by $Ig\text{-}1^b/Ig\text{-}1^e$ heterozygotes lacking the idiotype. Preliminary results of progeny testing of these animals indicate that the recombinants breed true. Possible mechanisms to account for these results are being assessed.

952 EVIDENCE FOR SHARED IDIOTYPY ON T AND B CELL RECEPTORS, Clifford J. Bellone and Charles A. Prange, St. Louis University School of Medicine, St. Louis, MO 63104

Shared idiotypy between B and T cell receptors specific for the antigen L-tyrosine-p-azophenyltrimethylammonium (tyr(TMA)) was studied in an antigen binding assay using idiotypic antisera. These idiotypic reagents were prepared by inoculation of rabbits with purified anti-tyr(TMA) antibody raised in strain 13 guinea pigs. The antisera blocked 75-81% of the antigen binding T cells (T-ABC) and 53-58% of the antigen binding B cells (B-ABC) from tyr(TMA) immune strain 13 lymph node cells (LNC). An excess of free strain 13 Ig in the ABC assay did not affect the ability of the idiotypic antisera to block T- and B-ABC. Nylon wool passed tyr(TMA) immune LNC were trypsin treated resulting in a 75% loss of T-ABC. The trypsin treated population was then cultured for 16 hours which resulted in a return of T-ABC to 92% of pre-trypsin values. Seventy-eight to 80% of these regenerated T-ABC could be blocked with idiotypic antisera. Specificity of the idiotypic antisera was tested in L-tyrosine-p-azobenzenearsonate (RAT) immune guinea pig LNC. Neither T- nor B-ABC were blocked in this heterologous system. Further blocking experiments were performed to characterize the nature of the T-ABC receptor. A variety of anti-Ig reagents, which block B-ABC, do not inhibit T-ABC. However, preliminary data indicate that a 2 anti-13 serum inhibits the T-ABC population but not the B-ABC. These data indicate that T cells synthesize receptors which share idiotypic determinants with anti-tyr(TMA) antibodies. The nature of the receptor on which the idiotypic determinants are found is open to question. Our data suggest that idiotypic determinants found on Ig-V regions may be linked to a histocompatibility gene product on T cells.

954 EXPRESSION OF A CROSS REACTIVE IDIOTYPE ON THE IgG2C SUBCLASS OF RAT ANTI-SACHO ANTIBODY, Gerrie A. Leslie, Dept. of Microbiology & Immunology, Univ. Oregon Health Sciences Center, Portland, OR 97201.

Eight inbred rat strains when hyperimmunized with Group A Streptococcal vaccine (GASV) produced only low levels (0-1.7 mg/ml) of precipitating anti-streptococcal carbohydrate antibody (anti-SACHO). In contrast, partially inbred Sprague-Dawley-derived rats (HPR) produced in our laboratory produce 5-36 mg/ml precipitating anti-SACHO. A high percentage of immunized animals possessed a cross-reactive idiotype, Id-1. No correlation was found between Ag-B haplotype and the expression of Id-1 nor the magnitude of the anti-SACHO response. Preliminary F1 and backcross data suggest dominance of the high-responsiveness.

Specifically purified precipitating IgG anti-SACHO from 6 inbred strains and HPR were almost totally of the minor IgG2c subclass. Of 16 animals examined the total serum IgG2c increased up to 100 times over that found in preimmune serum e.g. 0.24 mg/ml pre to 25.0 mg/ml post-immunization. In contrast the total IgG2a and IgG2b were rarely significantly changed. These data suggest that Id-1 is preferentially expressed on IgG2c anti-SACHO.

Studies are in progress to assess the effect of anti rat IgM (μ) and IgD (δ) class and anti-IgG2c subclass suppression on anti-SACHO idiotype and immunoglobulin expression. Initial studies have shown that neonatal administration of anti-μ suppresses IgM, IgD, IgA and IgG synthesis.

955 CHAIN SPECIFIC IDIOTYPES IN STUDIES ON THE INHERITANCE OF RABBIT ANTIBODIES, T.J. Kindt, J.A. Sogn, and M. Yarmush, The Rockefeller University, New York, NY 10021.

Idiotypic antisera (anti Id) in the rabbit normally recognize only a specific H-L combination. While idiotype inheritance has provided unique immunogenetic information, the utility of such studies is limited because genes at two loci are required for expression of a given idiotype. This reduces the likelihood of observing inheritance and complicates its interpretation. The requirement for genes at two loci is eliminated by the use of anti Id specific for H (anti HId) or L (anti LId) chain idiotypic determinants. Such reagents have been used to screen for the presence of antibodies with idiotypic similarities. Use of an anti LId preparation specifically isolated from an idiotypic antiserum specific for 4539 Ab revealed that 56% of related rabbits produced crossreactive antistreptococcal antibodies, while no crossreactions were found in nonrelated rabbits subjected to similar immunization protocols. An anti HId specific for the H chain of 4135 Ab has been prepared and used to show crossreactivity in 45% of immunized related rabbits. Furthermore, the HId was shown to be linked to the a3 allotype present in a specific allogroup (J). Antiserum from all but one of the related rabbits with the J allogroup inhibited the binding of 4135 Ab to the anti HId reagent. One rabbit lacking the a3 allotype produced significant amounts of HID-reactive antibodies. Upon isolation, these antibodies were shown to express latent a3 as well as the allotype d11, which is characteristic of the J allogroup. Such linked expression of latent allotypes and idiotype suggest the involvement of complex regulatory mechanisms governing the occurrence of nominal and latent allotypes.

**956** ISOLATION OF FUNCTIONALLY HOMOGENEOUS IDIOTYPIC ANTIBODIES. J.A. Sogn, M. Mudgett, J.E. Coligan, and T.J. Kindt. The Rockefeller University, New York, N.Y. 10021

Precise genetic information can be obtained by studies on idiotype (Id) inheritance using idiotypic antibodies (anti-Id) with specificity for limited and defined portions of an antibody V region. Antisera specific for Id determinants limited to a single antibody chain have been prepared by immunization of rabbits with appropriate hybrid immunoglobulin molecules. These antisera have been useful in genetic studies, and the finding that such anti-Id contain a limited number of specific antibody components has made it possible to obtain even more restricted antisera using three different approaches. First, preparative isoelectric focusing in Sephadex gel of anti-Id antibodies isolated by immunoadsorbent techniques has allowed the isolation of several monoclonal antibodies from a single antiserum. Second, inheritance studies using chain-specific anti-Id antisera have identified a large number of sera cross-reactive with the proband antibody in a homologous inhibition of binding radioimmunoassay. Studies on the ability of mixtures of sera to inhibit idiotypic binding have revealed that a small number of independently inherited determinants are recognized by the anti-Id antiserum. Absorption of the antiserum with appropriate crossreactive sera can yield an anti-Id antiserum specific for a single determinant. Third, antisera have been fractionated on the basis of differential hapten inhibitability by absorbing specific anti-Id antibodies to an immunoadsorbent column containing the proband antibody and eluting first with hapten reactive with the proband antibody and subsequently with a nonspecific chaotropic agent. The fractions obtained differ electrophoretically and appear to recognize two distinct classes of idiotypic determinants.

**957** STRUCTURAL ANALYSIS OF THE BINDING SITE REPERTOIRE OF RABBIT ANTIBODIES SPECIFIC FOR PNEUMOCOCCAL POLYSACCHARIDES, L. E. Cannon, M.N. Margolies and E. Haber, Mass. Gen. Hosp. and Harvard Medical School, Boston, MA 02114

The primary structures of the variable (V) domains ($V_H$ and $V_L$) of three homogeneous antipneumococcal type III polysaccharide (S3) antibodies have been determined. These antibodies were elicited in outbred rabbits and selected for study solely on the basis of specificity for the S3 antigen. The antigen complementarity-determining regions of these and six other anti-S3 antibody light (L) chains demonstrate marked differences in both amino acid sequence and length. Though less marked, sequence differences are also found among these $V_L$ framework structures. Diversity among complementarity-determining regions is also found for the heavy (H) chains of anti-S3 antibodies, including differences in length at positions 95 to 110. In contrast to $V_L$, the $V_H$ framework is highly conserved for a given group a allotype. Analysis of these $V_L$ and $V_H$ structures indicates: (1) a diverse spectrum of B cell products with specificity for the same antigen, (2) a greater diversity of framework structures for $V_L$ than for $V_H$, suggesting a larger number of structural genes for Kappa $V_L$ than for $V_H$, and (3) diversification of antigen complementarity-determining regions greatly exceeds that of the V region framework with respect to amino acid substitutions, deletions or insertions, and the frequency of two and three nucleotide base change events. These findings are most easily accommodated by a somatic mechanism for the generation of V region diversity.

## 809 REGULATION OF IDIOTYPES EXPRESSED ON RECEPTORS OF PHOSPHORYLCHOLINE-SPECIFIC B AND T LYMPHOCYTES

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Immunoglobulin (Ig) receptors on phosphorycholine (PC)-specific B cells bear predominantly the idiotype characteristic of the Balb/c PC-binding myeloma protein TEPC 15(T15), i.e. antibodies directed to the T15 idiotype specifically inhibit induction of an anti-PC response (1). To determine whether this "idiotypic homogeneity" is also characteristic of receptors on PC-specific T cells, we developed a functional assay for PC-specific helper cells. Balb/c mice were immunized with PC coupled onto isologous myeloma proteins. Splenic T cells were transferred along with BSA-primed B cells into irradiated syngeneic recipients which were then challenged with PC-BSA. Our results indicate that PC-specific helper T cells collaborate with BSA-specific B cells in the induction of an anti-BSA PFC response. Their helper activity was completely inhibited when the irradiated recipients were injected with anti-T15 antibodies prior to cell transfer. Furthermore, we have been able to prime PC-specific T cells by injecting Balb/c mice with low doses of anti-T15 antibodies. Therefore, in parallel with their B cell counterparts, receptors on T cells bear predominantly the T15 idiotype.

Injection of Balb/c mice with anti-T15 antibodies at birth changes the idiotypic pattern of the anti-PC antibodies from T15-positive ($T15^+$) to T15-negative ($T15^-$), i.e. at the B cell level, the majority of PC-specific precursors bear Ig receptors which express $T15^-$ idiotypes (2). If neonatal suppression of the T15 idiotype also induces a parallel change in the idiotypic pattern of receptors expressed on PC-specific T cells, the helper activity of PC-primed T cells from $T15^-$ mice should not be inhibited by anti-T15 antibodies. To this end, we transferred PC-specific T cells from $T15^-$ mice along with BSA-primed B cells from $T15^+$ mice into $T15^+$ irradiated recipients which were challenged with PC-BSA. The anti-BSA PFC response obtained was not inhibited by pre-treatment of the irradiated recipients with anti-T15 antibodies. Thus, PC-specific T cells from neonatally suppressed mice bear $T15^-$ receptors. This parallel behaviour of PC-specific B and T cells from normal and neonatally suppressed Balb/c mice suggests that both lymphocyte lineages are governed by similar regulatory mechanisms. These results shall be considered along with evidence indicating that autologous anti-idiotypic antibodies can regulate the idiotypic pattern of the response to PC.

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## 810 IDIOTYPIC ANALYSIS OF T CELL RECEPTOR VARIABLE REGIONS.

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T cells interact with antigen using thus for unidentified receptor molecules which do not appear to possess classical immunoglobulin structure (1). By a variety of techniques it can be shown that T cell receptors interact with anti-idiotypic antibodies prepared against isolated serum antibodies, suggesting that certain parts of the variable regions are shared between T cell receptors and serum antibodies: Experiments exploiting the genetic polymorphism of idiotypes (2) and immunochemical experiments utilizing anti-idiotypic antisera with preferential reactivity towards either the $V_H$ or the $V_L$ region, have suggested that the structural basis for the idiotypic similarity between T and B cells is the $V_H$ region.

The present experiments represent a continuation of these studies, using anti-idiotypic antisera prepared against antibody molecules artificially constructed from the H and L chains, respectively, from a monoclonal antibody(A5A) with specificity for Group A streptococcal carbohydrate and from pooled mouse immunoglobulin. These antisera exclusively react with the V region of the polypeptide chain derived from the A5A antibody, and are therefore entirely specific for either its $V_H$ or its $V_L$ region. These antisera are referred to as anti-HId and anti-LId, respectively. The analysis of such antisera is based on the previously described induction and inhibition of helper activity using Group A streptococci as carrier to which a hapten has been coupled (3,4). Induction of helper activity was achieved with either of two different anti-HId antibodies whereas one of two anti-LId antibodies gave no and the other marginal helper induction. Similarly, complete inhibition of helper activity in vitro was achieved with both anti-HId antibodies, whereas both anti-LId antisera caused slight inhibition also seen with normal control serum. Taken together, the data strongly support the concept that the immunoglobulin $V_H$ region is part of the T cell receptor whereas $V_L$ regions do not appear to be present on T cells.

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958 ROLE OF MEMBRANE LIPID COMPOSITION AND FLUIDITY IN IMMUNE REACTIVITY. Stanley Shimizu, Gail Mandel, Chris Puchalski, Ronald Gill and William Clark. Department of Biology and the Molecular Biology Institute, UCLA, Los Angeles California 90024.

A number of investigators using a variety of approaches have suggested that alterations in plasma membrane lipid composition and fluidity are an early consequence of lymphocyte activation. However, the role of such alterations in either the generation or expression of immune function has been difficult to define. Recently, we have successfully adapted techniques originally developed for fibroblasts, to manipulate in a controlled way the fatty acid composition (determined by GC analysis) and the fluidity(determined by ESR) of lymphoid cell plasma membranes.Under appropriate conditions we are able to achieve extensive substitution of fatty acids of controlled chain length and saturation into the membrane phosphatides of both restinglymphocytes, and of various lymphoid tumor cells used as targets in immune assays. These alterations have profound and interesting effects on immune reactivities. Decreasing the membrane fluidity of EL-4 target cell membranes decreases their susceptibility to lysis by cytotoxic effector cells. This is accompanied by a concommitant decrease in formation of visible effector-target cell conjugates. Cytolysis and conjugate formation can both be restored to normal by adjusting the temperature to a point where the normal fluid state of the membrane is restored. Complement-mediated lysis of EL-4 cells, on the other hand, is completely unaffected by identical alterations in membrane fluidity.We are currently examining the effect of altered plasma membrane structure and fluidity on the ability of T cells to become sensitized in vitro and to express cytotoxic function.

959 THE EFFECT OF LIPID SUBSTITUENTS ON ANTIGEN STIMULATION OF LYMPHOCYTES, Sidney Leskowitz and Daniel Mattern, Tufts University School of Medicine, Boston, Mass. 02111.

In Vitro stimulation of lymphocytes from guinea pigs sensitized to N-acetyl tyrosine azobenzenearsonate (ABA-tyr) exhibits dose response curves that show maximal stimulation at about 100 µg with no real inhibition at the largest doses studied (400 µg).

A number of derivatives were synthesized in which the $NH_2$ group of ABA-tyr was reacted with fatty acids of chain lengths $C_6$, $C_{12}$, $C_{16}$. All of these derivatives had roughly equivalent ability to sensitize in vivo however their ability to stimulate sensitized lymphocytes in vitro was markedly different. As the hydrocarbon chain length increased the optimal dose for stimulation decreased to 2 µg for the $C_{16}$ compound; larger doses were toxic.

The tendency for these lipid derivatives to stick to cell membranes was confirmed by testing passive sensitization of red cells for hemolysis with antibody to the ABA hapten. Only the $C_{12}$ and $C_{16}$ derivatives were active.

We are now trying to determine whether these substances can trigger lymphocytes directly by their ability to insert into membranes as well as to react with specific receptors or whether (since this response is macrophage-dependent) attachment to macrophages is a pre-requisite.

960 FUNCTIONAL RELATIONSHIP OF CHOLESTEROL AND CYTOSKELETON COMPONENTS IN MAMMALIAN PLASMA MEMBRANES, Hans-Jörg Heiniger, Andrew A. Kandutsch, and Harry W. Chen, The Jackson Laboratory, Bar Harbor, Maine 04609.

The sterol in mammalian plasma membranes can be depleted up to 50% of the original content under appropriate culture conditions by blocking the de-novo synthesis of cholesterol with certain oxygenated derivatives of cholesterol which specifically depress the activity of the rate limiting enzyme, HMG-CoA reductase, in the cholesterol synthetic pathway (1). We have demonstrated that such a depletion of membrane sterols in mouse L-cells reduces or abolishes endocytosis, i.e. the uptake of solutes by formation of membrane vesicles (2). This loss of endocytosis was prevented or reversed if the cells were incubated with cholesterol, desmosterol, or mevalonic acid (the product of HMG-CoA reductase). In L-cells with a normal sterol content endocytosis is strictly temperature dependent and no breaks in the Arrhenius plots are detectable, i.e. no temperature-related phase transitions are evident. In L-cells with reduced sterol content a break in the Arrhenius plot is observed within a temperature range of 16°C to 19°C. Below this temperature range the rate of endocytose is similar between the control and sterol deficient cells but above this temperature the rate in sterol-deficient cells is greatly reduced. Cells treated with drugs which affect the cytoskeleton (microfilaments and microtubules) also severely reduce endocytosis. However unlike cells treated with the sterol inhibitor no breaks in the Arrhenius plot are observed (3). These results lead us to hypothesize that cholesterol through its modulation of the fluidity of the plasma membrane may create an "anchor-environment" required for the cytoskeleton to execute membrane deformation (endocytosis, exocytosis). Experiments to substantiate this hypothesis are underway.

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961 DIFFERENTIAL EFFECT BY LPS ON THE MOBILITY OF IgM AND IgG RECEPTORS OF MOUSE B LYMPHOCYTES. K.A. Krolick, B.J. Wisnieski, and E. E. Sercarz. Anti-Ig induced redistribution of different surface Ig (sIg) classes was studied as a function of temperature, and correlated with membrane phase transitions as revealed by electron spin resonance spectroscopy (ESR). FITC-anti-IgG2 and -IgM induce patching and capping which proceed with increasing rates from 2° to 40°C (measured at 2° intervals). Characteristic temperatures mark the onset of discontinuities in such rate changes. IgG2-bearing cells display discontinuities at 14°, 22°, 28° and 36°C, while IgM-bearing cells display discontinuities at 18°, 24°, 32°, and 38°C. ESR studies employing the spin label 5N10, a nitroxide substituted decane, indicate that these temperatures are a function of phase transitions in the B cell membrane. Furthermore, treatment with mitogenic doses of lipopolysaccharide (LPS) has direct, but differential effects on the mobility of sIgM and sIgG2. After 15 minute exposure of IgM+ cells to 50μg/ml LPS at 37°, a shift of receptor activity is observed toward lower temperatures, indicating a direct fluidizing influence by the mitogen. IgG2+ cells demonstrate a shift toward higher temperatures, implying that LPS has a direct rigidifying effect at the cell surface. We conclude that membranes of IgM cells are less fluid than IgG2 cells, and consequently require higher temperatures to allow comparable lateral IgM receptor movement. In addition, LPS interacts with both populations, but activating ability may rest on how the mitogen influences the physical state of the cell membranes involved.

962 RESPONSE PATTERN OF B CELLS AFTER POLYCLONAL ACTIVATION, Eva Gronowicz, Samuel Strober and Wes van der Loo, Stanford University School of Medicine, Stanford, California 94305.

The characteristics of murine spleen cells responding to polyclonal B cell activators were investigated. Cells were cultured either in serumfree medium in high cell densities, or in low cell densities in medium containing fetal calf serum and 2-mercaptoethanol. Lipopolysaccharide (LPS), purified protein derivative from tuberculin and dextran-sulphate activated B cells in both culture systems, and at least in the high cell density cultures activated distinct subsets of B cells. LPS activation in serumfree cultures resulted in only IgM secretion, whereas cells activated in low cell density cultures secreted IgM, $IgG_2$ and $IgG_1$ antibodies. Evidence will be presented that the same subset of B cells is activated by LPS in the two culture systems. These results suggest that the functional performance of B cells can vary with different culture conditions.

963 IgM AND IgG SECRETION AFTER LPS ACTIVATION OF MOUSE SPLEEN CELLS. Wessel van der Loo, Eva Gronowicz, Jim P. Schröder, Depts. of Genetics and Medicine, Stanford University School of Medicine, Stanford, CA, 94305.

Mouse spleen cells were activated by lipopolysaccharide (LPS) in low cell density cultures in medium containing fetal calf serum and 2-mercaptoethanol. Cells from C3H mice gave a higher response than cells from SJL or (SJL x SJA)$F_1$ mice. The response was characterized by a rapid appearance of IgM secreting cells, while IgG secreting cells appeared some days later. In all strains most of the IgG was of the $IgG_2$ subclass, and only a small number of $IgG_1$ positive cells were detected ($IgG_3$ was not tested). At the time the $IgG_1$ positive cells appear in the cultures, approximately half of these were also positive for IgM. In contrast, the number of cells synthesizing $IgG_2$ and IgM simultaneously was less than 5% of the total $IgG_2$ producing cells throughout the culture period. The mitotic index was high (>10%) at the peak of activation, indicating that the large majority of the cells were actively proliferating.

964 POSSIBLE IMPORTANCE OF UNSATURATED FATTY ACIDS IN LYMPHOCYTE AND MAST CELL ACTIVATION, Charles W. Parker, Timothy J. Sullivan, and James P. Kelly, Dept. of Med., Washington University School of Medicine, St. Louis, MO 63110

Human lymphocyte responses ($^3$H thymidine uptake) to 4 different classes of mitogens (periodate, lectins, anti-thymocyte globulin, and A23187) were essentially unaffected by indomethacin and aspirin, which inhibit the cyclo-oxygenase pathway of arachidonic acid (AA, 5,8,11,14-eicosatetraenoic acid) metabolism. By contrast, 4-100 μM 5,8,11,14-eicosatetraynoic acid (ETYA), an AA analogue with triple bonds at the 5,8,11,14 positions which inhibits both the cyclo-oxygenase and lipoxygenase pathways, was inhibitory. AA partially or completely reversed the ETYA inhibition. AA markedly and rapidly stimulated cAMP accumulation (measured by radioimmunoassay) in intact lymphocytes whereas other long chain fatty acids were less effective. AA also potentiated and prolonged the early cAMP response to mitogenic lectins. AA and other fatty acids were shown not to interfere in the cAMP radioimmunoassay. ETYA lowered basal cAMP levels and inhibited the AA response. The mechanism of the AA induced increase in cAMP in lymphocytes is presently unclear. Direct addition of AA or ETYA to lymphocyte subcellular fractions fails to increase or decrease adenylate cyclase activity. However, modest increases in enzyme activity are seen when lipid extracts from cells pre-stimulated with PHA or con A are used. ETYA also markedly inhibits immunologically and non-immunologically stimulated histamine release from purified rat mast cells whereas AA (30-100 μM) enhances these responses. Unsaturated fatty acids may play an important role in immunologic (and other cell) activation analogous to that postulated previously for cyclic nucleotides and calcium.

965 EFFECT OF MITOGENS ON IN VITRO LYMPHOCYTE-MACROPHAGE INTERACTIONS. C. A. Abel, C. A. Stoecker and B. M. Rickard. National Jewish Hospital and Research Center, Denver, Colorado 80206.

The effect of mitogens on in vitro murine lymphocyte-macrophage interactions was studied by comparing the number of T or B cells bound to macrophages in the absence and in the presence of mitogens. Con A, a T cell mitogen which binds equally to T and B cells, increased the binding of T cells to macrophages (25-fold) when used in mitogenic doses. Non-mitogenic doses of Con A (20-50 μg/ml) did not enhance the binding of T cells, while identical doses of Succinyl-Con A (A-Con A), induced T cell mitogenesis and also increased significantly the binding of T cells to macrophages. PHA, which in mitogenic doses agglutinates lymphocytes, also increased the binding of T cells to macrophages (35-fold). Neither Con A nor PHA increased the binding of B cells. Lobster Agglutinin (LAg 1), a B cell mitogen, which agglutinates both T and B cells, increased the binding of B cells (50-fold), but not of T cells. However, LPS, another B cell mitogen, did not increase the binding of either T or B cells to macrophages. These observations indicate that Con A, S-Con A, PHA and LAg 1 specifically enhance intercellular contacts between responder lymphocytes and macrophages, and suggest that stable interactions between these cells are important in the induction of lymphocyte mitogenesis.

966 PROTEOLYTIC ACTIVITY AT THE SURFACE OF GLASS-ADHERENT LEUKOCYTES AND ITS POSSIBLE ROLE IN CELL COMMUNICATION, Zoltán A. Tőkés and Richard O'Brien, Cell Membrane Lab, LAC/USC Cancer Center, Depts. Biochem. & Path., USC School of Med., Los Angeles, CA 90033

Lymphocyte transformation by phytohemagglutinin or by periodate treatment requires the presence of glass-adherent leukocytes (GAL). Contact with periodate-treated macrophages transforms peripheral T lymphocytes. The molecular bases of this cell communication are not established. We have developed a method to estimate proteolytic activity at viable cell surfaces. Radioactive substrate, $^{125}$I-labeled casein, is covalently linked to modified latex beads, which are rolled over the surfaces of cells. Proteolysis is expressed by delocalization of labeled peptides from the solid support. Using this method we have measured available proteolytic activity on human peripheral lymphocytes and GAL prior to and after periodate oxidation. Under the same conditions used for obtaining lymphocyte transformation, low levels of proteolysis were detected on both the nonadherent and on the adherent populations of cells prior to the treatment. Within 30 min after treatment, the nonadherent population expressed about 15-25% increased proteolysis. The adherent cell population, however, had a marked increase of 250-300% of proteolytic activity which persisted for several hours. No corresponding increase in released proteolytic activity was observed nor was cell viability diminished. Activity detected at the surface was not inhibited by a ten-thousand fold excess of unlabeled protein in solution. The increased activity was not inhibited by cyclohexamide, indicating that an enzyme activation may occur. Since proteases have been shown to transform lymphocytes we suggest that peripheral lymphocytes may be modified by mitogen-activated proteolytic enzymes at the GAL surfaces, which may result in their further differentiation.

967 SECRETED PRODUCTS ASSOCIATED WITH HUMAN BETA$_2$-MICROGLOBULIN FROM ACTIVATED LYMPHOCYTES. Vernon C. Maino, Ralph T. Kubo and Howard M. Grey. National Jewish Hospital & Res. Ctr., Denver, CO. 80206

Studies with mitogen activated human lymphocytes indicated that activated T cells were capable of synthesizing and secreting large molecular weight proteins associated with beta$_2$-microglobulin ($\beta_2$m). Analysis of lactoperoxidase- or metabolically-labeled tonsil lymphocytes by specific immune precipitation and SDS polyacrylamide gel electrophoresis revealed a single 45,000 dalton HL-A component associated with $\beta_2$m. Recent demonstrations that $\beta_2$m may additionally be associated with soluble products (for example, the allogeneic effect factor) prompted us to examine secreted $\beta_2$m from mitogen-activated lymphocytes. Maximal secretion of $\beta_2$m from PHA-activated cells internally labeled with $^3$H-leucine was found 48 to 72 hours following stimulation. Analysis of anti-$\beta_2$m immune precipitates on reduced SDS polyacrylamide gels revealed three components of 70,000, 45,000 and 25,000 daltons plus the 12,000 dalton $\beta_2$m. Furthermore, at least two of these components were secreted by isolated T cells; the "B" cell population on the other hand did not secrete significant amounts of these products. Additionally, these $\beta_2$m-associated proteins could not be immunoprecipitated with rabbit anti-HL-A or anti-Ia sera. These studies suggest secreted $\beta_2$m may exist as a subunit of products other than the major histocompatibility antigens. The identification of these proteins and their relationship to T cell function is currently being investigated.

968 STIMULATION OF MURINE B LYMPHOCYTES BY Fc FRAGMENTS OF IgG AND IgM. Monique A. Berman and William O. Weigle, Scripps Clinic and Research Foundation, La Jolla, CA. 92037

B lymphocytes have been shown to have affinity for the Fc portion of antibodies, antigen-antibody complexes, and aggregated Ig. Our studies show that normal mouse spleen cells can be activated in vitro with soluble Fc fragments of human, rabbit, or goat IgG, as well as Fc from a human IgM myeloma. Blast transformation, increased $^3$H-thymidine incorporation (up to 150-fold), and polyclonal antibody synthesis are optimal between days 4 and 5 of culture. Fab or F(ab')$_2$ fragments are not stimulatory, and incubation with intact heat-aggregated human IgG (HGG) results only in a small increase in the rate of DNA synthesis. The response to Fc fragments can be shown in serum-free medium or medium supplemented with fresh or heated (56°C, 30 min) autologous mouse serum, but is inhibited by small amounts of mouse anti-HGG serum. After reduction and alkylation, Fc fragments show a reduced stimulatory activity when tested at low concentrations. The findings that the mitogenic response to human Fc is not impaired in HGG-tolerant mice, and that no detectable PFC specific for HGG are induced by Fc in normal spleen cell cultures, suggest that the response is not related to the immune response of mice to HGG as an antigen, but rather represents a general phenomenon (clonal expansion) of lymphocytes reacting with the modified Fc portion of Ig molecules (i.e., as in AgAb complexes). The responding cells, presumably Fc receptor lymphocytes, are not anti-T cell serum sensitive, are nylon wool adherent, can be rosetted with RBC coated with goat F(ab')$_2$ anti-mouse Ig, and are present in the spleens of nude mice.

969 IMMUNE COMPLEX-INDUCED CHANGES IN THE SURFACE PROPERTIES OF MONOLAYERS OF MACROPHAGE MEMBRANES, Po-Shun Wu, Mark W. Cowden and Sorell L. Schwartz, Georgetown University, Washington, D.C. 20007

Transduction of agonist/membrane interactions to cellular responses has received wide interest in areas of hormone/receptor and drug/receptor interactions as well as in membrane signaling in immunology. Possible mechanisms, among others, include: direct conversion of receptor to effector protein (e.g., direct enzyme activation or inactivation); receptor ---➤ effector activation; receptor ---➤phospholipid ---➤ effector activation. With regard to the latter, we have prepared lyophilized fractions of membranes from normal (NM) and BCG-induced (BCGM) rabbit alveolar macrophages. The total lipid fractions of both membranes were about the same. However, BCGM had about a two-fold higher fraction of lipid phosphate and cholesterol. Monolayers of each membrane fraction were spread on an automated Langmuir trough and area changes measured at a constant surface pressure (25 dynes). The following were added to the subphase: egg albumin (EA)/rabbit anti-EA serum (IRS) [EA-IRS]; EA/non-immune rabbit serum [EA-NRS]; EA/fetal calf serum [EA-FCS]. All of the mixtures caused a non-specific protein induced expansion. EA-IRS caused a substantial increase in the rate of expansion of BCGM as compared to EA-NRS and EA-FCS. This differential was not seen following the use of IRS alone nor could it be demonstrated in NM. Further experiments showed BCGM to be more responsive to membrane expanding agents such as chlorpromazine. It has been known for a few years that enzymes within phospholipid membranes can be activated by viscotropic changes induced in the membranes. Such a possibility might exist for a means of transduction of membrane contact to membrane contraction in phagocytosis by certain cells.

970 ACTIVATION OF MURINE LYMPHOCYTES BY ANTI-IMMUNOGLOBULIN. Donna G. Sieckmann, Richard Asofsky, Donald E. Mosier and William E. Paul, NIAID, NIH, Bethesda, Md. 20014.

In an effort to delineate further the molecular requirements for B lymphocyte activation, we have reinvestigated the capacity of anti-immunoglobulin (Ig) antibodies to stimulate in vitro DNA synthesis by mouse spleen cells. We find that a goat anti-mouse μ antiserum, and specifically purified goat anti-mouse IgGκ and rabbit anti-mouse κ antibodies cause proliferation of spleen cells from adult $BDF_1$ mice. $^3H$-TdR uptake in 64 hour microtiter cultures to which anti-Ig was added was 20-60,000 cpm with non-stimulated controls of 1-5,000 cpm; in parallel cultures LPS caused a response of 60-100,000 cpm. The anti-Ig sera and antibodies had been rendered specific by absorption with mouse tissues and/or passage over immunoadsorbant columns. The proliferation induced by these reagents could be inhibited by incorporating myeloma proteins of the appropriate class in the culture; these proteins had no effect on LPS induced proliferation. In addition, specifically purified goat anti-ferritin antibody had no stimulatory effect. In contrast to the responsiveness of spleen cells, neither thymocytes nor nylon-wool column purified T lymphocytes responded to anti-Ig, suggesting that the response was dependent on B cells. The capacity of spleen cells to respond to anti-Ig was age-dependent in that cells from $BDF_1$ mice less than four weeks of age show little response; in contrast to a previous report (Weiner, et al. J. Immunol. 116: 1656, 1976), spleen cells from mice of 8 to 12 weeks of age respond well to anti-Ig. We suggest that anti-Ig stimulates proliferation of a mature subpopulation of B lymphocytes. This concept is supported by the failure of spleen cells from immunologically defective $(CBA/N \times DBA/2)F_1$ male mice to respond to anti-Ig. Our results suggest that Ig receptors have a functional role in controlling B cell activation.

971 STIMULATION OF MOUSE AND HUMAN B LYMPHOCYTES BY CLASS-SPECIFIC INSOLUBLE ANTI-IMMUNOGLOBULINS, David C. Parker, The University of Massachusetts Medical School Worcester, MA 01605

Human and mouse B lymphocytes can be stimulated to proliferate by insolubilized anti-immunoglobulin (anti-Ig) (D.C. Parker, Nature 258 (75) 361). In these two species, soluble anti-Ig is not mitogenic, and can inhibit stimulation by other mitogenic agents. Stimulation by insolubilized anti-Ig can be blocked by either excess free Ig or by soluble anti-Ig at concentrations which strip surface Ig from B cells. Although insoluble anti-Ig is comparable to LPS in its ability to stimulate cell proliferation in mouse B cells, (unlike LPS) it fails to stimulate an increase in numbers of Ig-secreting cells. Using this system, various supernatants of activated T cells and macrophages have been assayed for "second signals" required for B cell differentiation towards antibody synthesis. Using class-specific antibodies to human IgM and IgD in soluble and insoluble form, it will be determined whether the two major classes of surface immunoglobulin can be distinguished functionally, either in the ability to trigger proliferation or to inhibit stimulation.

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972 ANTI-Ia ANTISERUM INHIBITS B CELL INDUCTION BY ANTIGEN, Daniel M. Keller, Philippa Marrack, and John Kappler, Department of Microbiology, The University of Rochester, Rochester, N.Y. 14642.

Spleen T lymphocytes stimulated by Concanavalin A produce a non-specific mediator (NSM) which is required by B cells in the production of an antibody response to heterologous erythrocytes. NSM is not required for the first 24 hours of culture, and antigen alone is sufficient for B cell proliferation during the first 24 hours. Anti-Ia antiserum added at the beginning of the culture period inhibits the response of B cells when they are cultured in the presence of antigen whether NSM is added at 0 or 28 hours, but anti-Ia is ineffective if it is added to culture at 24 hours even if NSM addition is delayed until 28 hours. B cells continue to show Ia determinants at 24 hours as shown by the cytotoxicity of anti-Ia and complement at that time. We conclude that B cells remain Ia positive during the initial phase of induction and that Ia is not the receptor for NSM.

973 DIFFERENTIAL ABILITY OF BUTANOL-EXTRACTED LIPOPOLYSACCHARIDE TO MODULATE B-CELL RESPONSES IN THE C3H/HeJ MOUSE. Michael G. Goodman, D. Elliot Parks and William O. Weigle. Scripps Clinic and Research Foundation, La Jolla, CA 92037

The lipopolysaccharide (LPS)-polypeptide complex extracted from the cell wall of E. coli K235 by the butanol-water technique has been shown to evoke a mitogenic response in B lymphocytes from C3H/HeJ mice resistant to LPS extracted with phenol. Therefore, the comparative ability of butanol and phenol LPS extracts to modulate other C3H/HeJ B-cell functions was investigated. Both butanol-extracted (LPS-B) and phenol-extracted (LPS-P) LPS preparations activated responder C3H/St spleen cell cultures to polyclonal antibody production, while only LPS-B activated C3H/HeJ spleen cells. Both LPS-P and LPS-B acted as adjuvants when injected with aggregated human gamma globulin (HGG) in C3H/St mice, but neither preparation was effective as an adjuvant in C3H/HeJ mice. LPS-P injected with deaggregated HGG (tolerogen) into LPS sensitive mice has been shown previously to inhibit the induction of tolerance to HGG. In the present studies it was shown that LPS-B, but not LPS-P, was able to abrogate tolerance induction to HGG in the C3H/HeJ. LPS has also been shown to bypass tolerant T cells late in tolerance to HGG in LPS sensitive mice when B cells are responsive. However, in the C3H/HeJ, neither LPS-B nor LPS-P was capable of this function. Thus, in the C3H/HeJ, LPS-B stimulates mitogenesis, polyclonal B-cell activation, and inhibition of tolerance induction, but cannot act as an effective adjuvant or as a bypass mechanism to activate B cells in the presence of tolerant T cells.

974 CELL MEDIATED MITOGENIC RESPONSE (CMMR) IN MOUSE LYMPHOCYTES, Keiko Ozato, Carnegie Institution of Washington, Baltimore, Md. 21210

Cortisone resistant thymocytes (CRT) exhibit a proliferative response to syngeneic mitomycin C treated spleen cells when either cell has been modified at its surface by pretreatment with a lectin such as Con A (J. Exp. Med. 143, 1; 1976). This response was characterized as CMMR since lectin modification itself is not mitogenic and the response requires cell contact between stimulator and responder; no soluble mediator has been found. Agglutination by itself is not sufficient since only mitogenic lectins (Con A, leucoagglutinin, lentil lectin) are capable of producing CMMR. The level of DNA synthesis is comparable to the mitogenic response to soluble mitogens, and the response occurs in allogeneic as well as syngeneic combinations. CMMR does not lead to development of cytotoxic cells towards syngeneic target cells. In allogeneic combinations this lectin modification of the cell surface suppresses the generation of cytotoxic cells. Population analysis indicated that only Ig positive lymphocytes with an intact membrane can generate CMMR. B cells are incapable of responding. Lymphoblasts generated from CRT in CMMR carry undegraded $^{125}$I-mitogens which must have been transferred from the stimulator to the responder. Autoradiography showed that $^{125}$I-mitogens are transferred via cell contact within 60 minutes.

975 DICHOTOMY OF LPS RESPONSIVENESS IN NUDE AND LITTERMATE MICE. D. Elliot Parks, Michael V. Doyle and William O. Weigle, Scripps Clinic & Research Fndn., La Jolla, CA 92037

Spleen cells of athymic, nude (nu/nu) mice on a C57BL/6J background and their heterozygous (nu/+) littermates, when stimulated with bacterial lipopolysaccharide (LPS), respond (1) mitogenically, (2) with polyclonal antibody to a variety of erythrocytes, and (3) with hemolytic plaque-forming cells (PFC) specific for LPS. Tolerance to human gamma globulin (HGG) can also be established and maintained for months in these animals with a single injection of 2.5 mg deaggregated HGG (DHGG). Furthermore, the injection of LPS 3 hrs after DHGG will inhibit the induction of tolerance in the splenic B cells of both nude and (nu/+) mice when assayed in primed and irradiated (nu/+) recipients. However, this inhibition of tolerance is not accompanied by the appearance of PFC to HGG in the intact nude animals, as it is in the (nu/+). The observation that LPS does not render the response to antigen T-independent in nude mice is further confirmed in that PFC are not stimulated in nude animals even after two injections of antigen and LPS. In contrast, LPS increases PFC to sheep erythrocytes or HGG when injected with antigen into intact (nu/+) mice and overcomes unresponsiveness in adult thymectomized, irradiated and bone marrow reconstituted (B) mice. These observations suggest that the splenic B cells of nude mice may be incapable of fully responding to LPS; reflecting a defect in the B cells or possibly in another cell population which interacts with LPS in normal mice. Additionally, these findings demonstrate that the ability of LPS to render a T-dependent antigen T-independent is distinct and separable from the capacity of LPS to generate mitogenic and polyclonal B-cell responses and to inhibit the induction of tolerance.

976 MEMBRANE EVENTS LEADING TO B CELL TOLERANCE, R.B. Bankert, G.L. Mayers and D. Pressman, Roswell Park Memorial Institute, Buffalo, NY 14263

B cell activation was interrupted by an antigen which binds covalently as well as specifically to antigen specific membrane-associated receptors. Thus oxidized dextran B1355 which binds to dextran specific receptors via an antigen antibody interaction also binds covalently with amino groups in or near the receptor via the reactive aldehyde groups of the dextran molecule. Native dextran alone induces a substantial immune response in BALB/c mice while a marked suppression (>95%) of the response to dextran was observed when the oxidized dextran was given at various times before, or up to four days after immunization with native dextran. Suppression was antigen specific, dose dependent, and stable after cell transfer. The events at the membrane which preceeded this suppression were investigated using cells of the myeloma MOPC104E tumor. These cells possess dextran binding receptors and secrete immunoglobulins which are structurally indistinguishable from primary response anti-dextran antibodies. Rosette and fluorescent label experiments demonstrate that tumor cells pulsed with unoxidized dextran clear their membranes of the dextran within hours and subsequently reexpress their receptors. Cells pulsed with oxidized dextran, however, fail to reexpress receptors after clearing their membranes of antigen. The in vivo observations of the normal antibody-forming cell and the in vitro observations of the neoplastic antibody-forming cell suggest the following sequence of events leading to dextran specific B cell tolerance; (1) crosslinking of receptor with the multivalent antigen (2) covalent binding of the antigen to the receptor or to adjacent membrane proteins (3) removal of the receptor-antigen complex from the surface and (4) failure to reexpress the receptor.

977 POSSIBLE ANTI-PROLIFERATIVE EFFECTS OF DBcAMP ON THE HUMORAL IMMUNE RESPONSE IN VITRO. Scott W. Burchiel and Kenneth L. Melmon, Dept. of Medicine, Div. of Clinical Pharmacology, University of California San Francisco School of Medicine, San Francisco, CA 94143

DBcAMP can exert a biphasic effect on the primary PFC response of murine spleen cells to sheep red blood cells (SRBC) both in vivo and in vitro. The response is augmented if DBcAMP is present at the time of immunization with SRBC in vivo, or if it is present only during an early phase in vitro. Inhibition results if DBcAMP is present during the later stages of immune induction. Since the concentration of DBcAMP required for these modulatory effects on the humoral immune response is about the same that would inhibit the proliferation of cultured lymphoid cells, we postulated that other drugs that could inhibit cell division, but not effect the concentration of cAMP, might mimic the actions of DBcAMP. We tested 3 agents that share a common ability to inhibit cell division reversibly, but each by a different biochemical mechanism. The Mishell-Dutton method was used to study drug effects on a primary response to SRBC and DNP-Lysine-Ficoll (DLF), a T independent antigen. Colcemid (C), cytosine-arabinoside (CA), and hydroxy urea (HU) each produced a significant dose-dependent augmentation (if added from hour 0-18) and inhibition (if added after hour 18) of the SRBC-PFC response. Effects were maximal when C was at $10^{-6}$M, CA was at 5 X $10^{-5}$M and HU was at $10^{-3}$M. Only DBcAMP augmented the DLF response indicating that simple inhibition of proliferation cannot account for all of its effects.

978 MECHANISM OF REGULATION OF THE SECONDARY ANTIBODY RESPONSE IN VITRO BY B CELLS AND LIPOPOLYSACCHARIDE (LPS). Sharyn M. Walker and William O. Weigle, Scripps Clinic and Research Foundation, La Jolla, CA 92037

LPS from E. coli has numerous effects on the immune response. We have been observing its effect on the in vitro generation of antibody producing cells. Spleen cells from mice primed with turkey gamma globulin (TGG), upon challenge in vitro with TGG, generate over 6 days a high number of indirect plaque-forming cells (PFC). However, addition of a mitogenic dose of LPS suppresses the response 70 to 95%. The mechanism of suppression was studied using a procedure we developed which separates non-TGG-specific B cells from TGG-primed spleen cells. It is known that immunoglobulin (Ig)-bearing cells can be removed from murine spleen cell preparations by rosetting with red blood cells coated with anti-mouse Ig. The Fc end is removed to prevent rosetting to Fc binding cells. Ig-rosetted cells are separated from non-rosetted cells by centrifugation on ficoll-isopaque. This procedure eliminated the generation of PFC to TGG. If, however, the spleen cells were appropriately treated with TGG before rosetting (30 min at 4°C at 100 µg/ml, washed and incubated 37°C for 1.5 hr and washed), the TGG-specific B cells were not removed; non-TGG-specific B cells were removed. Inhibition by LPS of the generation of PFC was mediated through the non-TGG specific B cells. That is, removing the non-TGG specific B cells, while retaining the TGG specific B cells, permitted a normal response regardless of LPS addition. These results show that suppression is not due to a direct effect of LPS on either T cells, macrophages, or progenitors of TGG-specific PFC. Rather, suppression is mediated through B cells without specificity for TGG.

**979** ALLOANTIGEN SPECIFIC T LYMPHOCYTE RECEPTORS AS ANALYSED BY ANTI-IDIOTYPIC ANTIBODIES, Hans Binz and Hans Wigzell, Institute for Medical Microbiology, Zürich, Switzerland, and University of Uppsala, Sweden. Anti-idiotypic antibodies raised against alloantibodies or purified peripheral T lymphocytes can be shown to react with normal immunocompetent B and T lymphocytes. Such anti-idiotypic antibodies suppress in a specific way T lymphocyte functions such as MLC and GvH reactions. Idiotypic, alloantigen reactive T and B cell derived molecules can be detected in normal sera and purified by the use of anti-idiotypic antibodies. B cell derived molecules consist of 7 to 8 S IgM and T cell derived molecules can be found in three different forms, namely with a molecular weight of 150,000 daltons, 75,000 daltons and with a molecular weight between 30,000 and 40,000 daltons as analysed on SDS gels. The molecules forming the 150,000 daltons peaks can be split into two single polypeptide chains with a molecular weight of about 70,000 daltons. The molecules with a molecular weight between 30,000 and 40,000 daltons are a degradation product of the 70,000 daltons molecules. No serological determinants of conventional immunoglobulins nor determinants coded for by the genes of the major histocompatibility locus could be found on the T lymphocyte derived molecules. Isolation of alloantigen binding molecules from normal or specifically activated T lymphocyte membranes showed a similar pattern.

**980** INTERACTION OF ANTIGEN WITH B CELL Ig RECEPTOR IS NOT AN ABSOLUTE REQUIREMENT IN THE INDUCTION OF IN VITRO HAPTEN SPECIFIC IgG RESPONSE, Salvatore Cammisuli, Dept. of Bact. and Immunol., Univ. of California, Berkeley, Ca. 94720

While interactions between antigen and B cell immunoglobulin receptor determine the specificity of the immune response, it is not clear whether they are directly involved in B cell activation. We report an experiment which indicates that antigen-Ig receptor interaction is not essential for generating an hapten specific IgG response. Keyhole limpet hemocyanin (KLH) has been concentrated on the membrane of azophenyllactoside-KLH (lac) primed Balb/c spleen cells via either one of the following hapten bridges: 1) Goat anti-mouse light chain antibodies to which azophenylarsonate (ars) groups were coupled by amidination, followed by rabbit anti-ars antibody conjugated with KLH (anti-ars-KLH); 2) C3H/He anti-Balb/c antibodies (anti-$H2^d$) similarly coupled with ars, followed by anti-ars-KLH. After treatment, the lac primed spleen cells were washed and azophenylglucoside-KLH (azoKLH) primed Balb/c spleen cells were added as a source of KLH specific T helper cells. The cells were then cultured for five days under Mishell-Dutton conditions. In both cases a substantial and highly significant indirect plaque forming response against azophenylactoside-sheep red cells was detected. The extent of this response is directly related to the lac primed: azoKLH primed spleen cell (B:T) ratio. At 1:30 B:T ratio this response is over 1/3 of the response achieved in the positive control lines by conventional immunization with lac. Since in our experimental design KLH was concentrated on all spleen cells from the lac primed donor, independently of their specificity, it is suggested that the hapten specific response observed mirrors an ongoing polyclonal activation.

**981** EFFECT OF B-CELL TOLERANCE AND EPITOPE DENSITY ON ANTIGEN INDUCED CAPPING. R. F. Ashman and D. Naor. UCLA School of Medicine, Los Angeles, CA 90024.

Spleen lymphocytes with receptors for TNP determinants detected by rosetting with TNP conjugated erythrocytes can be demonstrated to be specific for TNP by hapten inhibition. Immunized and unimmunized rosettes cap with similar kinetics, but rosettes from animals tolerized to TNP at the B cell level by the Fidler and Golub (trinitrobenzene sulfonate) method show deficient antigen induced capping and receptor regeneration after pronase treatment. Together with data from other laboratories concerning the capping behavior of tolerogens this suggests the unresponsiveness of the tolerized B cell may lie in an inability to clear its membrane and regenerate a new crop of receptors.

Though densely hapten conjugated red cells employed either as immunogen or as antigen produce more rosettes than lightly conjugated red cells, preferential binding of cells resembling the original immunogen in density of TNP determinants is observed. Tested with the same population of lymphocytes, densely conjugated red cells induce faster capping of receptors than lightly conjugated cells, probably because more efficient local crosslinking of receptors results in earlier transduction of the "signal" to cap.

(Supported by NIH grant CA 12800)

982 MACROPHAGE MEMBRANE STRUCTURES INVOLVED IN ANTIGEN BINDING, R.B. Hester, R.O. Endres, R.T. Kubo and H.M. Grey, Nat'l Jewish Hosp. & Res. Cntr., Denver, CO 80206

The role of macrophage-associated antigen in the immune response has been well established. In the present study, the murine macrophage-like cell line, J774, was used in an attempt to identify cell-surface antigen-binding structures. In tests to determine if antigen handling by J774 cells was similar to mouse peritoneal exudate cells, the uptake and persistence of keyhole limpet hemocyanin (KLH) by these two cell populations were compared and found to be similar. The amount of KLH which escaped rapid degradation was directly related to the pulse duration. Thus, although similar amounts of cell-bound KLH were present after a 30 min or 6 hr pulse, cell-bound KLH present 24 hr after pulsing represented only 8% of the 30 min uptake in contrast to 44% of the 6 hr uptake. Cell-surface antigen-binding structures were characterized by radiodination of J774 cells using the lactoperoxidase technique, followed by incubation of these cells with KLH for 1 hr on ice, lysis of the cells in 0.5% NP-40 and treatment of the lysates with a rabbit anti KLH antiserum. Two per cent of the TCA insoluble radiolabel was precipitated with anti KLH, whereas 0.8% was precipitated with a normal rabbit serum control. SDS-polyacrylamide gel electrophoresis of reduced anti KLH immune precipitates indicated a major peak of radioactivity with an approximate molecular weight of 45 - 50,000 daltons. Additional peaks of radioactivity with approximate molecular weights of 12,000, 15,000 and 20,000 daltons were also present. These results indicate that it is possible to define macrophage cell-surface structures involved in the binding of antigen. Studies to investigate the relation of these structures to major histocompatibility gene products are in progress.

983 MEMORY CELL SUBPOPULATIONS AS CHARACTERIZED BY THEIR SUSCEPTIBILITY TO SPECIFIC ANTI-IMMUNOGLOBULIN SERA. Mary Jane Potash and Paul Knopf, Division of Biology and Medicine, Brown University, Providence, RI 02912

Evidence is presented for the unique sensitivity of memory cells bearing surface $IgG_1$ to functional elimination with anti-Immunoglobulin and complement before adoptive transfer. Treatment of cells for transfer with anti-$\gamma_1$, anti-K, or anti-Immunoglobulin reduces the subsequent $\gamma_1$ PFC found in the adoptive host several days after transfer and challenge. There is no net decrease in the total PFC response. Antisera directed against other isotypes show no effect. The depletion of $\gamma_1$ PFC requires complement. Experiments are in progress to identify the cells affected as B cells or T cells.

984 DETECTION AND ISOLATION OF ANTIGEN-BEARING CELLS(ABC) DURING TOLERANCE. D. Scott, M. Venkataraman and M. Cohn, Duke U., Durham, NC 27710

Adult mice injected with deaggregated fluorescein-sheep-γ-globulin(FL-SGG) possess detectable splenic ABC for five days; no ABC are seen by day 7. Upon reinjection with FL-SGG at this time, normal numbers of ABC are found in the spleen despite the fact that these mice appear tolerant and their spleen cells remain tolerant in adoptive irradiated hosts challenged with "T-independent" FL-ficoll. However, when challenged with FL on a T-dependent carrier or when assayed for SGG-helper cell tolerance, DBA/2 and C3HxDBA $F_1$ mice were tolerant but BALB/c and C3H/st mice were not. Since the same ABC pattern is seen in all strains, it is unlikely that these ABC are non-tolerant T helpers or T-dependent B cells. These fluorescent ABC may be B cells capable of binding antigen but unable to be triggered specifically and/or they may be suppressor cells. Isolation of ABC, using anti-fluorescein affinity columns, has been used to determine the function of these ABC in "tolerant" mice. In contrast to the adult pattern, tolerogen-injected neonatal mice show reduced numbers of ABC upon reinjection with FL-SGG. This suggests that either clonal deletion or the failure to resynthesize receptors may be the mechanism of neonatal tolerance to this antigen. (Supported by USPHS grant # AI-10716.)

985 ISOLATION OF ANTIGEN BINDING CELLS FROM IMMUNE AND NONIMMUNE MICE, James J. Kenny, Dept. of Microbiology and Immunology, UCLA School of Medicine, Los Angeles, CA 90024

A two step centrifugation procedure has been established to isolate large numbers of antigen binding cells (ABC). The first step of the procedure involves separating ABC-rosettes from unrosetted lymphocytes based on their density. Lymphocytes are rosetted with sheep red blood cells (SRBC) at 50xg and then placed onto Ficoll-Hypaque and separated according to Parish et al. (1). The rosettes, lymphocytes and SRBC that pass through the Ficoll-Hypaque are then placed on a 40 ml linear (5-10%) Ficoll gradient and spun at 50xg for 10 min. The bottom 25 ml of this gradient is collected as the ABC rich fraction. Up to $10^9$ spleen cells have been processed from 5 day immune and nonimmune mice yielding 4.5 x $10^6$ ABC from the immune cells and 1 x $10^5$ from the nonimmune. The ABC from immunized animals are obtained at 50 to 100% purity while those from nonimmune animals are between 30 and 40% pure. Approximately 30 to 40% of the original ABC population is obtained in the enriched fraction which is composed of 0.5% or less of the original cell population. Of the ABC isolated from 5 day immune spleens, 0.1% are also plaque forming cells.

1. Parish, C.R. et al. 1974. Eur. J. Immunol. 4:808.

986 PREPARATIVE SCALE SEPARATION OF LYMPHOCYTES WITH AND WITHOUT SURFACE IMMUNOGLOBULIN BY DIRECT OR BY INDIRECT IMMUNOADSORPTION TO POLYSTYRENE PETRI DISHES COATED WITH SPECIFICALLY PURIFIED ANTI-IMMUNOGLOBULIN. Michael Mage, Thomas L. Rothstein, Louise McHugh, and James Mond. Lab. of Biochemistry, NCI, and Lab. of Immunology, NIAID, Bethesda Md. 20014

Mouse spleen cells could be directly separated into immunoglobulin-positive (Ig+) and immunoglobulin-negative (Ig-) populations by incubating as many as 2 x $10^8$ cells per 100 mm diameter petri plate coated with specifically purified goat anti-mouse immunoglobulin. The non-adherent population was 95% or more Ig-, and posessed graft versus host and cytotoxic effector activities, as would be expected of T cells. It could also generate a MLR and CML on culture with irradiated allogeneic cells in vitro. The adherent cells could not be released undamaged from plates coated with undiluted anti-Ig, but could be released from plates coated with a 1/4 or 1/10 dilution of anti-Ig in an irrelevant antibody. The released cells (over 90% viable by trypan blue staining, with 94% or more of the viable cells Ig+) responded to LPS, and generated antibody plaque-forming cells to TNP-Brucella abortus and to TNP-Ficoll in vitro. The non-adherent population responded to PHA and Con A. It was depleted of complement-receptor lymphocytes and was poorly responsive to LPS.

Indirect separation (potentially a general cell separation procedure) was done by incubating spleen cells with the FAb fragment of goat anti-mouse immunoglobulin, removing unbound FAb by centrifugation, and incubating the washed cells in petri plates coated with specifically purified rabbit anti-goat FAb. The non-adherent population was depleted of Ig+ cells, had GVH activity, and could generate a MLR and CML on culture in vitro.

987 ORGANIZATION OF $IgG_1$ ON THE CELL SURFACE, Alan M. Stall and Paul M. Knopf, Brown University, Providence, RI. 02912

P3, a cloned myeloma (MOPC-21) maintained in culture, secretes $IgG_1$ (K) and is sensitive to complement-dependent lysis by antibody directed against $\gamma_1$ or K. Sensitivity is attributed to the presence of immunoglobulin on the surface membrane, designated Ig-mem. This sensitivity is abolished by antigenic modulation of Ig-mem. By measuring the recovery of sensitivity to complement-dependent lysis of modulated cells, it has been possible to ascertain the effect of various drugs on the rate of Ig-mem acquisition.

Treatment of modulated cells with 2, 4 DNP or cytochalasin B allows recovery at control rates. Treatment with cycloheximide (Cx), Anisomycin (An) or Pactamycin (Pc), each a specific inhibitor of a different step in protein synthesis, produces significant reduction in rate of recovery. Paradoxically, Puromycin (Pm), also a specific protein synthesis inhibitor, has no effect on recovery. However, treatment of modulated cells with puromycin prevents subsequent inhibition of recovery by Cx, An, or Pc. Conversly, Cx, An, or Pc, when added prior to Pm decrease the rate of recovery to the same extent as if added alone. A model will be presented involving the organization of immunoglobulin at the cell surface.

988 THE DISTRIBUTION OF SURFACE IMMUNOGLOBULIN ON B LYMPHOCYTES, Alan S. Perelson, Theoretical Division, University of California, Los Alamos Scientific Laboratory, Los Alamos, New Mexico 87545

Studies of B lymphocytes have shown that they have immunoglobulin (Ig) receptors on their surfaces. Maps of the spatial position of surface Ig on murine B lymphocytes obtained by Abbas et al (1975), using a two step indirect labeling procedure designed to avoid cross-linking of receptors, were analyzed mathematically. An analysis of the radial distribution function of the maps indicates that the receptors are non-randomly distributed and tend to form clusters, confirming the initial conclusions of Abbas et al (1975). A two-dimensional power spectrum analysis of the maps indicates that the receptors may become oriented along lines, as would be the case if they were associated with an assembly of microtubules and microfilaments. Given maps of the surface Ig distribution at different times during the processes of patch and cap formation such mathematical analyses may prove instrumental in determing mechanisms involved in receptor aggregation and movement.

Abbas, A. K., Ault, K. A., Karnovsky, M. J. and Unanue, E. R. (1975) J. Immunol. 114, 1197.

989 DIFFERENCES IN ULTRASTRUCTURAL APPEARANCE AND MODULATION OF ENDOGENOUS AND EXOGENOUS SURFACE IMMUNOGLOBULIN OF RABBIT LYMPHOCYTES, D. Scott Linthicum, Raja M. Bahu and Stewart Sell, Dept. Pathology, UC San Diego, Ca and Northwestern University, Chicago.

Endogenous and exogenous surface immunoglobulin of rabbit blood lymphocytes differ in ultrastructural appearance and modulation characteristics. Endogenous surface immunoglobulin occurs as small patches on 70-80% of freshly prepared lymphocytes after specific mixed anti-globulin immunoferritin labelling. Endogenous patches are closely associated with the surface membrane and readily undergo endocytosis when labelled cells are warmed to 37°C. The binding of exogenous immunoglobulin may be demonstrated by exposure of lymphocytes to normal allogenic or xenogenic serum in vitro. Aggregated IgG is responsible for the binding of exogenous serum Ig; deaggregated IgG and Fab' fragments do not bind. Exogenous Ig exists as large aggregates precariously attached to the cell surface on 50-70% of the lymphocytes. Exogenous Ig does not undergo endocytosis at 37°C but is sloughed from the cell surface.


990 PARTIAL CHARACTERIZATION OF MEMBRANE IMMUNOGLOBULINS ON RAINBOW TROUT LYMPHOCYTES, Karen Yamaga, Ralph T. Kubo and Howard M. Etlinger, National Jewish Hospital and Research Center, Denver, Colorado 80206.

The reported presence of membrane immunoglobulins (Ig) on thymic cells of primitive vertebrates and the current controversy concerning the nature of the T-cell antigen receptor led us to the characterization of membrane structures that were reactive with rabbit anti-Ig reagents on thymic and splenic lymphocytes from rainbow trout. By immunofluorescence staining, greater than 90% of both splenocytes and thymocytes bound to anti-Ig sera with an apparent equivalent intensity. Quantitative estimates of Ig found on these cells by a radioimmuno-inhibition assay, however, revealed that thymocytes possessed 8 to 10-fold lower amounts of Ig than splenocytes. Characterization of the membrane Ig determinants by the lactoperoxidase iodination method and by SDS-polyacrylamide gel analysis showed the presence of 3 major components on splenocytes with approximate molecular weights of 100,000, 65,000 and 22,000 daltons. In contrast, thymocytes contained the 100,000 dalton component and a minor 40,000 peak but lacked radioactive peaks in the 65,000 and 22,000 dalton regions. The exact nature of the 100,000 dalton component is as yet unknown but it may represent a structure intimately bound to membrane Ig, a cross-reactive specificity or a surface contaminant unrelated to Ig. Experiments are currently in progress in an attempt to distinguish among these possibilities. Thus, although thymus cells react with anti-Ig reagents, the molecular structure of thymic Ig reactive determinants appear to be distict from those found on splenic lymphocytes.

991 NONE-IMMUNE ANTI-SELF RECEPTORS ON LYMPHOCYTES, Hubert Kolb Department of Biology, University of Konstanz, Konstanz, W.Germany. Lymphocytes from several species tested (mouse, rat, rabbit) carry receptors for syngeneic, allogeneic or closely related xenogeneic erythrocytes. Rosette formation is probably due to sterospecific cell surface receptors since erythrocytes of distant xenogeneic origin were not recognized. Such receptors can be demonstrated by spontaneous rosette formation analogous to that described for human T-cells and sheep red blood cells. Rosette formation could not be ascribed to a distinct lymphocyte subpopulation since a large population of spleen cells, thymus cell as well as spleen cells from thymusless (nude) mice formed rosettes. Serum from animals of the same or closely related species is highly inhibitory, serum from more distant species is less inhibitory. Analysis of normal sera reveals activity in the $\alpha,\beta$ -globulin fraction and none in the $\gamma$-globulin fraction.

The physical parameters of rosettes are comparable to those of human T-cell rosettes. In contrast, dead cells as well as live cells are able to form rosettes, $NaN_3$ and cytochalasin B were only weakly inhibitory. The receptor on the lymphocytes is not an immunoglobulin and sensitive to trypsin treatment. The physiological role of non-immune anti-self receptors will be discussed.

992 IMMUNORECOGNITION AND IMMUNE SURVEILLANCE IN CORALS, W.H. Hildemann, R.L. Raison, C.J. Hull, L. Akaka, J. Okumoto and G. Cheung, University of California, Los Angeles, CA 90024 and University of Hawaii, Hilo, HI 96720

Transplantation immunity in corals is characterized by cell-surface recognition with exquisite discrimination. Whereas intracolony or syngeneic transplants of pieces of living coral are consistently compatible, intercolony allografts and interspecific xenografts are invariably incompatible. At least four levels of immune surveillance, with manifestations ranging from mild to severe, are distinguishable: 1) contact avoidance reactions, 2) allogeneic contact incompatibility, 3) chronic xenogeneic incompatibility, and 4) acute interspecific cytotoxicity. Contact avoidance reactions despite soft tissue proximity were seen early preceding both allogeneic and xenogeneic cytotoxicity in areas of forced contact. This suggests sensitive immunorecognition which precedes cytotoxic reactions requiring a longer period of contact sensitization. Bilateral cytotoxic allograft reactions in contact zones occur in Montipora verrucosa with a median reaction time of 22.0 (19.2-25.3) days. Repeat allografting regularly yields accelerated rejection, while reaction times for unrelated third-party allografts ranged from 7 days, characteristic of a prompt secondary reaction, to 33 days typical of primary reactions. This indicates specific immunologic responsiveness with at least short-term memory. Extensive polymorphism of histocompatibility antigens is also revealed. Xenogeneic incompatibilities ranging from hyperacute and severe to chronic and weak are demonstrable in sympatric Hawaiian corals. Recognition signals, receptors, effector cell types and molecular pathways are now under investigation in these "lower" invertebrates.

993 CYTOCHROME c AS A PROBE FOR LYMPHOCYTE SPECIFICITY. G. Corradin and J. M. Chiller, National Jewish Hospital and Research Center, Denver, Colorado 80206

The specificity of lymphocyte function has been studied using cytochrome c (Cyt) as a molecular probe. These small protein molecules offer the advantage that they are well characterized in terms of amino acid (a.a.) sequence and three dimensional structure. Cleavage of Cyt with CNBr yields two peptide fragments (1-65 and 66-104) which can be recombined by condensation to yield a fully active protein. It is thus possible to generate a number of hybrid molecules using peptides derived from Cyts of different species. Using various Cyts which differ at only a few a.a. positions, T lymphocyte specificity was determined by a lymph node (LN) proliferation assay which measures specific antigen induced in vitro $^3$HTdR incorporation of primed T cells. $BDF_1$ mice were primed with either beef (B), horse (H) or rabbit (R) Cyt, and LN cells from each group were tested with each of the 3 antigens. LN cells from mice primed with B Cyt responded to B, poorly to H and not to R Cyt. Cells from H primed mice responded to B and H but not to R while R primed cells responded to R and B but only partially to H Cyt. Also, hybrid molecules made of peptide 1-65 from H and 66-104 from B stimulated cells from B primed mice. Correlation of these data to the sequence of the Cyts used suggests that a single a.a. difference at position 89 may be critical to the observed specificity of stimulation. B lymphocyte specificity was determined in PFC obtained from LNs of mice injected with a $2^o$ challenge. Crossreactivity at the B cell level tested by antigen inhibition of PFC revealed a pattern of specificity different from that observed in the LN proliferation assay. (Supported by AHA 76 849, NIAID 13131 and 00133.)

**811** MOLECULAR INTERACTIONS ACROSS MEMBRANES. S. J. Singer, J. F. Ash, D. Louvard, and L. Bourguignon. Department of Biology, University of California at San Diego, La Jolla, California 92093

We have obtained direct evidence for physical linkages produced between specific components on two sides of a plasma membrane. A normal rat kidney (NRK) cell line in monolayer culture was used. The distribution of Concanavalin A (Con A) on the cell surface, and of intracellular myosin-containing filaments were simultaneously determined using fluorescein-conjugated Con A (F-Con A) and a rhodamine indirect immunofluorescence method with antibodies directed to smooth muscle myosin. Initially, the F-Con A was uniformly distributed over the cell surface, while the myosin was present in extended filaments inside the cell. After 20 min at 37°, however, the F-Con A on the cell surface was often found in linear arrays that were superimposable on the arrays of the intracellular myosin-containing filaments. This strongly suggests that Con A induced a local redistribution of some Con A receptors on the cell surface which led to the receptors becoming physically linked to myosin-containing filaments inside the cell. These results were not affected by $10^{-5}$ M colchicine; microtubules are not implicated in this phenomenon. By contrast, another NRK cell surface marker, the enzyme aminopeptidase, was redistributed into patches when its specific antibodies were bound to the cell surface. Parallel experiments with mouse lymphocytes have been performed and will be discussed.

**812** HOW CONTRACTILE PROTEINS REGULATE PHAGOCYTIC CELL FUNCTIONS. Thomas P. Stossel, John H. Hartwig, Wayne A. Davies, Medical Oncology Unit, Massachusetts General Hsopital, Boston, Massachusetts 02114.

Phagocytosis is an example of surface-to-cytoplasm communication. Contact of an appropriate particle with the plasmalemma of phagocytes elicits pseudopod formation, endocytosis and secretion of lysosomal enzymes into phagosomes and the extracellular medium. A molecular explanation for these events is emerging from the systematic study of contractile proteins in macrophages and granulocytes.

A high molecular weight actin-binding protein (ABP) promotes the temperature-dependent gelation of filamentous (F)-actin which comprises ca 10% of the total cell protein (1-3). Myosin, in the presence of $Mg^{2+}$ATP and a protein cofactor contracts actin-ABP gels (2). We hypothesize: a) A submembrane ABP-actin gel stabilizes the membrane. b) Particle contact alters ABP-membrane association. c) Activated ABP promotes increased focal gelation of F-actin. d) Since the myosin:actin ratio of phagocytes is low compared to muscle, ABP enhances the efficiency of contraction by cross-linking actin filaments. Contraction of ABP-actin gels forms pseudopods. e) Sustained contraction locally disrupts the F-actin-ABP gel, permitting lysosome:membrane fusion as well as blebbing due to membrane destabilization.

Tests support this theory of a gelation-contraction-disruption cycle: a) The extractibility of ABP from macrophage membranes is greater in phagocytosing than resting cells (2). b) $>10^{6}$M cytochalasin B destroys pseudopods, induces surface blebs, inhibits phagocytosis and enhances secretion. $>10^{-7}$M cytochalasin B reversibly dissociates ABP from actin, concomitantly dissolving ABP-actin gels without impairing actomyosin contraction (4). c) Macrophages exposed to nylon wool secrete lysosomal enzymes and bleb, as a hyaline barrier between lysosomes and plasmalemma dissolves. Cytoplasmic extracts of nylon wool-exposed macrophages have reduced ABP and myosin content and capacity to gel, although their F-actin content is similar to that of resting cells.

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813 MEMBRANE EVENTS IN CELL SIGNALING, Martin C. Raff and Durward Lawson, Department of Zoology, University College London, London WC1E 6BT, England

The plasma membrane receives and transduces a large number of different signals in the cell's environment and plays an important role in the secretion of chemical signals. We will discuss three aspects of membrane events in such cell signaling: (i) the behavior of cell surface receptors following liquid binding; (ii) how membrane receptors transduce extracellular signals into intracellular signals and (iii) the molecular events in membrane fusion occuring during exocytotic secretion of histamine by mast cells.

**994** ENRICHMENT OF TARGET-SPECIFIC CYTOTOXIC LYMPHOCYTES BY SEPARATION OF ANTIGEN-BINDING CELLS. Elizabeth Grimm and Benjamin Bonavida. UCLA, Dept. of Microbiology and Immunology, Los Angeles, CA. 90024

The nature of the receptors on effector and memory cytotoxic thymus-derived lymphocytes (CTL) can best be studied utilizing cultures of purified populations of target-specific CTL. This report presents a technique that enriches antigen-binding CTL to greater than 80%. Enrichment is achieved by sedimentation on a fetal calf serum (FCS) gradient. The average frequency of CTL in effector cell populations from in vivo and in vitro murine lymphocytes sensitized to allografts is 20% as estimated by target conjugate formation (antigen-binding). Enrichment is performed by layering the heterogeneous effector-cell population containing target conjugates onto a discontinuous FCS gradient, allowing sedimentation at 1 x G for 75 minutes. After one passage the bottom fraction contains 60% to 90% target-binding lymphocytes. A single-cell cell-mediated cytotoxicity assay was used to verify cytotoxic function: 90% of these enriched binding lymphocytes kill their specific targets, thus yielding a 3 to 5-fold enrichment of target-specific CTL. Conditions for culture and activation of these CTL will be presented.

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**995** CHARACTERIZATION OF A CLONAL ASSAY FOR PRECURSORS OF CYTOTOXIC LYMPHOCYTES, H.S. Teh, E. Harley, R.A. Phillips, and R.G. Miller, The Ontario Cancer Institute, 500 Sherbourne Street, Toronto, Ontario, Canada M4X 1K9.

We have developed a microculture system for estimating the frequency of precursors of cytotoxic lymphocytes (CLP). Cytotoxic T lymphocytes (CL) were generated by culturing limiting numbers of RNC ($H\text{-}2^k$)-nu/+ lymph node (LN) cells with irradiated C3D2F1 ($H\text{-}2^{k/d}$) spleen cells in the presence of RNC-nu/nu spleen cells in microtiter trays. After a 7 day culture period, individual wells were assayed for cytotoxic effector cells directed against $^{51}Cr$-P815 ($H\text{-}2^d$) target cells. The effector cells generated under these conditions were shown to be T cells and the process of differentiation from precursors to CL is radiation sensitive ($D_o \sim$ 180 rads). The frequency of CLP to $H\text{-}2^d$ was calculated using Poisson statistics. In RNC-nu/+ LN, the frequency of CLP to $H\text{-}2^d$ using irradiated C3D2F1 spleen cells was found to be 1/776 ± 65. In combination with a visual assay for individual CL, it was found that the average clone size per precursor after 7 days in culture is 1043 ± 128. Our $^{51}Cr$ release assay could detect clones with as few as 40 CL per culture. We further showed that clones derived from single CLP are specific. Thus, RNC mice stimulated simultaneously by both $H\text{-}2^b$ and $H\text{-}2^d$ antigens produce clones which are specific for either $H\text{-}2^b$ or $H\text{-}2^d$ but not for both. Clonal specificity is also maintained when Con A is used to activate the precursors. Supported by the Medical Research Council (Grant MT-3017) and by the National Cancer Institute of Canada.

**996** A METHOD TO TEST THE SPECIFICITY OF THE CLONES OF H-2 STIMULATED CYTOTOXIC T LYMPHOCYTES, Joseph A. Coha and Richard W. Dutton, Dept. of Biology, Univ. of California, San Diego, La Jolla, CA 92093.

We have approached the question of the specificity of the T cell recognitive receptor by utilizing a system in which low numbers of splenic T lymphocytes are plated together with allogeneic mitomycin C-treated stimulator cells. Following five days in culture, the cells are assayed against the appropriate $^{51}Cr$-labeled target. Titrations of the number of lymphocytes initially plated indicate that the lymphocytes capable of developing H-2 directed cytotoxicity occur in a frequency range of $0.2 - 1 \times 10^{-3}$. This is in agreement with the estimates in the literature (Skinner and Marbrook, E.J.I. 143:1562). In order to study the target specificities to which an individual alloreactive clone is directed, the cells from one responding culture were split and assayed on two separate targets. The two responders chosen were $H\text{-}2^q$ and $H\text{-}2^k$. The stimulators were $H\text{-}2^b$ and $H\text{-}2^d$. According to Peavy and Pierce (J.I. 115:1515), $H\text{-}2^q$ develops no cross-reactive killing, i.e., stimulation by one haplotype results in killing directed solely toward that haplotype. On the other hand, $H\text{-}2^k$ stimulated with either stimulator cell type generates a substantial amount of killing directed toward the other haplotype. In this limiting dilution culture system, individual clones of $H\text{-}2^q$ and $H\text{-}2^k$ effectors stimulated against both stimulators could be isolated which have cytotoxicity directed against only one of the stimulating haplotypes. These results suggest that the recognitive receptors on the cell surfaces of a clone of cytotoxic T lymphocytes are able to distinguish antigenic specificities restricted to a given haplotype. (Supported by USPHS AI 00453-05.)

997 SPECIFICITY OF INDUCED GVH RESISTANCE IN RATS, Donald Bellgrau and Darcy B. Wilson, University of Pennsylvania School of Medicine, Philadelphia, Pa. 19174

It is known that $F_1$ rats, sublethally irradiated, are extremely susceptible to systemic graft-versus-host (GVH) disease caused by inoculation of small numbers of parental strain lymphocytes, mortality generally occurring within 2 - 3 weeks. However, recent studies in our lab indicate that a specific and highly effective state of systemic GVH resistance can be induced in $F_1$ rats by prior "immunization" with parental strain thoracic duct lymphocytes (TDL). Requirements for the induction of such resistance include the presence in the immunizing TDL populations of parental strain T cells having immunocompetence for host strain alloantigens, presumably bearing anti-host T cell idiotypes. Once induced, this specific GVH resistance is radioresistant, and it can be adoptively transferred with spleen cell suspensions to otherwise untreated $F_1$ recipients; however, thus far, we have not been able to detect anti-idiotypic antibodies in the sera of such donors. Genetic studies of the specificity of this resistance in A/B $F_1$ animals indicate that it extends to T lymphocyte populations of the immunizing strain (A) and, surprisingly, also to other $F_1$ T lymphocytes sharing the relevant parental genome (A/C). This may indicate either (i) that the anti-host idiotypes present on $F_1$ T lymphocytes (A anti-B, and C anti-B) are cross reactive, or (ii) that they are present on the same T cell subpopulation -- i.e., not allelically excluded. (Supported by U.S. Public Health Service Grants AI-10191, CA-15822 and CA-09140).

998 THE M-LOCUS ANTIGEN AS A TARGET FOR CYTOTOXICITY. Daniel L.Kastner and Robert R.Rich. Baylor College of Medicine, Houston, TX 77030.

A number of investigators have hypothesized that most of the cells which proliferate in mixed lymphocyte reactions(MLR)are responding to antigens different from those which serve as targets of T-cell mediated cytotoxicity. Central to this issue is the notion that activation by the M-locus(Mls)antigen in mice results in proliferation without the development of cytotoxicity. Therefore, we designed experiments to investigate the question of whether cytotoxic responses are ever generated against these antigens. In primary BALB/c($H\text{-}2^d$,$Mls^b$)vs. DBA/2($H\text{-}2^d$,$Mls^a$)mixed lymphocyte cultures, small but significant cytotoxic activity was detected against the DBA/2 tumor line P815. However, BALB/c spleen cells incubated alone also generated anti-P815 activity, sometimes equalling the killing generated in the presence of antigen. In contrast, utilizing several strain combinations, spleen cells primed in vivo with *H-2* identical, *Mls* different splenocytes generated strong secondary cytotoxic responses in vitro against Con A and LPS blasts of the sensitizing strain. Genetic experiments were designed to investigate the antigenic target for this killing. In accordance with the *H-2* restriction of killing against minor histocompatibility antigens, CBA($H\text{-}2^k$,$Mls^a$)cells primed in vivo and cultured with C3H($H\text{-}2^k$,$Mls^c$)did not lyse C3H.SW($H\text{-}2^b$,$Mls^c$)targets. However, C3H cells primed in vivo with AKR($H\text{-}2^k$,$Mls^a$)and cultured with C3D2$F_1$($H\text{-}2^{k/d}$,$Mls^{c/a}$)cells lysed AKR targets, and AKR-primed C3H cells cultured with AKR cells lysed C3D2$F_1$ targets. These data are compatible with M antigen directed killing, and do not permit genetic discrimination between the generation of proliferative and cytotoxic responses at the *Mls*. (Supported by USPHS N01-AI-42529 and HL-17269 and NASA NAS9-14368).

999 AUTOMATED FLUORESCENT ANALYSIS FOR CYTOTOXICITY ASSAYS. P. K. Horan and J. W. Kappler, Departments of Pathology and Microbiology, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642 U.S.A.

Classical measurements of cytotoxicity using dye exclusion and microscopic evaluation are both time-consuming and inaccurate. Using a two parameter (one color fluorescence, light scatter) cell sorter (TPS) a single dye system has been developed which stains live and compliment killed cells with different fluorescence intensity. After exposure of target cells to antibody and compliment, Ethidium Bromide is added to the target cells at a high enough concentration to stain compliment killed cells very intensely. The cells are then diluted and lysed to produce single nuclei, permitting live cells to be stained but less intensely than the dead cells. Fluorescence intensity is measured on single nuclei at a rate of 10,000 per minute. For these studies anti-T antisera was titrated for compliment dependent cytotoxic activity using normal mouse spleen cells, spleen cells from an anti-thymocyte serum treated mouse, and nylon wool purified mouse splenic T-cells. This procedure makes possible, reliable, and reproducible measurement of cytotoxic activity on 10,000 cells per determination.

**1000** SEPARATION AND FUNCTIONAL EVALUATION OF LYMPHOID CELLS FROM HETEROGENEOUS RETICULUM CELL SARCOMA OF SJL/J MICE. Marilyn Owens, Neelam Aggarwal and Benjamin Bonavida. Dept. Microbiol. and Immunol. U.C.L.A. Los Angeles, Calif. 90024

SJL/J mice have a characteristic high frequency of spontaneous reticulum cell sarcoma, a heterogeneous neoplasm arising in lymphoid organs. We have previously determined that these animals retain their immunocompetence and can respond to homogeneous syngeneic RCS culture lines in vitro. The role of lymphoid cells at the site of tumor growth, however, is not known and is crucial to the understanding of tumor progression. In this study, normal cells from involved lymph nodes and spleen of tumor-bearing mice were separated by a density gradient of ficoll and sodium metrizoate. Eight density solutions were loaded by steps, allowed to diffuse into a continuous gradient ($\rho$ range 1.06 - 1.08) and centrifuged for cell separation. Preliminary results reveal a single peak of normal cells ($\rho$ = 1.071) compared to two separate peaks of putative tumor cells ($\rho$ = 1.069 and 1.061). Further studies are in progress to determine the immunological reactivities of these normal cells from tumor nodes and their functional role in immune surveillance.

Supported by NIH grant CA 19753-01.

**1001** THE CONSEQUENCES OF IMMUNE SURVEILLANCE DURING CHEMICAL CARCINOGENESIS: PATHOGENESIS OF METASTASIZING MAMMARY TUMORS(MT). U. Kim, Roswell Park Mem.Inst., Buffalo, NY

The immune surveillance doctrine on cancer has been challenged by some investigators. However, a biweekly monitoring of MT development and their progression over an 18 month period in 3-methylcholanthrene fed young adult W/Fu female rats, verified much of the basic theorem of the doctrine. Thymectomy (Tx), splenectomy (Sx) or Tx + Sx increased MT incidence by 20-45 % over the intact control. Out of a total of 213 MT induced, 13 regressed spontaneously before reaching 10 mm in diameter. When an early appearing, putatively more antigenic MT was excised, 16 additional MT as large as 12 mm regressed. When MT were 10-15 mm, 93 of 213 MT were transplanted into syngeneic rats, and 3 of them were rejected. Out of 90 MT successfully transplated, 29 MT metastasized spontaneously to lymph nodes, and 37% of them came from the immuno-suppessed groups in which the excision of another MT was carried out. The excision caused some of dormant MT to grow faster, and such MT tend to metastasize and be nonantigenic, though they are histo-incompatible in allogenic rats. This experiment seems to reveal 4 classes immunologically distinct tumors: one that has antigen incompatible in autochthonous hosts (some may sneak through, but can be eliminated by immuno-stimulation of the host); one that grows well in autochthonous host but rejected by syngeneic rats; one that grows well in syngeneic rats and does not metastasize and carries TSTA, and one that metastasizes spontaneously in syngeneic rats and non-immunogenic. Thus, most human cancer that metastasize spontaneously may also have come through similar immunoselection as seen here. Characteristics of metastasizing and non-metastasizing MT cells will also be discussed.

**1002** EVIDENCE FOR THE PRESENCE OF ANTIBODY FORMING CELLS AGAINST SYNGENEIC THYMOCYTES. Yvonne E. McHugh and Benjamin Bonavida. Department of Microbiology and Immunology, U.C.L.A., Los Angeles, California 90024

Lipopolysaccharide (LPS), a polyclonal B cell activator, has been used as a probe to determine whether clones of B cells with specificities for syngeneic transplantation antigens are present in normal spleen. LPS activated spleen cells from various strains of mice, including BALB/c, C3H/HeJ and BALB/c nu/nu were tested for syngeneic and allogeneic reactivities in a modified plaque assay. Similar numbers of plaque forming cells were seen against syngeneic and allogeneic murine thymocytes regardless of the θ, TL or $G_{IX}$ antigens present and haplotype of the target donor. An additive effect was not observed when thymocyte targets from various strains were mixed although the LPS response has been shown to be polyclonal in our system. These results indicate that LPS activation of normal B cells generates antibody forming cells with reactivity against self antigens highly cross reactive with alloantigens other than those listed above. Further experiments were performed to examine the nature of the anti-thymocyte response. Spleen cells from murine strains other than C3H/HeJ generated the same reactivity described when activated with protein-free LPS; the C3H/HeJ strain could respond in a like manner only when regular LPS was used. These results indicate that the reactivity detected for thymocytes is probably not generated by activation of B cells with specificities cross reactive with the protein moiety of LPS. The in vivo state of these B cells with specificities for thymocyte antigens, and the immunoregulatory role they may play is not clear. Supported by Grant CA12800 from the NCI, NIH.

1003 ADHERENT CELL REGULATION OF MLC REACTIVITY IN AUTOIMMUNE MICE, C. Lopez, R. Wilmot, and N.T. Berlinger. Sloan-Kettering Institute, New York, N.Y. 10021

A/J strain mice demonstrate an increased incidence of autoimmune disease with increasing age. Splenic cells from A/J mice of various ages were cultured with autologous or allogenic irradiated spleen cells. The in vitro $^{3}$H-thymidine uptake increased significantly with increasing age. The high levels of $^{3}$H-thymidine uptake of splenic cells from aging mice could be reversed by thymic transplantation with thymuses from young adult A/J mice. Because thymic transplantation has been shown to reverse autoimmune disease in these mice, these data support the possibility that the $^{3}$H-thymidine uptake in spleen cell cultures may reflect cell-mediated autoreactivity. Removal of an adherent cell population by Sephadex G-10 filtration, reduces significantly the capacity of spleen cells from young adult A/J mice to maintain low $^{3}$H-thymidine uptake in cultures with cells from aging mice. If $^{3}$H-thymidine uptake reflects cellular autoreactivity, then the control system effected by the adherent cell may be important in the regulation of homeostasis of the cell-mediated immune response.

1004 SURVEILLANCE MECHANISMS RESPONSIBLE FOR THE SUPPRESSION OF THE AUTOIMMUNE RESPONSE TO MODIFIED SELF ANTIGENS. D. Noar, Immunology Dept., Hebrew University, Israel.

NZB and NZB/W mice have reduced anti-SRC and TNP PFC responses with age after injection either the thymus dependent antigen TNP-SRC or the thymus independent antigen TNP-MRC. However, the thymus dependent response reduced much faster than the thymus independent response. As a consequence, young New Zealand mice have higher anti-TNP response after injection of TNP-SRC than after injection of TNP-MRC, while old New Zealand mice have higher anti-TNP response after injection of TNP-MRC than after injection of TNP-SRC. The avidity of PFCs of NZB.W mice injected with TNP-SRC diminished with age, while the avidity of PFCs of mice injected with TNP-MRC did not change with age. Young NZB/W did not produce anti-MRC PFC after injecting either TNP-MRC or TNP-SRC. Old NZB/W mice had few spontaneous anti-MRC PRCs. The number of anti-MRC was increased 4 to 10 times after injecting old mice with either TNP-SRC or TNP-MRC.
It is suggested that surveillance mechanisms are responsible for suppressing the autoimmune response to modified self antigens. The unregulated immune system of NZB and NZB/W mice appear to be an expression of the impairment of such a hypothetical surveillance mechanism. This conception is supported by the facts that both aged (B10C3)$F_1$ and nude mice produced self anti MRC response after injection with TNP-MRC, while young (B10C3)$F_1$ mice and the littermate controls of the nude mice did not.

1005 MONOCLONAL ANTIBODIES TO XENOGENIC CELL SURFACE ANTIGENS, Ronald Levy and Jeanette Dilley, Stanford University School of Medicine, Stanford, CA 94305

The cell surface antigens of human lymphoid cells are being analyzed by monospecific antibodies generated in vitro. Mice were immunized with homogeneous populations of human B cells, peripheral blood lymphocytes from patients with chronic lymphocytic leukemia (CLL). Limiting dilutions of spleen cells from immunized mice were transferred intravenously to syngeneic, irradiated recipients. Spleen fragments from recipient animals were cultured in vitro by the method of Klinman and stimulated with CLL cells. These cultures produced antibody to human cell surface antigens. The antibodies produced were monoclonal by the following criteria: (1) the frequency of antibody producing fragments was linearly related to the number of immune cells transferred; (2) isoelectric focusing analyses showed the antibodies to be of extremely limited heterogeneity; (3) their reactivity patterns against panels of target cells, including normal and CLL cells from other individuals, defined unique reactivities. The availability of such monoclonal, and therefore monospecific, antibodies should provide a new approach to the analysis of cell surface antigens. (Supported by the American Cancer Society Grant Nos. 782 and IM 114)

**1006** SPLENIC REGULATION OF A CYCLICAL PFC RESPONSE IN RABBITS. C. G. Romball, R. J. Ulevitch and W. O. Weigle, Scripps Clinic and Research Foundation, La Jolla, CA. 92037

The PFC response in rabbits to a single intravenous injection of aggregated human gamma globulin (AHGG) provides an example of the complex factors regulating immune responses. In this response, PFC appear in the spleen in a cyclical manner, with a peak on day 5 and a second peak on day 13. In contrast, subcutaneous footpad injection of antigen results in a linear appearance of PFC in the draining node during the same time period. However, the node response can be converted to a cyclical response by simultaneous intravenous injection of antigen in normal, but not splenectomized rabbits, demonstrating a major role of the spleen in the regulation of this response, even in distal lymphoid organs. Cyclical variations in the immune response might be expected to play a role in conserving the immune potential toward those antigens which persist in the body for prolonged periods of time. In fact, a correlation between the continued presence of radiolabeled HGG in the follicles of the spleen and the appearance of the second peak of PFC has previously been observed. Since injection of the anticomplementary protein, cobra venom factor (CoF), has been shown to interfere with localization of AHGG in spleens of mice, the effect of prior injection of CoF was investigated in this system. Direct and indirect PFC to AHGG in CoF-treated rabbits did not differ significantly from control rabbits on day 5, although C3 levels were depleted to less than 10% of pretreatment levels. However, PFC on day 13 were markedly reduced in CoF-treated rabbits, although the cyclical nature of the response was maintained. Day 13 PFC were not decreased in CoF-treated rabbits if injection of CoF was delayed until 3 days after antigen injection, when antigen localization has already occurred.

**1007** CAN THE COLLABORATION BETWEEN ANTIGEN SPECIFIC LYMPHOCYTES IN VIVO BE EFFECTED BY DIRECT CELL CONTACT? George I. Bell, Los Alamos Scientific Laboratory, University of California, Los Alamos, New Mexico 87545

Data on lymphocyte traffic within lymphoid tissue have been reviewed in order to estimate the rate at which a recirculating lymphocyte will collide with relatively sessile cells in vivo. In a lymph node, a rate of about 200 encounters per cell per hour is obtained. Assuming that antigen-specific B and T cells represent $10^{-4}$ of their respective populations, a collision frequency between antigen specific T and B cells, of $\lesssim$ 400 per hour per gram of lymph node tissue is obtained. This could be sufficient to ensure collaboration in a single sheep node or a whole mouse but may not suffice with a single mouse node. It is suggested that mobile B cells interact with T cells, which have become stuck by antigen to reticular cells in the paracortex of a node.

A few antigen bridges between two lymphocytes can establish a firm enough bond to resist hydrodynamic forces in vivo or in vitro. However, other forces between the cells may be important. In particular, a differential adhesiveness between lymphocytes based on their histocompatability might be important and would lead to differences in collaboration between lymphocytes from inbred and outbred animals, as well as between histocompatible and incompatible cells.

**1008** IMMUNE REGULATION IN "VIRGIN" LYMPHOCYTE POPULATIONS: EXPERIMENTAL FACTS AND THEORETICAL MODELS. Garnett Kelsoe and Jan Cerny, Depts. Trop. Pub. Health and Micro., Harvard School of Public Health, Boston, Massachusetts 02115.

We demonstrate the existence of active regulator cells in non-immunized mice by the use of Marbrook cultures containing splenic lymphocytes from two individual, syngeneic mice (A and B). The primary plaque forming cell (PFC) response of mixed cultures (5 x $10^6$ A cells plus 5 x $10^6$ B cells) to immunization with Ficoll(AECM)-TNP was compared to the response of cells from each individual alone ($10^7$ A cells or $10^7$ B cells). Contrary to the expectation, the response in the mixed cultures was always non-additive (not equal to a response $[\overline{A}+\overline{B}]$x0.5), i.e. being either suppressed or enhanced as compared to the averaged response from the individual cultures. Experiments with several pairs of mice revealed that: (1) suppression occurs at 70% and enhancement at 30%, approximately; (2) in titration experiments, it appeared that cells from the higher responder were suppressive and those from the lower responder were "enhancing"; and (3) the regulation was independent for two non-cross-reacting antigens. These results can be explained solely by postulating a continuous dynamic equilibrium between responder and regulator cells, each cell population oscillating through time about an ideal value. Mathematical treatment of these sinusoids generates predictions on the relationship between responder and regulator cells following antigenic stimulus and the nature of immunological memory. Further, the existing theories of regulation by linear immune networks has been modified by postulating small, circular subunits in the network. Mathematical and immunological considerations of models utilizing units of two, three, or four mutually reactive cells will be discussed.

**1009** DEFICIENCES OF IMMUNOREGULATION IN HAMSTERS AND THE MAJOR HISTOCOMPATIBILITY COMPLEX
By: J.W. Streilein, W.B. Duncan, and D. Lause, UTHSCD, Dallas, TX 75235

Developing notions about mechanisms of immunoregulation emphasize the important roles of subsets of lymphocytes and macrophages as well as the cell surface determinants which are thought to govern their various interactions. In Syrian hamsters, several aspects of regulation appear to be defective: (1)the anamnestic response to sheep RBCs is comprised of excessive numbers of IgM secreting cells, and relatively fewer cells secreting IgG antibody; (2) specifically sensitized lymphocytes respond to strong alloantigenic stimulators with unrestricted clonal expansion, and no evidence for suppression of this response in GVHR or MLR can be found; (3)hapten modification of syngeneic target cells fails to elicit the production of specific effector T cells,or does so only very poorly. Each of these deficiencies involves the interactions of at least two categories of cells, interactions which are promoted in one way or another by gene products encoded for by the major histocompatibility complex(MHC). Our evidence concerning the MHC in hamsters (Hm-1) suggests that all hamsters currently in experimental use are either identical for serologically defined determinants (analogous to murine K and D), or fail to express these determinants appropriately. Moreover, the alloantigens which are encoded for by alleles at Hm-1, and give rise to skin graft rejection,GVHR and MLR, appear to be expressed only on thymus derived cells. We can not find them on fibroblasts,tumor cells, or B lymphocytes. We propose that the ineffective cellular interaction which characterizes the hamster immune response is directly related to the apparently capricious expression of MHC gene products on some T lymphocytes, and not on B lymphocytes and perhaps other T lymphocytes.

**1010** REGULATION OF HUMAN IMMUNOGLOBULIN PRODUCTION BY T HELPER AND T SUPPRESSOR CELLS.
Ronald H. Stevens and Andrew Saxon*. UCLA School of Medicine, Los Angeles, Ca. 90024.

Human peripheral blood lymphocytes can be stimulated by pokeweed mitogen to produce IgM, IgG, IgA immunoglobulins (Ig). This Ig production was shown to be totally T cell dependent and macrophage independent. T lymphocyte help was not dependent on DNA synthesis and was mediated through both B cell clonal expansion and increased B cell maturation. Suppression of Ig production by T cells was also effected through the same pathways, but required prior DNA synthesis. Allogeneic and autochthonous T lymphocytes were equally effective in providing both helper and suppressor function. Isolated T cells pre-activated for 5 days by pokeweed mitogen functioned as helpers in B cell Ig production in the absence of further stimulation with pokeweed mitogen. Under the same conditions suppressor cells did not appear unless a second stimulation with mitogen was given when the T cells were added to B cells. These results demonstrate that T helper and T suppressor lymphocytes are distinct populations in normal human peripheral blood and that they differ in the conditions necessary for final differentiation and functional expression. Immature T cells (thymocytes) were unable to mediate either helper or suppressor function.
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**1011** REPRESSION AND DEREPRESSION OF IgM SYNTHESIS. Harvard Reiter, Pei-Ling Hsu, and Sheldon Dray. Dept. Microbiol. Univ. of Illinois Med. Ctr., Chicago, IL 60680.

We found that IgM synthesis was 90% suppressed in cultures of rabbit lymph node or spleen cells treated for 20 hr with 40 μ/ml antibody to the b4 allotype of κ light chains (anti-b4 Ab). IgG synthesis was not greatly affected. Acrylamide gel analysis of the residual Ig synthesized showed that anti-b4 Ab suppressed both light (L) and heavy (H) chain synthesis. Similarly, antibody to the a-allotype of heavy chains also suppressed both L and H chains. The number of cells committed to the suppressed state increased between 4 to 12 hr of treatment, and there was a transient increase in protein synthesis during the 6 to 20 hr interval of treatment. After 20 hr, total non-Ig protein synthesis and the synthesis of a specific non-Ig protein, adenosine deaminase (ADA), was only slightly higher in treated cultures than it was in control cultures. ADA synthesis was the same in isolated B cells from suppressed or control cultures. Almost all adsorbed $^{125}$I-labeled anti-b4 on treated cells was degraded and released into the medium. The cells retained a small amount of labeled Ab fragments bound mostly to the cell membranes. Pronase stripped 75% of the bound $^{125}$I from the cells, and it also reversed suppression. We conclude that there is a specific, coordinate repression of L and H chain Ig synthesis in antibody-treated IgM synthesizing cells. Derepression may have occurred because of the removal of repressing molecules, or because of a membrane stimulus that overrode an internal represser signal. This "on-off" system provides a model for the study of the molecular events that regulate Ig synthesis.

**1012** INDUCTION OF ALLOTYPE SUPPRESSING CELLS IN BALB/c MICE, Raimund Di Pauli and Cornelia Kolb*, The Salk Institute, San Diego and *University of Konstanz, West Germany.

Spleen cells producing antibodies against the polysaccharide from S. Strassbourg were transferred from BALB/c-$Ig^b$ either into normal syngeneic mice or into BALB/c-$Ig^a$ mice. The two strains are congenic and differ only at the immunoglobulin allotype locus. The recipients were challenged with the antigen and the production of donor antibody judged by isoelectric focusing. After about four weeks the BALB/c-$Ig^a$ recipients had no antibody at all, according to the IEF, in their serum, whereas the syngeneic BALB/c-$Ig^b$ showed clearly the donor IEF spectrotype. The BALB/c-$Ig^a$ also showed no donor $Ig^b$ allotype in their serum. Lymphoid cells from the BALB/c-$Ig^a$ recipients were transferred into different $Ig^b$ mice congenic with BALB: a) irradiated BALB/$Ig^b$; b) newborn (BALB/c-$Ig^a$xBALB/c-$Ig^b$) F1; c) BALB/c-$Ig^b$ nudes. Group a) and b) were injected with spleen cells only. Nude mice received either spleen cells or thymus cells. In all groups the level of the $Ig^b$ recipient allotype dropped a 100 to a 1000-fold from the original level and remained at these undetectable levels for over 9 months. Controls receiving normal BALB/c-$Ig^a$ cells did not show any change in their own allotype level. We measured mainly the $IgG2a^b$ level, but could also not demonstrate any $IgG1^b$ in the suppressed mice. This suggests that in this system more than one class is suppressed. Since we could transfer the suppressive activity with thymus cells, it is probable that the suppressing cells are thymus derived.

**1013** GENETIC DIFFERENCES RESPONSIBLE FOR POSITIVE ALLOGENEIC EFFECTS. Peter Panfili and R. W. Dutton, Dept. of Biology, Univ. of California, San Diego, La Jolla, CA 92093.

The effect of allogeneic T cells in the generation of a primary anti-SRBC antibody response in vitro is being investigated.

The basic system employs cocultivation of B cells (anti-mouse brain-treated spleen), T cells (nylon wool passaged spleen) and SRBC in microtest plate wells. Direct PFC are assayed on day 4.

Under these conditions allogeneic T cells can provide help. Such help may be different from that delivered by syngeneic T cells. More PFC are generated in the presence of allogeneic T cells than with identical numbers of syngeneic T cells. Furthermore, allogeneic help dilutes out much later than does syngeneic help. Nor is allogeneic help diminished by pretreatment with mitomycin C. To distinguish this from syngeneic help, this allogeneic help is termed a Positive Allogeneic Effect.

Velocity sedimentation of T cells, which separates on the basis of size and has been used to separate functional subpopulations of T cells, shows similar positive allogeneic effect activity in all gradient fractions.

Minimum genetic differences necessary to generate a positive allogeneic effect are being investigated. An H-2 region difference appears necessary and sufficient. Curiously, K and/or D gene differences alone generate positive allogeneic effects.

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**1014** GENETIC REGULATION OF IgE ANTIBODY HETEROGENEITY, David G. Marsh and Nicholas Willcox, Johns Hopkins Univ. Sch. of Med., Baltimore, Md 21205 and Univ. of Newcastle, England

Total serum IgE in man appears to be regulated primarily by a single autosomal gene, R/r, where the low IgE trait is dominant over the high (Marsh et al, PNAS 71:3588, 1974). We now propose that the IgE-regulator allele (R) limits the number of IgE antibody-producing clones that are stimulated to respond to antigen, resulting in a restriction in the heterogeneity of the antibody response. Our evidence is as follows: (i) Associations between specific HLA types and specific IgE antibody responses to highly purified allergens (2 cases) are much more pronounced in allergic people with low total IgE levels (below 100-130 U/ml) than those with high IgE levels. Probably, only one HLA-associated Ir gene allows recognition of antigen in the former case, but several different Ir genes are involved in the latter. (ii) Allergic subjects with high total IgE levels are sensitized naturally to a greater number of antigens, and are more easily sensitized artificially to new antigenic determinants, than are allergic people with low IgE levels. (iii) Recent experiments by Gleich et al. show that IgE antibody production is more readily suppressed by immunotherapy in patients with low than in patients with high IgE levels. (iv) Finally, a striking analogy can be drawn between the genetics of total IgE levels in man and the genetics of the heterogeneity of IgG antibody responses in rabbits to streptococcal polysaccharide antigens (cf. Eichmann et al, JEM 134:48, 1971). High IgE levels and heterogeneous IgG responses both appear to be inherited as autosomal recessive traits. Recognition of the importance of these regulatory phenomena should greatly facilitate genetic studies of specific immune responses in outbred species.

**1015** CELL MEDIATED IMMUNE RESPONSE IN HUMANS AFTER VACCINATION WITH VACCINIA VIRUS, L. H. Perrin, J. M. Hull, R. M. Zinkernagel and M. B. A. Oldstone, Scripps Clinic and Research Foundation, La Jolla, Ca. 92037

To study the role of T cells and major histocompatibility markers in human viral infection, 10 humans were vaccinated with vaccinia virus. All showed primary like responses with local induration and lymphadenopathy. Their PBLs were tested sequentially from day 0 to 12 after vaccination against $^{51}Cr$ labeled autologous or homologous fibroblasts and heterologous mouse L cells infected or not with vaccinia virus. A peak of specific lysis of the vaccinia infected cells occurred between day 5 and 10. Specific lysis was not dependent on the presence or absence of HLA-A or HLA-B compatibility between effector lymphocytes and target cells; neither was the specific lysis increased in autologous conditions. Infected murine L929 cells were also lysed. Specific lysis was detected at PBLs to target ratio as low as 2.5:1 in 8/10 individuals and greater than 40% specific lysis in 6/9 at 10:1 ratio. T cells isolated by sheep erythrocyte rosetting technique in 4 individuals induced minimal lysis whereas the fraction of depleted T cells showed a 2-4x enrichment over the lytic activity of the unfractionated PBLs. We conclude that the effector cell present in PBLs of humans after vaccination with vaccinia virus is a non T cell. Other experiments showed that the addition of IgG purified from individuals recently vaccinated against vaccinia induced a specific lytic activity by normal adult HPLs against vaccinia infected cells, whereas no detectable killing occurred after absorbtion of specific Ig or when either immune IgG or HPLs alone were added to virus infected targets. (Supported by USPHS AI-07007, NS-12428 and Swiss M. S. Society)

**1016** COMPARISON OF THE IN VITRO ADJUVANT ACTIVITIES OF BACTERIAL LIPOPOLYSACCHARIDE AND A BACTERIAL CONDITIONED MEDIUM (BacCM). S. Shiigi, R. Capwell, K. Grabstein, and R. Mishell. Dept. of Bacteriology and Immunology, Univ. of Calif., Berkeley, Calif.

We hypothesized that bacterial contamination of FCS during processing prior to membrane filtration plays a major role in determining which sample of sera support in vitro primary humoral responses. A Gram-negative, psychrophilic (cold growing) bacteria was isolated from a supportive lot of sera. Growth of this bacteria in a deficient, non-supportive sera converted the sample into a supportive sera. We then compared the in vitro adjuvant and mitogenic activities of medium conditioned by the growth of the bacteria (BacCM) with that of bacterial LPS to determine whether the active factor in BacCM was LPS. Both BacCM (1% to 10% dose range) and LPS ($10^{-4}$ to $10^{-1}$ ug/ml dose range) significantly stimulated the humoral responses of $BDF_1$ spleen cells cultured in deficient serum. This stimulation was shown to be antigen dependent. Although optimal doses of BacCM and LPS stimulated in vitro humoral responses equally, BaCM was significantly less mitogenic than LPS in cultures of $BDF_1$ spleen cells. We then showed that BacCM strongly stimulated the humoral responses of $C3H/HeJ$ spleen cells cultured in deficient serum. These spleen cells were unresponsive to LPS. We therefore conclude that the adjuvant activities of BacCM are due to bacterial products other than LPS. This adjuvant appears to be significantly less mitogenic than LPS and may therefore act via different mechanisms than does LPS.

**1017** SOMATIC CELL HYBRIDS EXPRESSING BOTH T CELL AND I-REGION MARKERS, Richard A. Goldsby, Barbara A. Osborne, Donal Murphy and Leonard A. Herzenberg, Ames Research Center, Moffett Field, CA., and Stanford University School of Medicine, Stanford, CA.

Hybrid populations have been obtained by PEG 1000 assisted fusion of BW5147, a HAT sensitive mouse lymphoma, with $(SJL \times BALB/c)F_1$ normal spleen cells and subsequent selection in HAT medium containing $3.3 \times 10^{-4}$ M ouabain. Microcytotoxicity tests demonstrated that several of the hybrid populations were of the T cell type bearing the theta antigen of both parents (Thy 1.1 of BW5147 and Thy 1.2 of the normal parent). Interestingly, a search for the presence of I-region markers of the s haplotype using microcytotoxicity employing A.TL anti-A.TH plus rabbit complement revealed that these same populations expressed I-region markers on their surface. The specificity of the A.TL anti-A.TH killing was confirmed by absorption of A.TH anti A.TH on tissue from B10.S(7R) and B10.BR. Absorption on B10.S(7R) removed all cytotoxic activity whereas absorption on B10.BR failed to remove any cytotoxic activity. These studies demonstrate that lymphoma x normal spleen cell somatic cell hybrids provide a means of producing pure populations of cells expressing various T cell surface markers including I-region markers of a specific haplotype. Such cell populations should facilitate biochemical studies of these markers. Furthermore, T cell hybrids may be expected to be of assistance in studies of the detailed cell biology of T cell function.

1018 FETAL THYMUS FUSIONS:EFFECTS OF ALLOGENEIC VERSUS SYNGENEIC FUSIONS.

D. DeLuca, G.A. Luckenbach, T. Mandel and M. Kennedy, Walter and Eliza Hall Institute of Medical Research, Melbourne, Victoria, Australia.

Fetal thymus lobes from 14d CBA or BALB/c mice have been grown as fusions in organ culture. After 24 hr in vitro no obvious separation is found between the lobes in histological sections. Syngeneic (CBA/CBA or BALB/BALB) or allogeneic (CBA/BALB) fusions have been used in an attempt to study the generation of self-tolerance by a developing lymphoid organ in vitro. Syngeneic fusions produce two peaks of lymphoid cell growth.The first peak, seen at days 5-7, has been shown in single lobe cultures to consist primarily of cortisone sensitive, "high Θ", "high TL" cells; and the second peak (days 10-12) is primarily cortisone resistant, "low Θ", and TL negative. $^{125}$IUDR incorporation studies show that a burst of DNA synthesis precedes each lymphoid peak. Allogeneic fusions have a normal first peak of cell growth, but consistently lack the second peak. Semi-allogeneic fusions (e.g. CBA vs. CBA/BALB $F_1$) can produce a normal second peak of growth, but the cells in this peak are primarily of the parental type, as assessed by immunofluorescence with anti-H-2 sera.