IMMUNOGLOBULIN IDIOTYPES AND THEIR EXPRESSION

Charles Janeway, Eli E. Sercarz and Hans Wigzell, Organizers

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Keynote Address

001 STRUCTURES OF IDIOTYPES, Michael Potter, National Cancer Institute, National Institutes of Health, Bethesda, MD 20205

Structural diversity of immunoglobulins is primarily generated by the multiplicity of V-region genes and combinations of V_L and V_H genes, but is amplified by rearrangements of V-region genes and somatic mutations. Two products of structural diversity are the diversity of binding sites and the formation of potential antigenic structures (idiotypes). Idiotypes are useful markers but also have important biological properties, as potential autoantigens. The principle of conformational homology of V-region domains makes it possible to generate plausible hypothetical models of Fab regions. We have recently made a number of models on the Evans and Sutherland 3-dimensional picture system. We describe how the models are made. We will describe with space filling 3-dimensional models the putative idiotypic structures associated with β1,6D galactan, inulin, phosphorylcholine and α1,3 dextran binding myeloma proteins in mice. In each of these systems the primary structures of two or more closely related proteins are available. The models permit possible identification of some of the epitopic regions associated with the primary structural changes.

Immunoglobulin Genes: Rearrangement and Transcription

002 MOUSE AND HUMAN IMMUNOGLOBULIN LIGHT CHAIN GENES. P. Hieter*, S. Korsmeyer+, J. Maizel*, E. Max*, T. Waldmann+ and P. Leder*. *NICHD and +NCI, National Institutes of Health, Bethesda, Maryland 20205.

Kappa light chain diversity arises in large part from a site specific DNA recombination event that joins one of several hundred germline V-region genes to one of four active J segments. The combinational power of this system is increased by a recombination mechanism which allows variation in the crossover point. Although the flexible nature of this recombination event generates increased diversity in a coding region critical to the formation of the antigen combining site, it also leads to aberrantly rearranged genes which cannot produce a functional light chain product. We have extended our studies to the human κ and λ light chain gene systems since, in contrast to the mouse, the λ gene system contributes significantly to antibody diversity in man. The κ and λ light chain genes of man, including several genes in their active (rearranged) configuration, have been cloned using corresponding mouse gene segments as probes under low stringency hybridization conditions. The mouse and human genes have selectively conserved sequences which are thought to be involved in DNA and RNA splicing reactions. The human λ light chain genes constitute a complex locus that includes at least four λ constant region genes encoding the four non-allelic human λ light chains. In addition we have identified several polymorphisms in restriction sites surrounding these genes that should provide useful human gene markers. The cloned human gene segments have been used as probes to study the arrangement of light chain genes in a variety of human B cell lines and lymphocytic leukemias that represent discrete stages in the development of an immunocyte. The results suggest that the process of light chain gene rearrangements, which appears to involve the frequent deletion of the κ constant region gene, may be ordered so as usually to progress from the κ to the λ genes.

003 SOMATIC GENERATION OF IMMUNOGLOBULIN GENES, Susumu Tonegawa, Hitoshi Sakano, Yoshikazu Kurosawa, and Richard Maki, Basel Institute for Immunology, Postfach, 4005 Basel 5, Switzerland

It has been well established that complete immunoglobulin genes are generated by somatic DNA recombination(s) that occurs during the development of B lymphocytes. In the light-chain genes of the mouse, in both λ and κ types, the last 13 CO_2H terminal residues of the variable region are encoded by a separate DNA segment called J DNA (J for joining) in the germ-line genome. Coding information for the rest of the V region is contained in an embryonic V DNA segment. Accordingly, somatic recombination occurs between the 3' end of the V DNA and the 5' end of the J DNA to generate a complete light-chain V gene. In contrast, a heavy-chain V region is encoded in three separate DNA segments, V, D and J, in the germ-line genome and an assembly of the three DNA segments is necessary for generation of a complete V gene. The D DNA segment codes for the major portion of the third hypervariable region or complementarity-determining residues. Recently we have identified a series of germ-line D DNA segments and characterized the structure and organization of these DNA segments. We will discuss the role of the D DNA segments in somatic diversification of antibody repertoire.

While the aforementioned DNA rearrangement and the "switch" recombination play key roles in control of Ig gene expression in B cell development, differential processing of the primary RNA transcripts also regulates gene expression. We will describe this latter mechanism with respect to the simultaneous synthesis of two classes of immunoglobulins in a single cell.

004 THE REARRANGEMENTS OF ANTIBODY GENES, Leroy E. Hood, Stephen T. Crews, Philip W. Early, Johanna A. Griffin, Henry V. Huang, Stuart K. Kim and Carol W. Readhead, Division of Biology, California Institute of Technology, Pasadena, CA 91125

Antibody molecules are comprised of light (L) and heavy (H) chains which fold into discrete molecular domains which carry out two categories of functions—pattern recognition or antigen binding (the variable [V] domain) and effector functions such as complement fixation (the constant [C] domains). Light chains are encoded by several discrete coding segments or exons that are separated by intervening sequences—leader (L), variable (V), joining (J), and constant (C). Heavy chains are encoded by these exons as well as several additional exons—diversity (D) and membrane (M). Two distinct types of DNA rearrangements occur during the differentiation or antibody-producing or B cells. (1) V-J (or V-D-J) joining juxtaposes the various gene segments that encode the variable region in association with the C_μ gene. (2) Then C_H switching may displace the V region coding elements to any other class or subclass of C_H gene. The DNA recombination mechanisms that mediate each of these types of DNA rearrangements are quite distinct and clearly are developmentally controlled. In addition, several aspects of antibody expression require an RNA splicing mechanism. I will discuss our latest data on the organization and rearrangement of heavy chain gene segments and upon RNA splicing with special emphasis on the various combinatorial mechanisms the vertebrate immune system has employed to amplify information. I also will discuss some of the mechanisms whereby DNA rearrangements and somatic mutation lead to antibody diversification.

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005 THE CONTROL OF IMMUNOGLOBULIN HEAVY CHAIN GENE EXPRESSION BY RNA PROCESSING. Randolph Wall, Carla Carter, Linda Clayton, John Rogers and Lawrence Souza. Molecular Biology Institute and Department of Microbiology and Immunology, UCLA School of Medicine, Los Angeles, Ca. 90024.

DNA rearrangements are clearly involved in the changing patterns of immunoglubulin gene expression during B-lymphocyte development. Here we describe recent findings which indicate that RNA processing also plays an important role in controlling immunoglobulin gene expression in the development of the immune response. This was first seen in studies which showed that the expression of membrane or secreted IgM is regulated by RNA processing events which generate μ mRNAs with alternative 3'-sequences coding for either membrane (μ_m mRNA) or secreted (μ_s mRNA) C-termini (1,2). In the μ gene, the μ_s C-terminal coding sequences are contiguous with the C_H4 domain, while the μ_m C-terminal sequences (the M exons) are located approximately 2 kb (kilobases) on the 3'-side of the C_H4 domain. In the nuclear processing pathway for μ_m mRNA, the M exons are spliced into the sequence GGT-AAA (coding for Gly-Lys) at the end of the C_H4 domain, thereby replacing the μ_s C-terminal sequences. We have proposed that the production of μ_m or μ_s mRNA is regulated by the choice of alternative poly(A) addition sides in the μ transcription unit.

Because all immunoglobulin heavy chain chains now sequenced at either the DNA or protein level have GGT-AAA or Gly-Lys at the end of their final domain, we previously predicted that all heavy chains might use the RNA processing mechanism just described to generate membrane or secreted forms. We have now confirmed that all subclasses of mouse γ heavy chains have membrane C-terminal gene segments located several kb to the 3'-side of the different C_H3 domains.

Finally, our recent findings indicate that the simultaneous expression of μ and δ chains in IgM and IgD on the surface of B lymphocytes is also achieved through RNA processing mechanisms. The mouse C_δ gene has been isolated (3) and used to map RNAs in μ-, δ- producing mouse cell lines. We find that these cells produce both μ_m and μ_s mRNAs and two δ mRNAs which contain alternative 3'-sequences derived from separate, noncontiguous gene segments located 3-7 kb beyond the 3' end of the δ C_H3 domain. These four immunoglobulin mRNAs appear to be generated from a single large transcription unit through post-transcriptional processing choices of alternative poly(A) sites and alternative RNA splicing pathways.

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Immunoglobulin Diversity: Sequence and Idiotype

006 IDIOTYPIC ANALYSIS OF MURINE ANTI-ARSONATE ANTIBODIES, Mark Siegelman, Clive Slaughter, Pila Estess, Larry McCumber, Antonio Pacifico, Debbie Jeske, Stella Robertson and J. Donald Capra, Department of Microbiology, The University of Texas Health Science Center at Dallas, Dallas, Texas 75235

Efforts in our laboratory over the past ten years have been devoted to the serological and structural dissection of an induced antibody system in an attempt to understand its molecular and genetic basis. After the complete sequence analysis of the induced serum anti-arsonate antibodies was completed, we turned our attention to the products of B cell hybridomas. At the present time, the amino acid sequence of four CRI positive and one CRI negative B cell hybridoma products is underway. These studies are being done in an attempt to localize the idiotypic determinants in this inherited idiotypic system.

T cell hybrids have also been produced between spleen cells from A/J mice suppressed for arsonate DTH and the BW51 thymoma cell line. Analyses of the T cell hybrids and their non-immunoglobulin, antigen binding products will be presented.

007 MURINE ANTI-DEXTRAN ANTIBODIES: SEQUENCES AND IDIOTYPES, J. Davie, B. Clevinger, J. Schilling and L. Hood, Washington University, St. Louis, MO 63110 and California Institute of Technology, Pasadena, CA 91125

Mice of the $IgCH^a$ allogroup, after immunization with dextran B1355, produce large amounts of IgM and IgG3 antibodies specific for $\alpha(1\rightarrow3)$ dextran. Analysis of serum anti-dextran antibodies showed them to be a heterogeneous but related family of molecules; essentially all possessed λ light chains and half shared a cross-reactive idiotypic determinant (IdX) with the dextran-specific myeloma proteins, M104 and J558. Individual idiotypic determinants, IdI(M104) and IdI(J558), were found in most antisera, but accounted for a small minority of the antibodies. Furthermore, amino-terminal sequence analysis of the heavy chains from IdX positive and IgX negative pools of antibody showed identity through the first hypervariable region. If λ light chains are relatively uniform as thought, most diversity in the anti-dextran repertoire was expected to be found in the carboxy-terminal half of the V_H region (1).

Amino acid sequence analysis of hybridoma proteins specific for dextran has confirmed these conclusions and provided insight into the mechanism of diversification (2). Twenty IgMλ proteins have been studied. Amino acid sequence diversity patterns divide the V_H regions into three distinct segments - V segment (1-99), D segment (100-101) and J segment (102-117). These have been shown subsequently to be encoded by distinct genetic elements. The 20 V_H regions are composed of 4 V segments, 11 D segments and 4 J segments that appear to segregate randomly. Only 2 possible instances of single base mutation were found. This suggests that most antibody diversity arises from the combinatorial joining of germline genes and not from base changes within these genes.

Idiotypic determinants can be localized with precision (3). IdX was found associated with 2 amino acids in the second hypervariable region. Interestingly, one of these positions had an associated carbohydrate side chain. Three of four V segments possessed IdX determinants. The IdI(M104) and IdI(J558) determinants were localized to the D segment.

Thus, it is clear that the anti-dextran repertoire is very large (no two proteins have been found which were identical) and yet composed of proteins that share elements. It remains to be determined what the functional consequences of the limited diversity might be.

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008 1. SPONTANEOUS CLASS SWITCH VARIANTS IN MYELOMA AND HYBRIDOMA CELLS - A SUMMARY
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We have isolated by fluorescence activated cell sorting spontaneous somatic variants of myeloma and hybridoma cells with changed immunoglobulin isotype. The following "forward" switches were observed: $\mu \rightarrow \delta$; $\gamma 3 \rightarrow \gamma 1$; $\gamma 1 \rightarrow \gamma 2b \rightarrow \gamma 2a \rightarrow \alpha$. In addition, a variety of double producers and phenotypic revertants were found in the case of MOPC 21 variants. Forward switches occurred with a frequency of ca. 10^{-6} per cell per generation. Reversion was 10 times more frequent. The variants express a complete heavy chain constant region of a given isotype together with the V-D-J-region of the wild type, and at least some of the switches are accompanied by C gene deletion (K. Beyreuther et al., P. Schreier et al.). The mechanism of reversion is still a matter of speculation.

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2. NETWORK CONTROL IN THE EXPRESSION OF NP-SPECIFIC ANTIBODIES. K. Rajewsky, G. Kelsoe, M. Reth and T. Takemori, Institute for Genetics, University of Cologne, Cologne, F.R.G.

Antibody B1-8 is a $\lambda 1$-bearing IgM with specificity for the hapten 4-hydroxy-3-nitro-phenylacetyl (NP). Several classes of idiotopes can be defined on the B1-8 molecule by monoclonal anti-idiotope antibodies. These idiotopes are expressed in the anti-NP response of C57BL/6 mice in various combinations on a family of related, but individually distinct λ-bearing antibodies (c.f. Reth et al., this volume). The injection of minute amounts (in the nano-microgram range) of anti-idiotope antibodies (including autologous ones) results in striking enhancement or suppression of idiotope expression in a subsequent anti-NP response. These effects may depend upon: the dose and class of injected antibody, the time of injection in ontogeny and the interval between injection and challenge with antigen. The various idiotopes of B1-8 are largely co-regulated, but the pattern of co-regulation is complex and depends on both the specificity of the regulating anti-idiotope and the time of injection in ontogeny. The injection of 10 µg of B1-8 itself (instead of anti-B1-8 antibodies) results in enhancement of B1-8 idiotope expression in a subsequent anti-NP response - immunological memory determined by network interactions. The system allows us to study rules of network control as well as the development of the network and the expression of antibody diversity in ontogeny.

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009 ANTIGEN BINDING MUTANTS OF MOUSE MYELOMA CELLS, Matthew D. Scharff, Wendy Cook, Angela Giusti, Sau-Ping Kwan, Pallaiah Thammana, Dale E. Yelton, Donald Zack and Stuart Rudikoff, Department of Cell Biology, Albert Einstein College of Medicine, Bronx, New York 10461.

Mutations in immunoglobulin production arise spontaneously and frequently in cultured mouse myeloma cells and hybridomas. Many of these mutations result in changes in the structure of the immunoglobulin polypeptide chains. In order to determine if such somatic mutations could affect antigen binding, the S107 cell line, which produces an IgA kappa immunoglobulin that binds phosphocholine and bears the T-15 idiotype, was examined for variants with changes in PC-binding. Such variants arise with a spontaneous frequency of 0.1-1.0%. A family of these variants has been generated and the mutant proteins show changes in antigen and hapten binding, variable region serology and amino acid sequences. Most of the mutant proteins are of normal size and are normally assembled. They differ from each other by a few tryptic peptides in their heavy chain and in at least one case, a single amino acid substitution has occurred in the J segment. The mutation rate for one set of mutants has been determined by fluctuation analysis to be approximately 10^{-4}/cell/generation.

Idiotypic T Lymphocyte Receptors and Products

010 USE OF HETEROANTISERA TO T CELL ANTIGEN BINDING PROTEINS AS PROBES FOR THE T CELL RECEPTOR. R.E.Cone, J.H. Murray, R.W. Rosenstein, W. Ptak, G.M. Iverson and R.K. Gershon. Yale University School of Medicine, New Haven, CT. 06510.

Antisera to immunoglobulin isotypic determinants have facilitated the detection, isolation and identification of cell membrane immunoglobulins as B cell antigen recognition structures. We have used a similar logic in attempts to identify T cell membrane antigen units by immunization of rabbits with 68,000Mr, DNP-specific, murine T cell derived suppressor factor purified by hapten-affinity chromatography. By indirect immunofluorescence rabbit anti-TSF (RαTSF) stains 60-70% of murine splenic T cells and does not stain B cells. Within the splenic T cell pool, 100% of Lyt 2+ cells are stained brightly while 50% of Lyt 1+,2- cells are stained weakly by this reagent. Absorbtion of RαTSF with splenic T cells but not splenic B cells removes binding activity for both T cells and purified TSF. Cell surface radiolabeled proteins bound by RαTSF are resolved by SDS-PAGE under reducing conditions into 68,000(major) and 45,000 and 25,000Mr (minor) molecular species. Under certain immunoprecipitation conditions the 68,000Mr membrane molecules and TSF are degraded to 45,000 and 25,000 Mr products. Analysis of RαTSF-bound membrane proteins under non-reducing, denaturing or non-denaturing conditions indicates that these molecules may exist as monomers and non-covalently linked dimers. RαTSF also binds murine suppressor factors specific for sheep erythrocyte(SRBC) antigens and to rat T cell-derived, allospecific, idiotypic 70,000Mr molecules. Conversely, antisera to rat T cell antigen binding (allospecific) proteins(prepared by Drs. H. Frischknecht and H. Binz) and to SRBC-specific suppressor factors (prepared by Drs. J.Mattingly and C. Janeway Jr.) bind to DNP-specific TSF and to membrane proteins bound by RαTSF. These results suggest a commonality of antigenic determinants carried by many T cell antigen recognition structures. Moreover, (in collaboration with Drs. L.McVey-Boudreau and H. Cantor)RαTSF-IgG has been found to exert a powerful adjuvant effect on the generation of cytotoxic T cells during a mixed lymphocyte reaction. Taken together our results suggest that many T cell antigen recognition structures bear common (isotypic?) epitopes. Because membrane molecules bearing these determinants bear striking structural similarities to soluble, antigen specific T cell derived products it is probable that the membrane molecules represent T cell surface receptors for antigen.

011 ISOLATED HAPTEN-SPECIFIC T-CELL RECEPTOR MATERIAL, Matthias Cramer and Michael Reth, Institute for Genetics, University of Cologne, Weyertal 121, D-5000 Köln 41, F.R.G.

Hapten-specific receptor material can be isolated from splenic B and T lymphocytes on hapten-coupled nylon discs. Idiotypic analyses of material derived from thymocyte reconstituted chimeric nude mice demonstrated that the 4-hydroxy-3-nitrophenylacetyl(NP)-specific receptor material isolated from such functionally as yet undefined T cells is an endogenous T-cell product (1). The biochemical properties of isolated NP-specific T cell receptor molecules as revealed by a number of NP-specific techniques can be summarized as follows: 1) The molecule is oligo - most likely bivalent as far as antigen-binding is concerned (2). 2) Its molecular weight is 150 000 Daltons. 3) The molecule is composed of two types of polypeptide chains, 50 000 Daltons and 25 000 Daltons. - Serological studies had revealed that the variable region of immunoglobulin heavy chains (V_H) is the only structural element shown to be shared between B- and T-cell receptor molecules (3). That the entire V_H, i.e. both binding-site related and framework determinants are shared was demonstrated using three genetic V_H markers: heteroclicity, NP^b idiotype, and rabbit a-allotypes. A fourth V_H marker defined by a rabbit anti-V_H antiserum was also studied (3), but no genetics were performed here. We now verified that in parallel to antibody V_H and functional T-helper cell activity (4), AKR-derived isolated T-cell receptor molecules are devoid of the (allotypic) determinants detected by this particular anti-V_H reagent. - Monoclonal anti-idiotopic antibodies raised against an anti-NP hybridoma antibody (5) were used to analyse the extent of overlap between B-cell/antibody V_H and T-cell V_H as expressed in the anti-NP response of C57BL/6 mice. Since no significant differences in idiotope frequencies were observed when antibodies and T-cell receptor materials were compared for three idiotopes we may conclude at this stage, that, indeed, B- and T-cells make use of a very similar set of V_H genes.

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012 ANTI-IDIOTYPIC SPECIFICITY OF T CELL REPLACING FACTORS INDUCED BY CONCANAVALIN A, Joel W. Goodman, Daniele Primi and George K. Lewis, Department of Microbiology and Immunology, University of California, San Francisco, CA 94143

While membrane Ig-ligand interactions by themselves do not induce antibody (Ab) production by B cells, they do potentiate the response of B cells to signals delivered by intact T cells or by factors in Con A-activated T cell cultures (1). The T cell-replacing helper factors (TRF) in such culture supernatants could be resolved from suppressor factors, which were also present, on the basis of molecular size. Suppressor activity for the response to a particular antigen (Ag) was removed by and recovered from the Ag, whereas helper activity was unaffected by absorption with Ag (2). Moreover, TRF induced polyclonal Ab synthesis in cultures of spleen cells from normal or athymic mice in the absence of exogenous antigen (2). The hypothesis that the "non-antigen-specific" helper factors in TRF might recognize idiotopes on Ab molecules was tested using the anti-azobenzenearsonate (ABA) idiotypic response of A/J mice, designated the CRI (3). B lymphocytes from A/J mice cultured with various concentrations of syngeneic TRF mounted anti-ABA IgM PFC responses (500-600 PFC/10^6 cells) in the absence of Ag which were almost totally inhibited by rabbit anti-CRI Ab in the plaquing medium. This contrasts with the proportion of CRI+ PFC in Ag induced responses, which seldom exceeds 50-60%. In order to determine if the exclusively CRI+ response to TRF was induced by a CRI-specific factor, TRF was passed through a CRI affinity column. Absorption of TRF by CRI completely abrogated the CRI+ PFC response; CRI-inducing activity was recovered from the column by elution with thiocyanate. The findings provide compelling evidence that the helper factor in Con A-induced TRF for the CRI+ anti-ABA response recognizes and binds the idiotope(s). What, then, is the mechanism by which, during the course of the normal immune response, such idiotope-specific T cells become activated? One can visualize that contact with Ag causes expansion of specific B cell clones, perhaps with the assistance of antigen-specific T help, presenting an antigenic stimulus for T cells recognizing the expanded set of surface idiotopes. B cells from Ag-primed mice, when cultured with normal T cells, induced id-specific helpers in the absence of Ag, indicating that T_H activation by B cell idiotopes does indeed occur. Kinetic studies of the anti-ABA response support the concept that "minor" idiotypes arise relatively late, through the action of carrier-specific help exclusively, whereas "major" idiotypes assume early dominance through the combined action of carrier-specific and id-specific help.

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Immunoregulation: Cellular Circuits

013 BIOLOGICAL PROPERTIES OF A PURIFIED ANTIGEN-SPECIFIC SUPPRESSIVE GLYCOPROTEIN, Harvey Cantor, M. Fresno, G. Nabel and L. Boudreau, Department of Pathology, Harvard Medical School, Boston, MA 02115

We have developed methods that allow production of large numbers of continuously propagatable, antigen-specific inducer or suppressor T-cell clones (3). Analysis of these clones reveals that all Lyl^-2^+ clones tested carry suppressor but not helper activity and secrete a characteristic pattern of polypeptides that differs from other cloned T-cell sets. The ability to generate large numbers of cloned, antigen-specific suppressor T-cells has allowed us to analyze the structural basis of specific suppressive activity. We describe the characteristics of an antigen specific suppressor molecule (M_R = 70,000; pI = 5.0) that is secreted by one of these clones.

014 THE ROLE OF IDIOTYPE AND THE MHC IN SUPPRESSOR T CELL PATHWAYS, Ronald N. Germain, Michael H. Dietz, Mark I. Greene, Alfred Nisonoff,* Judah Weinberger, Shyr-Te Ju, Martin E. Dorf, Baruj Benacerraf and Man-Sun Sy, Dept. of Pathology, Harvard Medical School, Boston, MA 02115 and *Brandeis University, Waltham, MA 02254

A large series of experiments in models involving 4 different antigens (GAT: L-glutamic acid^{60}-L-alanine^{30}-L-tyrosine^{10}; GT: L-glutamic acid^{50}-L-tyrosine^{50}; ABA: azobenzenearsonate; NP: 4-hydroxy-3-nitrophenyl acetyl) have revealed immune suppression to be the result of a complex series of T-T interactions regulated by genes in the H-2 complex and involving idiotype-anti-idiotype recognition. The results in these various models are generally consistent with one another, and with those in the literature, and lead to the following scheme: 1) antigen triggers an Ly 1^+ first order suppressor cell (Ts_1), a process shown in the GT system to be under the control of H-2 linked immune suppression (Is) genes. Once activated, this cell has the ability to bind to the eliciting antigen without the apparent involvement of H-2 gene products, via a receptor which shares idiotype determinants (controlled by V_H linked genes) with antibody of the same nominal specificity (e.g., CRI determinants for ABA-Ts_1, NP^b for NP-Ts_1). These cells can only act to suppress in an afferent mode (i.e., at the time of immunization). They elaborate a soluble factor, TsF_1, which also bears idiotypic determinants, lacks C_H or C_L determinants, and possesses I-J subregion coded determinants, as shown in the GAT and ABA models. 2) This TsF_1 functions, together with very small amounts of antigen, by activating resting T cells to become second order suppressors (Ts_2). This induction occurs efficiently across H-2 and V_H differences between TsF_1 donor and recipient. These Ts_2, which appear to be Ly 2^+ when activated, are anti-idiotypic (anti-CRI in the ABA system, anti-NP^b in the NP model). They can bind to idiotype in the apparent absence of H-2 gene products. 3) The Ts_2, which act in an efferent manner, elaborate a second soluble factor, TsF_2, which has an anti-idiotypic binding specificity, lacks C_H or C_L determinants, but bears H-2 coded determinants. This factor, which has efferent suppressor properties, differs strikingly from TsF_1 in its inability to work in H-2 different animals, or animals lacking the appropriate idiotypic target. Studies currently in progress suggest that this idiotypic T cell target may be distinct from the bulk of the DTH effector cells, and perhaps constitutes a third Ts in the pathway.

The critical experiments supporting this scheme will be presented, and related to other studies in the literature, to try to draw the data on T cell suppression into a single framework. Particular attention will be paid to the critical roles of H-2 and V_H gene products at each step of the pathway.

015 A NEW VIEW OF HOW HETEROGENEOUS ANTIBODY RESPONSES ARE REGULATED, Leonore A. Herzenberg, Takeshi Tokuhisa, and Leonard A. Herzenberg, Department of Genetics, Stanford University School of Medicine, Stanford, CA 94305

Two regulatory T cell populations have basically captured the central stage as regulators of the heterogeneous antibody responses raised to hapten-carrier conjugates (e.g., DNP-KLH). Carrier-specific helper T cells, being required for successful responses to these "T-dependent" antigens, are generally viewed as stimulating and thereby directly controlling the expression of anti-hapten memory B cells. Carrier-specific suppressor cells, being capable of suppressing T-dependent hapten-carrier responses, are viewed as reducing the size or activity of the helper population and thereby limiting the amount of T cell help available for B cell responses. In addition, feedback loops that permit the helper cells to regulate suppressor activity have been implicated as potential homeostatic mechanisms. Thus, a complex but fairly consistent picture of response regulation has evolved which places carrier-specific control at the center of the immunologic universe (e.g., 1).

Nevertheless, as we shall show, this construct is based on data which can (and probably should) be interpreted quite differently. Our recent studies on the regulation of in situ responses to hapten-carrier conjugates (2) demonstrate that mechanisms akin to idiotype-specific and allotype-specific regulation play a central role to the regulation of hapten-carrier responses. Furthermore, our studies reveal unrecognized assumptions that mar previous conclusions concerning the role of the carrier-specific suppressor population and suggest a novel alternative for the contribution these cells make towards regulating antibody production. These observations therefore create a substantially new perspective on the regulatory interactions involved in heterogeneous antibody responses.

Our studies define a "hapten-specific" suppression mechanism capable of specifically controlling the individual antibody responses mounted to various determinants present on a complex antigen (e.g., DNP-KLH). Carrier-specific interactions contribute to the induction of this type of suppression, but are irrelevant to the operation of the suppression-effector mechanism once induced. The joint operation of these induciton and effector mechanisms, we suggest, provides a central regulatory system responsible (for example) for the non-genetic variability observed in antibody responses to common antigens.

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016 Immunological circuitry governed by MHC and V_H gene products, Tomio Tada, Kyoko Hayakawa, Ko Okumura, Gen Suzuki, Ryo Abe and Yoshihiro Kumagai, Department of Immunology, Faculty of Medicine, University of Tokyo, Tokyo, Japan 113

Our circuit model of the specific suppression of antibody response was based on the fact that I-J subregion gene products expressed on different subsets of T cells serve as the restricting elements for the consecutive activation of a series of cells involved in the suppressor pathway. Thus, a very strict genetic restriction has been observed in the interaction between the suppressor T cell factor (TsF) and responding cells. Our recent study, however, demonstrated that TsF has another polymorphic structure, IgVH, which is detectable by antibodies directed to the framework structure of Ig heavy chain V region (provided by Dr. D. Givol). This observation prompted us to explore whether or not VH also serves as an restricting element in the regulatory circuit.

Antigen-binding T cells specific for 4-hydroxy-3-nitrophenyl acetyl (NP) were isolated from C57BL/6 and B10.BR spleen cells primed with NP on nonimmunogenic carriers. A portion of NP-binding T cells carried the allotype-linked major crossreactive idiotype (NP^b) which was detectable by heterologous anti-id and monoclonal antibodies against NP^b idiotopes associated with the framework (group II) and antigen-binding (group I) structures of primary anti-NP antibodies (provided by Dr. M. Reth). None of the monoclonals against λ chain and Igh^b allotypes reacted with isolated NP-binding T cells. The staining of the cells with anti-id was inhibitable by NIP-BSA.

TsF from NP-binding T cells as well as from a T cell hybrid clone (7C3-13) made by fusion with BW5147 could suppress NP-specific antibody response. Furthermore, the product of 7C3-13 clone had a very high haptenated phage inhibition activity which was heteroclitic (examined by Dr. M. Kramer). There was an interesting genetic restriction in which either H-2 or VH match was necessary to induce the suppression of anti-NP antibody response. No suppression was observed in both H-2 and V_H incompatible combinations. In the case of VH compatible but H-2 incompatible combinations, the overall suppression was generally slight or even undetectable, while the expression of NP^b idiotype was selectively suppressed. These results suggest that there are at least two pathways in NP-specific suppression, in which either MHC or Ig gene products are the restricting elements. The former is via the activation of I-J compatible acceptor cells which finally lead to the overall suppression, while the latter is via the interaction with anti-idiotypic helper T cells required for the expression of major idiotypes. The superimposition of these two pathways makes up the circuitry of the idiotype and MHC governed regulations, and suggests that both antigen and idiotype 'presented' by TsF in association with I-J subregion products direct the response toward the suppression by selecting two second cell types, i.e., I-J recognizing antigen-specific acceptor T cells and anti-idiotypic T cells (Supported by a grant from the Ministry of Education, Culture and Science, Japan).

Immunoregulation: The Control of Immunoglobulin Quality by T Lymphocytes

017 THE INFLUENCE OF HELPER T CELL SUBSETS ON B CELLS RESPONDING TO T-DEPENDENT AND T-INDEPENDENT FORMS OF PHOSPHORYLCHOLINE, Kim Bottomly, Department of Pathology, Yale University School of Medicine, New Haven, CT 06510.

Optimal B cell activation by T-dependent (TD) forms of phosphorylcholine (PC) in BALB/c mice requires the presence of two subpopulations of helper T (Th) cells. The anti-PC antibodies produced bear predominantly the T15 idiotype (Id). These Th cells can be distinguished by the specificities they appear to recognize in interacting with a PC-specific B cell. The conventional Th cell set which is activated by the recognition of antigen in the context of self major histocompatibility complex (MHC) encoded determinants is clearly necessary for an adoptive secondary anti-PC response. The functional activity of this Th cell (ThMHC) set depends on hapten-carrier linkage to efficiently induce antibody production by B cells, and in the absence of the second Th cell set, the ThMHC cell set activates B cells according to their ability to bind PC. The anti-PC response induced by the ThMHC cell set is not dominated by the T15 Id. These findings can be duplicated in vitro by the use of ovalbumin (OVA) specific proliferating T cell clones as a source of ThMHC cell function. While T_{OVA} cloned cells induce a substantial B cell response to PC-OVA, this response is not T15 dominated. By contrast, the same B cell population when stimulated by PC on Brucella abortus, a T-independent antigen, produced mainly T15 bearing anti-PC antibody. The failure of ThMHC cells to induce a T15 dominated anti-PC response to PC-OVA has been shown to be due to a lack of an antigen specific Th cell set that is not MHC-restricted and is necessary for predominant T15 production (ThId). These data suggest that during responses to TD forms of PC, the clonal dominance seen is due to the presence of the ThId cell set. Yet by contrast, the T15 dominated anti-PC responses of PC-specific B cells to T-independent (TI) forms of PC are not influenced by the absence of the ThId cell set, and possibly the selective activation of T15 bearing B cells within the subpopulation of B cells responding to TI antigens is due to additional mechanisms as yet undefined.

018 REGULATION OF IDIOTYPY AND ANTIBODY SPECIFICITY BY T CELLS IN THE LYSOZYME SYSTEM. E.Sercarz, L.Adorini, B.Araneo, C.Benjamin, M.Harvey, M.Katz, R.Maizels, D.Metzger, A.Miller, L.Wicker, and R.Yowell. Dept. Microbiology, UCLA, Los Angeles, CA 90024

The response to HEL in H-2^a mice is quite heterogeneous at the levels of charge, affinity, and fine specificity. Nevertheless, almost all of the antibody molecules produced in the late 1° and 2° responses bear an idiotypic marker termed IdX-HEL, whereas earlier antibody lacks this marker. A large proportion of the antibodies are directed against a single determinant region on the molecule, at the N-terminal-C-terminal conjunction (N-C). We have been trying to identify the forces which contribute to the determination of idiotype and specificity in this system.

The MHC Directs the Specificity of the Response. One hypothesis to be developed is that the peptide specificity of the anti-HEL response is an indirect consequence of the MHC-directed determinant selection of particular Ag-specific Th cells (AgTh). Furthermore, if an epitope on peptide L2 (a.a. 13-105) is preferentially utilized by B10.A T cells, antigen-bridging constraints in T-B collaboration will lead to induction of antibodies directed against a determinant on the other side of the molecule (anti-N-C). Although antigen-presenting cells of a particular H-2 haplotype have the genetic potential to activate T cells of several peptide specificities, a type of pre-emption operates to restrict the repertoire actually employed.

Positive Selection of Predominant Idiotype. The anti-HEL first produced after HEL-CFA injection does not display IdX-HEL, even at the peak of the 1° response on day 9, as determined by PFC inhibition studies. However, by day 12, these PFC are replaced by an IdX-HEL$^+$ population. In earlier work (Adorini et al, EJI 9,906,1979) it was shown that 2 different Th were involved in the maturation of the anti-HEL response, one of which, the IdXTh, presumably exerting positive selection among B cells first activated by the other, MHC-restricted AgTh. Interestingly, the IdX-HEL determinant(s) can appear on hybridoma antibodies specific for different, non cross-reactive portions of HEL.

IdX-HEL on Ts vs. Th Cells. HEL-CFA induces Ts in B10 non-responder mice and all of the suppressive activity is removed with heterologous anti-IdX-HEL + C. Furthermore, positive adherence selection of HEL-specific Ts, restricted to H-2^b HEL-pulsed MØ monolayers, can be prevented by anti-IdX-HEL serum blocking. MHC restricted, HEL-specific Th or proliferative T cells are not affected in either of these systems by anti-IdX-HEL.

Therefore, the regulatory circuitry in the HEL system accommodates itself to this "asymmetric" situation where the AgTh belongs to a different idiotypic universe from that of the B cell, the IdXTh and the Ts. Supp. by NIH-AI-11183, CA-24442, and ACS IM-263.

019 T CELL CONTROL OF THE GROWTH AND DIFFERENTIATION OF TNP-SPECIFIC MYELOMA CELLS. R. G. Lynch, G.L. Milburn, R.G. Hoover, J.W. Rohrer and B. Dieckgraefe. Department of Pathology, Washington University, St. Louis, MO 63110

The TNP-binding murine myeloma MOPC-315 is both a target and an inducer of specific immunoregulatory signals. As a target MOPC-315 is responsive to a multiplicity of: i) idiotype (Id^{315})-specific, and ii) TNP-antigen specific immunoregulatory signals from T and B cells. These signals can regulate the growth and differentiation of MOPC-315 cells (reviewed in 1.). As an inducer the TNP-binding IgA protein (M315) released from MOPC-315 cells activates an extraordinary expansion of IgA-specific T cells that have many of the features of immunoregulatory cells(reviewed in 2.). The data to be presented will focus on recent studies that have begun to identify the cellular and molecular mechanisms involved in two separate aspects of myeloma immunoregulation.

One form is a T cell mediated, Id^{315}-specific inhibition of M315 secretion from MOPC-315 cells. The T cells that inhibit secretion: i) are θ^+, $Ly_1{}^-2^+$; ii) have surface membrane structures that recognize M315 idiotypes but not idiotypes on M460, another IgA anti-TNP myeloma protein; iii) do not affect MOPC-315 growth or viability; iv) do not alter M315 expression on the surface of MOPC-315 cells, and v) do not require macrophages at the effector stage.

The second form of immunoregulation involves IgA-specific T cells (Tα cells) that are expanded in mice with MOPC-315. We have observed that the Tα cells: i) are θ^+, $Ly_1{}^-2^+$; ii) are adult thymectomy sensitive; iii) are present in mice with several other secretory IgA myelomas, and iv) can be induced in normal mice by injection of large quantities of IgA myeloma protein. A similar expansion of Tγ cells accompanies IgG myeloma and Tμ cells are increased in mice with IgM myeloma. It is clear that the variable and constant regions of myeloma proteins play central but distinct roles in the immunobiology of myeloma. Since myeloma proteins have been extensively characterized in structural terms, myeloma provides a powerful experimental tool with which to explore fundamental immunoregulatory mechanisms.

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Numbers and Organization of V Genes in Germ Line DNA

020 NUMBERS AND ORGANISATION OF V GENES, Suzanne Cory, Jerry M. Adams and David J. Kemp, Molecular Biology Laboratory, Walter & Eliza Hall Institute of Medical Research, P.O. Royal Melbourne Hospital, Victoria 3050, Australia

The generation of antibody diversity remains one of the most intriguing issues in molecular immunology. It has been established that there are multiple variable region (V) genes in the germline for both kappa and heavy chains and that point mutations can occur. Moreover, since a complete V region sequence is assembled by somatic recombination between a specific V gene and one of four joining (J) genes plus, in the case of heavy chains, one of several diversity (D) genes, combinatorial joining of these elements substantially augments germline diversity. Nevertheless the extent of the germline repertoire and the role of somatic mutation remain matters of controversy which can only be resolved by more information on the number and nature of germline V genes. We have hybridised ten cloned V_κ and and three cloned V_H cDNA sequences to restriction fragments of mouse embryo DNA (Southern blots). Each probe labeled multiple fragments and indicated that the germline contains distinct sets of related V_κ genes and V_H genes. Both unique and overlapping patterns were observed. From the numbers of fragments observed, estimates can be made for the total number of V genes (Cory and Adams, in preparation).

To provide information regarding the organisation of V genes, we have cloned large fragments of mouse DNA bearing V_H genes (1). Analysis of the clones suggests that the V_H locus is a tandem array of genes which are separated by "spacer DNA" some 40 times the length of a V_H gene. Hybridisation studies of the clones with V_H probes representative of three unrelated germline V_H gene sets favours a model in which closely related genes are clustered rather than interspersed amongst non-homologous V_H genes. The sequence of spacer DNA between related genes is much less conserved than that of the genes themselves (Kemp et al., in preparation.

Since expression of light and heavy polypeptides is confined to one allele, productive V-(D)-J recombination would be expected to occur at only one allele. It is therefore surprising that in certain plasmacytomas, B and "pre-B" lymphoma lines, rearrangement of DNA has taken place at or near J_H genes on both alleles (2). Intriguingly, similar events occur in some T lymphoma lines (2). Current work is aimed at elucidating whether or not the rearrangements involve V genes and whether one allele has been functionally inactivated by aberrant recombination.

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021 GAPS IN UNDERSTANDING HOW ANTIBODY SPECIFICITY AND COMPLEMENTARITY ARE GENERATED. Elvin A. Kabat, Columbia Universty, New York, N. Y. 10032 and The National Cancer Institute, Bethesda, Maryland 20205.

"Generation of Diversity" is being used as equivalent to "Generation of Antibody Specificity and Complementarity". The two are not necessarily the same since diversity in amino acid sequence even in the complementarity determining regions (CDR) of the light (L) and heavy (H) chains does not necessarily cause changes in the specificity of antibody combining sites. Thus, nucleotide sequencing of the region in which V_L and J_L are joined to form a functional V gene, multiple recombinations of the nucleotides of the single V_L and J_L codon are possible to generate diversity in the amino acid at position 96. Does this diversity, generate differences in antibody complementarity or specificity and to what extent does this contribute to the structure of the antibody combining site? In only one of the five X-ray crystallographic structures, McPC603, is position 96 of V_L a contacting residue for the hapten phosphorylcholine. Five anti β1$\rightarrow$6 galactans had Ile at position 96 which could not be generated by the proposed intracodon recombination and these differed among themselves in binding constant whereas one had a binding constant identical with that of a sixth anti-β1$\rightarrow$6 galactan with Trp at position 96 and the two myeloma proteins showed the same specificity for 30 different haptens. The data clearly show that to form V_L, nucleotides coding for amino acids 1-95 are joined to a J minigene coding for amino acids 96-108 and that in V_H, nucleotides code for amino acids 1 through the end of framework (FR)3 and that a J minigene is also present. However, nucleotide sequencing of the 3' side of the V beyond the last residue of FR3 and those of the 5' side of J do not permit construction of the intact V_H gene; a segment which codes for from five to 14 amino acids, the D minigene, is missing. The existence of minigene segments was inferred from evidence indicating that independent assortment of FR segments occurred and by findings that an FR2 segment identical in amino acid composition was present in one human V_κI, 20 mouse V_κ and 13 rabbit V_κ chains. It becomes necessary to reconcile the assortment data especially those indicating that all the FR, CDR, and J segments of rabbit V_H chains assort independently with the findings that cloned genes from germ line DNA code contiguously through position 95 in V_L and through FR3 in V_H.

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022 ORGANIZATION OF KAPPA LIGHT GENES IN A MOUSE MYELOMA, Michael Komaromy and Randolph Wall, UCLA, Los Angeles, Ca. 90024

Kabat et al.(1) have proposed that variable region genes are assembled, during embryogenesis, from minigenes, each of which comprises either a framework region(FR) or complementarity-determining region(CDR). In the course of isolating kappa light chain genes from the mouse myeloma MOPC21(in a Charon 4A gene library) a large number--32--of clones were isolated which apparently contain only light chain variable region sequences with no constant region. The screening used a cDNA plasmid which includes the entire MOPC21 V region. Eight of the clones hybridize to a probe which represents the precursor region and the variable region from amino acids 1-23(FR1). Other probes representing different sequences--either amino acids 23-53(FR2,CDR1&2), 54-83(CDR2,FR3) or 96-108(FR4)--hybridize to different subsets of the original clones. Restriction enzyme analysis of the clones suggests that the large number of isolates from the library is not due to repeated isolation of a limited number of variable regions. Since Joho et al.(2) have determined that gross rearrangement of variable region genes does not occur during early embryogenesis, it is proposed that the observations may be accounted for by either evolution of the variable region genes or fine-structure rearrangements during B-cell differentiation which were not detected using the Southern blot methods(2).

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023 MAPPING OF Igh-V GENES BY SOUTHERN BLOT ANALYSIS OF Igh RECOMBINANT STRAINS, Peter H. Brodeur and Roy Riblet, Institute for Cancer Research, Philadelphia, PA 19111

The number and arrangement of genes coding for immunoglobulin heavy chain variable region is not known. To determine the fine structure of the heavy chain gene region of mouse chromosome 12, 24 inbred strains have been constructed which are genetic recombinants between the heavy chain constant region genes (Igh-C) and the heavy chain variable region gene involved in responses to dextran, Igh-DEX.

We are currently constructing a panel of cloned DNA probes for V_H genes isolated from BALB/c and NZB plasmacytomas of known antigen binding properties and inherited idiotypes.

Those V_H clones obtained thus far are being used to analyze the Igh recombinant strains by Southern blot hybridization. In addition, blot analysis of germline DNA from many inbred strains is underway. This approach should permit (a) an estimation of the V_H gene pool size and composition and (b) the assignment of the gene order for many V_H genes of known idiotype and/or binding specificity.

024 ORGANIZATION OF "AUTOANTIBODY" V_H GENES. Kathleen J. Barrett, Chester Andrzejewski, Jr. and Robert S. Schwartz, Tufts University School of Medicine, Boston, MA 02111

Hybridomas that produce anti-DNA autoantibodies were recently isolated from spleen cells of non-immunized MRL/l mice, a strain that spontaneously develops systemic lupus erythematosus (Andrzejewski et al., 1980). The idiotypes and antigen binding specificities of these autoantibodies have been analyzed (Andrzejewski et al., 1980, in press).

We are cloning the V_H genes of these autoantibodies from hybridoma mRNA in order to ask whether V_H structural gene information plays a role in this autoimmune disease. The cloned V_H probes are being used to examine the germ-line DNA of normal and autoimmune mice. Preliminary results on the number, relatedness and conservation of "autoimmune" V_H genes in these different strains of mice will be discussed.

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025 DIVERSITY OF IMMUNOGLOBULIN V GENES, Erik Selsing and Ursula Storb, Department of Microbiology and Immunology, University of Washington, Seattle, WA 98195

The diversity of antibody proteins rests in part on a multiplicity of V-region gene sequences encoded in the germline genome. In addition, during B-cell differentiation, the somatic rearrangement of V- and C-gene segments (V-J recombinational joining) into a contiguous unit has been implicated in additional generation of diversity in the third hypervariable region of the antibody polypeptide. A degree of play in the exact joining site can result in amino acid substitutions in this region. Evidence for joining site play is found in a non-functional rearranged C_k gene isolated from the myeloma, MOPC21. In addition, a somatic mechanism resulting in single base-pair differences between expressed variable regions and their germline counterparts seems also to contribute to antibody diversity. Nucleotide sequencing of the germline V_{k167} gene which is expressed in MOPC167 indicates that four base-pair differences have arisen during the differentiation of this plasma cell. Southern blot analysis rules out possible cloning artifacts or sequencing errors as the source of these differences, and, in addition, demonstrates that no germline gene corresponds to the expressed gene in MOPC167. Patterns of nucleotide substitutions in expressed immunoglobulin genes versus their germline counterparts suggest that an error-prone repair mechanism may operate during V-J joining to introduce somatic mutations.

026 CHANGES IN J CHAIN GENE METHYLATION DURING B CELL DIFFERENTIATION, Mayumi Yagi and Marian E. Koshland, University of California, Berkeley, CA 94720

The B lymphocyte responds to a primary antigenic stimulation by differentiating into a blast cell that assembles and secretes pentamer IgM antibody. This process has been shown to require de novo synthesis of a small protein, the J chain, which functions as the first crosslinking bridge in the polymerization of IgM. Studies of J chain induction in lymphoid lines suggest that J chain synthesis is controlled at the level of transcription rather than by RNA processing or translation. To study the mechanism of J chain gene transcription, we have analyzed the gene structure in cell lines representing various stages of B cell differentiation. In contrast to the Ig heavy and light chain genes, the J chain gene does not undergo a gross rearrangement during differentiation. However, significant changes occur in the degree of methylation of the gene as measured by digestion with the restriction endonucleases, Msp I and Hpa II. The chromosomal region containing the J chain is heavily methylated in cell types not expressing the gene (embryo, fibroblasts, and the B lymphoma WEHI 231) and much less methylated in cells synthesizing J chain (the IgM-secreting tumor cells, MOPC 104E, and the IgG-secreting myeloma MPC 11). Moreover, in an IgM-secreting hybrid line derived from the fusion of WEHI 231 and MPC 11 the J chain gene is less methylated than the gene in either parent. A similar correlation between demethylation and gene activation was observed for the IgM heavy (μ) chain which exhibits a different pattern of expression during B cell differentiation. These data indicate that the observed changes in methylation are associated with the expression of a specific gene product and do not represent a general differentiation-induced response.

027 THE IMMUNOGLOBULIN LAMBDA LIGHT CHAIN GENE FAMILY: DNA CLONING AND GENE MAPPING, James F. Miller and Ursula B. Storb, University of Washington, Seattle, Wa 98195.

During differentiation from a stem cell to an antibody secreting cell, the immunoglobulin genes within a B cell undergo a rearrangement which juxtaposes a variable region gene to a constant region gene. To analyze the genetic organization of an immunoglobulin gene family in nonrearranged, germline DNA, we have constructed a recombinant DNA library from randomly cleaved mouse kidney DNA fragments (Maniatis et.al. (78) Cell 15:687-701). From this library, we have isolated three overlapping recombinant clones containing the constant region gene for lambda$_I$ light chains ($C_{\lambda I}$). These clones, spanning 24.5 kilobase-pairs (kb) of mouse DNA, were aligned by restriction mapping and heteroduplex analysis. There are no variable region sequences located within these segments of DNA. Hybridization of these clones with lambda$_{II}$ cDNA (provided by A. Bothwell and D. Baltimore) demonstrated the presence of an additional gene located 3.2 kb 5' of $C_{\lambda I}$. The location of this gene was verified by R-loop analysis using RNA containing λ_I and λ_{II} mRNA sequences. This gene was identified as $C_{\lambda III}$ by partial nucleotide sequencing and additional restriction enzyme mapping. $C_{\lambda III}$ has recently been identified by H. Eisen (personal communication) and differs from $C_{\lambda II}$ by five to seven amino acids. We are presently isolating clones containing $V_{\lambda I}$, $V_{\lambda II}$, and $C_{\lambda II}$ genes to complete the physical map of the lambda gene family.

Organization of Immunoglobulin C Genes

028 STUDIES ON THE MOLECULAR STRUCTURE OF MOUSE Cδ, Phil Tucker, Robin Robinson, Hwei-Ling Cheng, University of Texas Southwestern Medical School, Dallas, 75235.

A single DNA fragment containing both μ and δ immunoglobulin heavy chain constant region genes has been cloned from normal BALB/c liver DNA (1). The DNA sequence of the Cδ region from both this gene and the IgD plasmacytoma TEPC 1017 indicated an unusual structure for the tumor δ chain (2). This structure has been extended by further sequencing studies to confirm (a) the lack of a conventional second C_H domain in the mouse germline, (b) the presence of a repetitive DNA sequence in the large intron between the hinge and Cδ3 domains that forms a stem-loop structure with an inverted complementary sequence in the 2.5 killobase pair intron between the μ membrane and Cδ1 exons, and (c) the presence of at least two potential exons (in addition to that termed CδDC (2) which encodes the 3' terminus of the TEPC 1017 major mRNA species) some 5 killobase pairs downstream from the Cδ3 domain which may code for alternative C-termini for membrane binding functions.

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029 ORGANIZATION AND REARRANGEMENT OF IMMUNOGLOBULIN HEAVY CHAIN GENES, T. Honjo, Y. Nishida, A. Shimizu, N. Takahashi, T. Kataoka, M. Obata, Y. Yamawaki-Kataoka, T. Nikaido, S. Nakai, Y. Yaoita and N. Ishida, Department of Genetics, Osaka University Medical School, Kita-ku, Osaka 530, Japan.

Mouse immunoglobulin ε chain gene was cloned from DNA of an IgE(anti DNP)-producing hybridoma. Since a given active C_H gene is linked to a J_H gene at its 5' side, the expressed C_ε gene of the hybridoma was cloned from a phage library containing partial Sau 3A digests of IgE hybridoma DNA using a J gene fragment as a probe. A partial nucleotide sequence determined predicts the amino acid sequence which resembles a part of the C_{H3} domain of human ε chain. Deletion profile of the C_ε gene in various myelomas expressing different C_H genes indicates that the C_ε gene is located between the $C_{\gamma 2a}$ and C_α genes.

With all the cloned C_H genes in hand, we have set out to directly determine the order of the mouse immunoglobulin C_H genes by molecular cloning of overlapping chromosomal segments. We have, so far, demonstrated that the order is 5'-C_μ-(4.5 kb)-C_δ-(unknown distance)-$C_{\gamma 3}$(34 kb)-$C_{\gamma 1}$-(21 kb)-$C_{\gamma 2b}$-(15 kb)-$C_{\gamma 2a}$-(14.5 kb)-C_ε-(12 kb)-C_α-3'. This order of C_H genes is perfectly consistent with our previous model (1) and the position of the C_ε gene is determined for the first time. Gel blotting and hybridization experiments indicate that there are no J regions at the 5' side of each C_H gene except for the C_μ gene and that these constant region genes seem to have repetitive sequences characteristic to S regions at their 5' side.

It is now established that deletion of a chromosomal segment accompanies the class switch (S-S) recombination (2). Two alternative models can be proposed to explain the mechanism for the C_H gene deletion. One model postulates that the S-S recombination takes place in a single chromosome by mutual recognition of two S regions. The intervening DNA segment is looped out and lost from the chromosome (looping-out model). The other model, called a sister chromatid exchange model, explains the deletion of DNA segment by an unequal crossing-over event between sister chromatids (3). An expressed γ1 gene of MC101 myeloma comprises five germline DNA segments, namely a variable region gene, a D segment, a segment of the 5' flanking region of the C_μ gene (containing J regions), a segment of the 5' flanking region of the C_α gene and the $C_{\gamma 1}$ gene with its flanking regions. The presence of the C_α gene-flanking region (S_α region) at the 5' side of the $C_{\gamma 1}$ gene-flanking region ($S_{\gamma 1}$ region) implies that the heavy chain class switch may not be mediated by step-wise linear deletion along the order of the C_H genes and that a sister chromatid exchange model is more favorable.

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030 THE ARRANGEMENT OF HUMAN IMMUNOGLOBULIN HEAVY CHAIN CONSTANT REGION GENES, Terence H. Rabbitts, MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, England

We have exploited the ability of mouse immunoglobulin gene probes to cross-hybridise to the homologous human genes in order to isolate a set of heavy chain constant region (CH) genes from foetal liver DNA. The human CH genes studied in detail include those of the $C\mu$ and $C\gamma$ class. Nucleotide sequencing studies of the $C\mu$ gene show that, as described for mouse, the gene is split into $C\mu 1$, $C\mu 2$, $C\mu 3$ and $C\mu 4$+tp as discrete domains separated by small intervening sequences. A set of JH segments occurs very approximately eight kilobases (kb) from the 5' side of the $C\mu$ gene, and to the 3' side of the $C\mu$ gene are the coding segments characteristic of the membrane form of IgM utilised by such cells as the DAUDI line.

In the intervening sequence between the human $C\mu$ and JH segments a sequence occurs which is very analogous to that reported in the mouse. This imperfect tandem repeat sequence (designated the S segment) has the basic unit G-G-G-C-T(G-A-G-C-T)3 and a similar sequence occurs adjacent to an isolated human $C\gamma$ gene clone and probably also near the other CH genes. Previous studies of mouse CH genes [1] has implicated sequences of this type in the mechanism of the class switch. The conservation of these sequences across species barriers indicates a true functional significance since intervening sequences in general seem to diverge more rapidly than coding sequences. The occurrence of tandemly repeated sequences in a region where the class switch occurs implies that a likely mechanism is homologous recombination [2]. The relatively imprecise nature of this recombination contrasts markedly with the process of V-J joining where defined sequences (particularly the sequence C-A-C-A-G-T-G) occur and where the joining is rather site-specific.

The co-expression of the two human CH genes, $C\mu$ and $C\delta$, does not appear to occur by either of these mechanisms and may well occur by co-transcription of two linked genes. Experiments on the DNA of cells expressing these CH genes will be discussed.

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031 EVIDENCE FOR TRANSCRIPTIONAL CONTROL OF J CHAIN EXPRESSION, George Lamson and Marian E. Koshland, University of California, Berkeley, CA 94720

The J chain protein required for pentamer IgM assembly has been shown to be expressed as a direct result of antigen encounter with a virgin B lymphocyte. The nuclear events leading to J chain expression were investigated by analyzing murine cell lines representing different stages in B cell differentiation. The nuclear poly(A)+ RNA was isolated from fully activated IgM or IgA-secreting plasmacytomas and from a resting, non-secreting B-cell lymphoma, and the J chain sequences present were identified by gel electrophoresis, blotting, and hybridization with cloned J chain cDNA. The plasmacytoma RNA preparations were found to contain a mature J chain message of 1.6 kb and at least five larger precursor species ranging in size from 14 kb to 2.8 kb. These findings indicated that the J chain gene spans a minimum of 14 kb, and, like other eukaryotic genes studied to date, the structural information is interrupted by a number of untranslated regions that are successively excised by RNA processing. On the other hand, the nuclear RNA prepared from the B lymphoma contained no detectable J chain sequences. The absence of precursor molecules as well as mature J chain message from these lymphocyte-like cells strengthens the evidence that J chain expression is controlled at the level of transcription rather than by RNA processing.

032 PREPARATION AND CHARACTERIZATION OF A RAT IMMUNOGLOBULIN EPSILON HEAVY CHAIN cDNA CLONE, Wayne Kindsvogel, Joan Moore and Charles Faust, University of Oregon Health Sciences Center, Portland, OR 97001

A poly(A)-rich fraction of total mRNA has been prepared from the rat myeloma, IR 162. This has been shown to contain biologically active mRNA sequences which code for rat immunoglobulin epsilon heavy chain and kappa light chain. The total mRNA fraction has been used to prepare a single-stranded cDNA population with reverse transcriptase. This cDNA was in turn made double-stranded with DNA polymerase I. All single-stranded DNA was eliminated with S1-nuclease, thereby generating a blunt-ended cDNA library ranging from about 400 to 2000 base pairs in length. The average size was about 1200 base pairs. This cDNA library was tailed with 10 to 20 dC residues, using terminal transferase. The cloning vector pBR322 was similarly tailed with dG residues in the Pst I restriction enzyme site of the ampicillan resistance gene. Following annealing of the tailed cDNA with the tailed plasmid, the host $\chi 1776$ was transformed. The ampicillan sensitive and tetracycline resistant clones were selected, and a series of replicate "Grunstein-Hogness" filters were prepared. These were screened with a panel of (^{32}P)-labeled cDNA probes to distinguish myeloma-specific clones from non-myeloma-specific clones. Several groups of clones could be identified and characterized based on this panel of hybridizations. All those examined could be excised with Pst I. One of these groups had the properties expected for a colony containing an epsilon heavy chain cDNA insert. Results of preliminary hybrid-selected mRNA analyses support this. Details on the identity and characterization of the clone will be reported. Supported in part by NSF PCM 79-05041 & MRF.

033 CHROMOSOMAL LOCALIZATION OF IG STRUCTURAL GENES IN THE MOUSE, Peter D'Eustachio, Dimitrina Pravtcheva, and Frank H. Ruddle, Dep't. of Biology, Yale University, New Haven, CT 06520

In order to determine unambiguously the chromosomal localizations of Ig structural genes, we have used recombinant DNA probes to screen mouse x hamster somatic cell hybrids for the presence of these genes. Our panel of hybrid cell lines has retained various numbers of mouse chromosomes together with a complete set of hamster chromosomes in each case. DNA from these hybrids was digested with restriction endonucleases, fractionated by agarose gel electrophoresis, and scored by the blotting procedure of Southern for the presence of mouse DNA sequences reactive with heavy and λ light chain cDNA probes. Correlating the presence or absence of these fragments in the cell lines of our panel with the presence or absence of the various mouse chromosomes in these cell lines allowed the assignment of the Cα, Cγ1, Cγ2a, Cγ2b, and Cμ (heavy chain) genes to chromosome 12, and of the CλI, CλII, VλI, and VλII genes to chromosome 16. Together with the previous assignment of κ light chain V and C genes to chromosome 6, these results complete the chromosomal localization of the Ig structural genes in the mouse, and represent the first such systematic mapping for any species.

034 TRANSCRIPTION OF δ CHAIN GENES IN MOUSE MYELOMA CELLS AND NORMAL SPLEEN, Leona Fitzmaurice and J. Frederic Mushinski, NIAID and NCI, National Institutes of Health, Bethesda, MD 20205.

A cDNA clone containing a major portion of the mouse δ chain constant region derived from mouse myeloma TEPC 1017 mRNA has been constructed and characterized [Mushinski, et al., P.N.A.S., in press; Tucker, et al., Science 209 1353-1360 (1980)] and used to identify genomic sequences in a mouse liver DNA library [Liu, et al., Science 209 1348-1353 (1980)]. Hybridization studies of mouse myeloma RNAs electrophoresed on methyl mercury hydroxide agarose gels have identified a major δ mRNA species (1750 ± 50 nucleotides) in both TEPC 1017 and TEPC 1033 RNA. Three larger species have been observed in TEPC 1017 RNA (approximately 2100, 2900, and 3200 nucleotides), and one larger species (approximately 3200 nucleotides) has been observed in TEPC 1033 RNA. Normal spleen cells appear to contain only one major δ mRNA species (approximately 3200 nucleotides); this mRNA species may be identical in size to that observed in the B cell lymphoma line BCL_1-5b. Studies of these various δ RNAs and their nuclear RNA precursors are in progress and will increase our understanding of δ chain gene expression in the mouse.

Molecular Mechanisms in the Regulation of V Gene Expression

035 THE ORIGIN OF NP^b IDIOTYPIC DIVERSITY: AN ASSESSMENT OF THE CONTRIBUTION OF SOMATIC AND COMBINATORIAL DIVERSITY DURING THE IMMUNE RESPONSE. Alfred Bothwell, Michael Paskind, Klaus Rajewsky+, Thereza Imanishi-Kari+, Michael Reth+ and David Baltimore, Department of Biology and Center for Cancer Research, M.I.T., Cambridge, MA 02139; +Institut fur Genetik, der Universitat zu Koln, 5 Koln - Lindenthal, Germany.

The response of C57BL/6 mice to the hapten NP results in the expression of a restricted set of serum antibodies which comprise the NP^b idiotype. These antibodies have characteristic isoelectric focusing patterns, antigenic determinants (public idiotypes) and patterns of binding to related haptens. The primary response is almost exclusively generated by μ or γ_1 heavy chains and a λ_1 light chain. Therefore, the major source of the idiotype diversity in the primary response is the variable region of the heavy chain.

Analysis of the immunoglobulin gene products was initiated by cloning the heavy and light chain mRNAs expressed in two idiotype positive hybridomas. Among a pool of 48 hybridomas these are serologically as distant as any pair of hybridomas and yet they still have idiotypic determinants. One was derived during a primary response (B1-8) and the second was generated during a hyperimmune response (S43). Nucleotide sequence analysis of the entire V coding sequence derived from the two cDNA clones indicated that the V regions were very similar. The two cDNA V sequences cross-hybridize to each other and indeed to at least eight germ line genes by Southern blot analysis.

The nucleotide sequence of seven genomic V genes isolated from a library of genomic DNA has revealed that one corresponds exactly to the V region sequence expressed in the primary response hybridoma. In addition, the V region sequences from the hyperimmune response hybridoma is closer to this germ line sequence than any other V gene. Therefore, the entire V_H contribution to the idiotype may be derived from this single V gene. These comparisons suggest that the diversity found in the antibodies of the primary response is generated primarily by combinatorial mechanisms resulting in sequence differences at the V-D and D-J junctions as well as in the generation of D itself. The higher affinity antibodies and new specificities characteristic of a hyperimmune response are a result of the primary response combinatorial diversity, but superimposed upon that is somatic mutation.

Since several of these cross-hybridizing V genes are physically linked to each other it is possible to derive some concept of the total informational content between them. To this end, repetitive DNA sequences and regions flanking V genes have been compared by restriction endonuclease mapping and DNA sequence analysis.

036 IMMUNOGLOBULIN GENE REARRANGEMENTS IN NORMAL B LYMPHOCYTES. I.L. Weissman, C. Nottenburg, R. Joho, A. Tsukamoto and H. Gershenfeld. Laboratory of Experimental Oncology, Department of Pathology, Stanford Medical Center, Stanford, CA 94305.

The rearrangement of immunoglobulin genes to form DNA segments coding for immunoglobulin heavy and light chains has been studied extensively (by others) using malignant myeloma and lymphoma cells. These tumors differ from normal B cells because they have gone through hundreds of cell divisions, and because they are generally aneuploid. Thus there is a danger that the analysis of chromosomal genes in these cells may not reflect the normal circumstance. For example, the control of expression of one immunoglobulin gene rather than its allelic counterpart in B cells (allelic exclusion) may be regulated at the level of gene rearrangement, gene transcription, and/or gene translation; and these processes may not be retained intact in malignant cells. We have examined allelic exclusion at the level of normal B lymphocytes, asking whether the rearrangements of variable region genes to J regions occurs only on the expressed chromosome, or on the nonexpressed chromosome as well. Splenic B lymphocytes expressing kappa immunoglobulins show chromosomal rearrangements in about half the amount of the cellular DNA. In contrast, $B\mu\delta$ lymphocytes expressing the Balb allotype on their cell surface show DNA rearrangements to the J_H gene region on both the Balb/c parent-derived heavy chain chromosome and the C57Bl-derived heavy chain chromosome. We are currently examining whether the H chain-chromosome has precise V/D/J rearrangements on both the expressed and nonexpressed chromosomes. Because H chain expression occurs developmentally prior to κ chain expression, and because H chain rearrangements require the translocation of the $V\kappa$ segment to the $J\kappa$ segment, it may not be surprising that these two independent events could involve distinct enzymatic mechanisms for rearrangement and/or control of transcription.

037 CHARACTERIZATION OF RABBIT SPLEEN mRNA AND PRODUCTS SYNTHESIZED IN A MESSENGER DEPENDENT CELL FREE SYSTEM, Andrea Pavirani, Leona Fitzmaurice and Rose Mage Laboratory of Immunology, NIAID, NIH, Bethesda, MD 20205.

Spleens from hyperimmunized rabbits were used as the source of poly A^+ RNA and lymphocytes for in vitro synthesis studies. Total RNA was isolated by 4 M guanidinium thiocyanate extraction and poly A^+ RNA selected by oligo-(dT)-cellulose chromatography. Total and poly A+ RNAs were characterized by electrophoresis on methyl mercury hydroxide agarose gels. In a rabbit reticulocyte translation system, the mRNAs directed synthesis of different ^{3}H-Leu labeled proteins which we characterized electrophoretically on SDS acrylamide gels. In addition, specific immunoprecipitation allowed us to recognize kappa light chains which carry specific b-allotypic markers in their constant regions, as well as μ and γ heavy chains. Comparisons were made between the electrophoretic patterns of the cell-free products and those from rabbit spleen cell cultures pulsed with ^{3}H-Leu for 5, 30 and 120 minutes. The different mobilities of the μ and light chains we observed were probably the result of synthesis of polypeptide chains with precursor (signal) peptides and non-glycosylated heavy chains in the cell-free translation system. The isolated mRNAs provide a source of material for construction of recombinant clones containing rabbit immunoglobulin heavy and light chain cDNAs. The efficient translation of mRNA for rabbit heavy and light chains of defined allotype in a messenger-dependent cell free system has provided us with a tool for use as an aid in the identification and characterization of cloned cDNAs as well as genomic sequences for rabbit immunoglobulin heavy and light chains.

038 REARRANGED κ CONSTANT GENES IN NONPRODUCING MPC-11 VARIANTS, Edmund Choi, Michael Kuehl and Randolph Wall. University of California, Los Angeles, CA 90024 and University of Virginia, Charlottesville, VA 22901

MPC-11 mouse myeloma cells express two differently rearranged C light-chain genes (1, 2). One chromosome undergoes normal rearrangement in which the MPC-11 V-region is joined to a J region. This rearranged κ gene produces a normal MPC-11 κ mRNA. The other chromosome is aberrantly rearranged such that a different V-region is joined to a site located in the intervening sequence between the J and C regions. The RNA transcript of this gene does not contain a J region. As a result, the 5' leader sequence is spliced to the C region forming an abnormal mRNA coding for a fragment light chain lacking a variable region.

Other variants of MPC-11 which no longer produce any full length light chain molecules have now been analyzed. The C region of the light chain gene is deleted in some of these variants. In another case, the sequences flanking the 5' side of the V region have been altered from that of the normal MPC-11 light chain gene. The promotor for transcription is inactivated or lost by this alteration since there are no transcription products from this gene. Another variant light chain gene examined showed no detectable differences from the restriction maps of the wild type gene. In these cells, the loss of normal light chain mRNA production appears to result from a splicing defect since a polyadenylated nuclear RNA transcript is synthesized in amounts comparable to normal MPC-11 cells.

1. Choi et al., Nature, 286:776 (1980)
2. Seidman et al., Nature 286:779 (1980)

039 REARRANGEMENTS AND EXPRESSION OF IMMUNOGLOBULIN κ GENES, Brian Van Ness, Robert Perry and Martin Weigert, Institute for Cancer Research, Philadelphia, PA 19111.
It is now clear that functional expression of immunoglobulin genes requires site specific recombinations of DNA in which elements coding for variable (V) and constant (C) regions are brought together within a single transcription unit. We have examined the DNA from mouse plasmacytomas expressing the V_κ21 light chain for gene rearrangements. As seen by blot hybridizations, rearrangements of a V_κ gene to one of the J_κ regions is reflected in an alteration in the size of a C_κ containing restriction fragment. Because blot hybridization demonstrates a restriction fragment whose size is characteristic of a particular productive V_κ21 rearrangement, this genetic analysis allows correlation of an expressed V_κ21 gene with amino acid sequence data we have obtained on over 40 V_κ21 expressing myelomas. It has recently been reported that the unrearranged (germline) κ locus produces a large (8.4 kb) transcript. However, RNA processing competence apparently has important structureal requirements which are met only in transcripts of correctly rearranged κ genes. The large germline transcript is not processed or expressed. Furthermore, a significant number of nonproductive rearrangements have been observed which are transcriptionally inactive. We are analyzing the requirements for gene expression by comparisons of functional and nonfunctional κ loci. Specifically, we are comparing clones from the germline κ locus, from a functionally rearranged κ gene, and from a nonproductively rearranged, transcriptionally inactive κ gene.

040 STRUCTURE AND EXPRESSION OF IMMUNOGLOBULIN GENES, U. Storb, J. Miller, R. Wilson, A. Walfield, E. Selsing and B. Arp, Department of Microbiology and Immunology, University of Washington, Seattle, WA 98195
The germline organization of λ genes determined from several clones of a genomic library will be shown. Furthermore the difference between functional and nonfunctional rearranged κ genes will be addressed. Restriction mapping, electron microscopy and DNA sequencing of κ genes cloned in Charon 4A showed that a nonfunctional gene had misaligned a V_κ gene with a J gene so that four nucleotides were deleted from the 3' end of the V gene. This nonfunctional gene codes for mature cytoplasmic κ mRNA which is not translated into kappa chains because of the frame shift. The chromatin conformation of κ genes was determined by mild DNase I digestion of nuclei of myeloma, other lymphoid and non-lymphoid cells. In cells which produce kappa chains, all C_κ genes are in a DNase I sensitive chromatin conformation, regardless of whether the C_κ gene is rearranged, or if rearranged, whether it is expressed into kappa chains. The altered chromatin state is localized around the rearranged V_κ and/or C_κ genes - V_κ genes in germline context on the same chromosome are not DNase I sensitive. Kappa genes are not DNase sensitive in cells which do not produce kappa mRNA. The results indicate that allelic exclusion does not operate on the level of chromatin conformation, but rather on the level of DNA organization: nonfunctional alleles are either in germline configuration or have undergone faulty rearrangement. It remains to be determined whether there exist positive control mechanisms which would prevent the formation of two different functional L or H chains by one cell.

041 INDUCTION OF LIGHT CHAIN GENE REARRANGEMENT IN A PRE-B CELL LINE BY FUSION TO MYELOMA CELLS, Sylvia C. Riley, Emily J. Brock, and W. Michael Kuehl, University of Virginia, Charlottesville, VA 22908

Murine pre-B cells synthesize μ heavy chain (H) in the absence of light chain (L) synthesis. An Abelson virus-transformed mouse cell line (18-81) has pre-B cell characteristics. When 18-81 is fused to variant myeloma cells which do not express L chain, 40% of the somatic hybrids express a κ L chain. Southern blots of DNA from 18-81 cells show that Cκ genes are on a 13kb Bam HI fragment which comigrates with the 13kb embryonic Bam HI fragment in splenic DNA. Southern blots of DNA from pre-B cell x myeloma hybrids which express κ L chain show that the 13kb Bam HI fragment disappears and a new Bam HI fragment appears which is not found in either 18-81 pre-B cells or any of the myeloma cells. Each independent hybrid has a different size de novo rearranged Cκ gene. Therefore, the 18-81 germline Ck gene can be rearranged to different Vκ genes. This data supports the hypothesis that a pre-B cell, which is committed to synthesis of a particular H chain, acts as a stem cell for expression of different L chains.

Ig expression in normal B cells is limited to one allele for both H and L chain in a single cell, i.e. allelic exclusion. In contrast to the results above, when 18-81 is fused to myeloma cells which express κ L chain, the pre-B cell κ L chain genes are not rearranged or expressed. Thus, rearrangement and expression of a pre-B cell L chain appears to be affected by the presence of another properly rearranged L chain in a pre-B cell x myeloma hybrid. This indicates that for κ L chains allelic exclusion is an active process.

042 IMMUNOGLOBULIN GENE REARRANGEMENTS IN NORMAL B LYMPHOCYTES, Carol Nottenburg and Irving L. Weissman, Stanford University, Stanford, CA 94305.

A strategy was devised to study immunoglobulin gene rearrangements in physiologically normal B lymphocytes. Restriction fragment length polymorphisms were correlated with heavy cahin allotypes and each heavy chain containing chromosome in an allotypically heterozygous F_1 mouse could be distinguished. B cells expressing one of the allelic pair or and genes (allotypically-excluded B cells) were isolated with a Fluorescence-Activated Cell Sorter. Hybridization of a C gene containing probe was performed on restriction enzyme digests of the allotypically-excluded B cells. Both chromosomes were rearranged in the J_H gene region. In contrast, asubstantial fraction of C_κ genes remained in germ-line (unrearranged) context. The nature of the rearrangement on the non-expressed heavy chain chromosome is currently being analyzed.

Molecular Biology of Non-Antibody Gene Systems in Immunology

043 DNA ANALYSIS OF ARSONATE-BINDING T CELL AND B CELL PRODUCTS, John E. Sims, Terence H. Rabbitts, Pila Estess, Antonio Pacifico and J. Donald Capra, MRC Laboratory of Molecular Biology, Cambridge, England, and Department of Microbiology, University of Texas, Dallas, Texas 75235.

Antigen-binding molecules produced by T cells are believed to contain immunoglobulin V_H segments attached to "C_T" segments of unknown nature. Recently a T cell hybridoma was made from A/J mouse spleen cells fused to the T lymphoma BW147; this cell line secretes a polypeptide which binds to arsonate and which carries both the arsonate cross-reactive idiotype (V_{ars}) and Ia antigenic determinants. Since the arsonate cross-reactive idiotype is also expressed by several B cell hybridomas, we have used one of these hybridomas (which makes an IgG1 idiotype-positive anti-arsonate antibody) to make cDNA clones containing V_{ars}. These V_{ars} cDNA probes have been used to study the complexity and rearrangement of the corresponding set of V_H genes in B and T cell DNA. In addition, the cross-reactive idiotype probe (V_{ars}) has been used to investigate the mRNA encoding the T cell hybridoma product as a prelude to the isolation and characterisation of T cell cDNA clones made from this mRNA.

044 IN VITRO TRANSLATION OF IL2 MESSENGER RNA, R. Chris Bleackley, Vern H. Paetkau, Calliopi Havele, Barry I. Caplan, R.G. Ritzel, Tim R. Mosmann and John J. Farrar, Departments of Biochemistry and Immunology, University of Alberta, Edmonton, Alberta, T6G 2H7, Canada.

Murine spleen cells stimulated in culture produce a 30,000 M.wt. protein called interleukin 2 (IL2). Partially purified IL2 stimulates thymocyte proliferation, supports the growth of T cell lines and replaces T helper cells in specific CTL responses. IL2 with these properties is also secreted by the T lymphoma line RBL4. Poly A^+ RNA was extracted from RBL4 cells which had been stimulated with phorbol-12-myristate-13-acetate (PMA). This RNA was translated in Xenopus laevis oocytes and in mRNA dependent rabbit reticulocyte lysates (MDL). The translation products were shown to contain biologically active IL2. There was a linear relationship between the amount of mRNA injected into each oocyte and the yield of IL2 over the range 20-200 ng. The in vitro synthesized IL2 was found to cochromatograph on a Sephadex G100 column with a sample of authentic IL2. (Supported by MRC and NCI Canada.)

045 ISOLATION OF GENOMIC CLONES OF THE MOUSE H-2 GENES, Kevin W. Moore, Michael Steinmetz, John Frelinger, Douglas Fisher, Leroy E. Hood, Division of Biology, California Institute of Technology, Pasadena, CA 91125

Using cDNA clones prepared by Steinmetz et al.[1] as probes for genes of the H-2 locus, we have isolated several genomic clones from a library of BALB/c sperm DNA. These clones are being characterized by R-loop mapping and restriction map analysis.

[1]Steinmetz, M., Frelinger, J., Fisher, D., Pereira, D., Weissman, S., Hood, L. (1981) in preparation.

046 THE ORGANIZATION OF THE β2-MICROGLOBULIN GENE(S). Jane R. Parnes, Baruch Velan, Adam Felsenfeld, Lata Ramanathan, Umberto Ferrini, Ettore Appella, and J.G. Seidman, National Institutes of Health, Bethesda, Maryland 20205

We have isolated three cDNA clones for β2-microglobulin, the small subunit of the major histocompatibility antigens. β_2-Microglobulin comprises less than 0.1% of mouse liver protein, and its mRNA is approximately 0.03% of liver poly(A)$^+$ mRNA. The cDNA clones were identified by screening 1400 cDNA clones made from 9-10S mouse liver poly(A)$^+$ mRNA. The procedure for screening the cDNA clones involved binding pooled plasmid DNAs to nitrocellulose filters and testing the ability of each filter to select β_2-microglobulin mRNA. The filter selected mRNAs were assayed for their ability to direct the synthesis of β_2-microglobulin in in vitro translation reactions. The isolated clones were shown by nucleotide sequence analysis to encode β_2-microglobulin. The clone screening procedure is a general one that allows the screening of large numbers of cDNA clones and should allow the isolation of cDNAs corresponding to any mRNA whose in vitro translation products can be immunoprecipitated. It is of particular value in the isolation of cDNA clones corresponding to rare species of mRNA.

We have used the β_2-microglobulin cDNA clones as probes to identify bacteriophage clones containing segments of mouse genomic DNA that encode β_2-microglobulin. Although β_2-microglobulin is a subunit of several different proteins, evidence from these genomic clones and from Southern blot analysis suggests that there is a single complete β_2-microglobulin gene with at least one, and probably two intervening sequences.

047 IN VITRO TRANSLATION AND PROCESSING OF H-2^b ANTIGENS, Penny J. Gilmer, Rebecca L. Croft and William R. Marzluff, Jr., Department of Chemistry, Florida State University, Tallahassee, Florida 32306.

Poly A-RNA from murine EL4 (H-2^b) tumor cells has been translated in vitro in a rabbit reticulocyte system both with and without the presence of dog pancreatic microsomal membranes. The translation products were immunoprecipitated with anti-H-2^b alloantiserum and rabbit anti-human β_2-microglobulin. The alloantiserum will only immunoprecipitate H-2^b antigens when they are associated with β_2-microglobulin after both have been correctly processed by membranes. We are comparing via two-dimensional gel electrophoresis the products synthesized in vitro with in vivo products isolated from the endoplasmic reticulum and the plasma membranes by EL4. The endoplasmic reticulum forms of H-$2K^b$ and -D^b are more basic and of slightly lower molecular weight than the corresponding final forms as present on the plasma membrane.

048 THE GENOMIC ORGANIZATION OF THE HUMAN MHC GENE LOCUS, P.Andrew Biro, Dennis Pereira, Ashwani Sood and Sherman M. Weissman, Yale University, New Haven, CT. 06510

We have been studying the genomic organization of sequences complementary to a cDNA clone of HLA-B mRNA isolated from an RPMI cell line. The positive clone was identified using an 11base synthetic oligonucleotide probe.

Southern blotting of human DNA isolated from a variety of sources shows multiple bands complementary to the HLA probe. Approximately 16 bands can be identified using any of a number of different restriction enzymes. Specific differences between individuals can be detected and data on the possible significance of such differences will be presented.

Genomic cloning of DNA from an HLA-expressing cell line shows that some of the more intense bands detected by blotting are composed of more than one type of fragment, suggesting some degree of long-range duplication of DNA sequences in the genome.

Probes from the 3' and 5' ends of the cDNA clone are being used to determine the number and lengths of the intervening sequences and also the number of HLA genes or gene segments present in the genome.

It will now be possible to compare the HLA with the immunoglobulin gene complexes and determine possible evolutionary relationships between them.

049 MOLECULAR CLONING OF HLA ANTIGENS, Hidde L. Ploegh, Harry T.Orr and Jack L. Strominger, Harvard University, Cambridge, 02138 Mass.

An HLA-A.B heavy chain specific cDNA probe has been constructed and characterized (1), employing standard molecular-biological techniques. This probe has been used in the isolation of HLA genes from genomic libraries established in bacteriophage lambda Charon 28. The characterization of these genes by nucleotide sequence analysis and restriction mapping will be discussed.

H.L.Ploegh, H.T.Orr and J.L. Strominger. Molecular cloning of a human histocompatibilty antigen cDNA fragment. Proc. Natl. Acad. Sci. USA (1980) 77 6081-6085.

050 MURINE T CELL HYBRID LINES PRODUCE POLYADENYLATED RNA WHICH HYBRIDIZES WITH A Cμ PROBE, M.C. Zuniga, P. d'Eustachio, N.H. Ruddle, Yale University, New Haven, CT 06510

Recent reports (Kemp et al. PNAS 77: 2876-2880, 1980; Forster, et al. Nature 286: 897-899, 1980) have demonstrated that the Cμ gene is rearranged in some murine T cell lymphomas and in two killer T cell clones. The Cμ sequences in these cells are transcribed to produce polyadenylated RNAs detectable by Northern blot analysis. These RNAs differ in size from the major RNA_μ species isolated from differentiated B cells and B lymphomas. We have performed similar studies with antigen specific cloned T cell hybrid lines (Ruddle, et al. Molec. Immun. 17: 925-931, 1980). Affinity purified polyadenylated RNA was denatured with glyoxal, fractionated electrophoretically, and transferred to nitrocellulose filters. Immunoglobulin-specific sequences were identified by hybridization with ^{32}P-labeled cloned probes. The azobenzenearsonate-specific suppressor T cell hybrid, Hyb 51H7D, produces a polyadenylated RNA of approximately 1.9 kb which hybridizes with a Cμ probe. Since the T lymphoma parent of this hybrid, BW 5147, has no Cμ-specific polyadenylated RNA, it is likely that the RNA is a transcript of DNA sequences donated by the antigen-specific parent T cell. This RNA might encode protein(s) important in T cell function such as component(s) of the T cell receptor for antigen. Further characterization of this RNA in Hyb 51H7D is in progress. In addition, other antigen-specific T cell hybrid lines are being examined for production of RNAs which hybridize with immunoglobulin-specific probes. These studies in conjunction with experiments on the protein level should determine the relationship (if any) of these RNAs to the T cell receptors for antigen. (Supported by NCI CA 16885, ACS FRA-196, and GM-09966. P. d'E is a special fellow of the Leukemia Society of America, Inc.).

Light Chain V Region Markers

051 ORGANIZATION OF V_K GENES ENCODING ALLELIC PHENOTYPES IN BALB/c AND AKR MICE, Virginia Campbell, Janet Stavnezer and J. Latham Claflin, University of Michigan, Ann Arbor, MI 48109 and Memorial Sloan-Kettering Cancer Center, New York, N.Y. 10021

Inbred strains of mice vary in their expression of L chain found in anti-phosphocholine antibodies carrying the T15 idiotype (Id). Genetic studies have demonstrated two phenotypes, Igk-Pc-A (AKR, C58, RF and PL) and Igk-Pc-B (BALB/c and other strains), which behave as co-dominant alleles controlled at a single locus on chr. 6 (1). T15 Id^+ hybridoma antibodies (HP) from AKR and BALB/c exhibit the expected phenotypic variation. We have begun an exploration of the V genes encoding T15 L chains (V_K-22) in AKR and BALB/c in an attempt to determine the genetic basis for this variation.

We have successfully completed a series of Southern blot experiments with enzymatically cut DNA from myelomas, hybridomas and liver which were probed with the T15 V region probe $pT15V_K$. Eco RI digestion yields 7 fragments which hybridize with the probe, the strongest appearing at 3.6, 4.3, 9.0 and 16 kb. Digestion with Hind III yields a major hybridizing fragment at 4.3 kb, 5-10 additional fragments which hybridize to a lesser degree. Neither endonuclease cuts the cloned cDNA in the V region. Thus, there are a large number of bands which hybridize with the probe, indicating that we are looking at a gene family. Second and more importantly, the banding patterns for germline DNA from both phenotypes are indistinguishable. This indicates that the V_K-22 gene families in the two strains are basically similar. Our current working model is that the two phenotypes arose from either allelic genes or pseudoalleles, rather than as a consequence of gene deletions or major compositional differences in the genes between the two strains.

[1]Claflin, J.L., et al. Immunogenetics 6: 379-387, 1978.

052 IDENTIFICATION OF KAPPA CHAIN SUBGROUPS INVOLVED IN LIGHT CHAIN IF-POLYMORPHISMS. D.M.Gibson, W.-T.Hum and C.Lazure,Département de Biochimie,Université de Sherbrooke Sherbrooke,Québec, Canada J1H 5N4

The light chains of normal mouse serum immunoglobulin can be reproducibly resolved into approximately 60-70 major and minor focusing 'bands' by isoelectric focusing (IF-). We became interested in the nature of the light chain bands after finding that some of the bands appear to be under Mendelian control by chromosome 6 loci closely linked to the Lyt-2,3 locus. We previously described two such loci, IgK-Ef1and IgK-Ef2, each of which determines the presence of certain bands in normal light chain IF- profiles (1,2). In the present work we report on the identity of some of the bands involved in the polymorphisms and on the nature of amino acid sequence diversity found within groups of light chains showing identical IF-behavior. The approach was to screen large numbers of randomly selected myeloma proteins from mice carrying the appropriate chromosome 6 markers in search of examples of light chains co-focusing with the polymorphic bands observed in normal light chain IF-profiles. This was possible in the case of the inbred strains NZB (IgK-Ef2^b) and BALB/c (IgK-Ef2^a) since myelomas can be induced directly in these strains. We are indebted to Drs M.Weigert and M.Potter for generously furnishing myelomas from these strains. Screening of the two myeloma collections led to the identification of seven BALB/c myelomas having light chains identical to the Ef2 marker bands present in normal BALB/c light chains (3). Complete sequencing of four of the light chains(FLOPC-1,TEPC-817,TEPC(CAL-20)-105 and TEPC-821) revealed that all four proteins could be considered as members of the same subgroup and could potentially be coded by a single Vk gene. Two of the proteins (T-817 and T-105) were identical throughout the V-region except at position 95 where the normally invariant proline was deleted in T-817. This is presumed to be a new example of V-J junctional variation. The remaining two proteins (FLOPC-1 and T-821) differed from the prototype sequence by 3 (2cdr,1fr) and 1 (fr) residues,respectively. Conventional interpretation of these data would indicate that the four chains must be coded by at least 3 genes, implying that the Ef2 marker involved a cluster of tightly-linked v-genes, some (eg.T-105 and T-821) differing by a single framework residue. Alternatively,if the polymorphism involves a single Vk gene, it would imply that somatic mutation may give rise to substitutions in framework as well as complementarity determining regions.

1. Gibson,D., J.Exp.Med. 144, 298 (1976)
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053 VκSER: A UNIQUE Vκ GROUP ASSOCIATED WITH TWO MOUSE L CHAIN GENETIC MARKERS, Paul D. Gottlieb, Dept. of Microbiology, University of Texas at Austin, Austin, TX 78712; David M. Gibson, Department de Biochimie, Universite de Sherbrooke, Quebec, Canada J1H SN4; and L. Edward Cannon, Rosenstiel Basic Medical Sciences Research Center, Brandeis University, Waltham, MA 02154. Different inbred strains of mice express different repertoires of Vκ regions, governed by genes closely linked to the Lyt-2 and Lyt-3 loci on chromosome 6. While Vκ and Cκ structural genes are known to be present on the same chromosome, it is not known whether the Vκ repertoire differences observed reflect structural or regulatory gene differences among the strains. The Igk-Trp^a allele, associated with the Lyt-3^a genotype, governs expression of a Vκ genetic marker, the I_B-peptide marker, detected by peptide mapping of normal serum Ig L chains.[1] To obtain myeloma L chains bearing the I_B-peptide marker, the Lyt-3^a and Igk-Trp^a alleles of the C58/J and AKR/J strains (non-inducible for myelomas) were bred onto the Balb/c genetic background (myeloma-inducible) to generate the C.C58 and C.AKR congenic strains, respectively. Two hundred myelomas were induced in these mice, and 25 C.C58 myeloma κ chains were purified and subjected to automated amino acid sequence analysis to obtain a sample of the C.C58 Vκ repertoire. One protein, C.C58 M75, had an unusual amino-terminal SER residue, and was unlike any Balb/c or NZB Vκ sequence known. Peptide mapping indicated that it contained the I_B-peptide marker. Isoelectric focussing of all 200 myeloma L chains revealed that C.C58 M75 and 7 other C.C58 or C.AKR L chains co-focussed with normal serum Ig L chain bands unique to the Igk-Ef1^a phenotype described by Gibson[2] and also determined by Lyt-3^a-linked genes. Amino acid sequence analysis of three of these from the C.C58 strain revealed the same amino acid sequence as C.C58 M75 for at least the first 49 residues. Thus they appear to belong to a new Vκ group, VκSER, apparently expressed by some strains of mice but not others. This VκSER group is responsible, in part, for both the Igk-Trp^a and Igk-Ef1^a phenotypes, but both phenotypes are heterogeneous and other Vκ regions are likely to be involved.[3] Amino acid sequence analysis of C.AKR κ chains with focussing properties similar to C.C58 M75 are in progress. In the one protein examined to date, C.AKR F17, the amino-terminal 40 residues are identical with the exception of one difference, surprisingly in the framework peptide which represents the I_B-peptide marker. Cloning of the Vκ regions of VκSER proteins using recombinant DNA technology has been undertaken. Use of such DNA segments to probe the genomes of strains which differ in expression of VκSER should allow us to determine whether failure to express VκSER is due to a structural gene defect or to activity of a regulatory gene. Work was supported by ACS grant IM-113B and NIH grant CA 15808 to P.D. Gottlieb.

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054 IDIOTYPIC ANALYSIS OF HUMAN ANTITETANUS-ANTIBODIES, Peter Altevogt, Michael Mann, and Hans Wigzell, Institut für Immunologie und Genetik,DKFZ, Heidelberg, FRG, and Biomedical Center, Uppsala.

An antiidiotypic antisera was raised in a rabbit against purified $F(ab)_2$ fragments of donor P.A. anti-tetanustoxoid (TT) IgG according to Geha et al. (J.Immunol.121,1518 (1978)). After appropriate absorption the sera was found to react in a RIA assay to anti TT $F(ab)_2$ fragments but not to anti PPD and anti diphteriatoxoid $F(ab)_2$ fragments of donor P.A. The binding of the sera could be partly blocked by soluble TT but not by an irrelevant antigen. The anti idiotypic sera also did not show crossreactivity when tested on a panel of anti TT $F(ab)_2$ fragments of unrelated donors.

The antiidiotypic sera was then used to study the inheritance of the recognized idiotypic determinant(s). Donor P.A.'s father's anti TT $F(ab)_2$ were found to bind while P.A.'s mother's anti TT $F(ab)_2$ fragments did not or only marginally bind to the anti idiotype.

In a second set of experiments radiolabelled anti TT reactive IgG molecules where compared from donor P.A. and his mother and father by 2D gelelectrophoresis. The heavy chains of all three anti TT antibody preparations did not show a high degree of variability and where almost superimpossible. However, the L chain patterns were identical between P.A. and his father whereas that of the mother was totally different. These results suggest that the L chains may play an important role in the construction of the recognized idiotype(s).

055 THE CONTEXT OF ANTIBODY VARIABLE AND CONSTANT REGION GENES IN MICE, Konrad Hüppi, Brian Van Ness, Cynthia Scott, Roy Riblet, Michael Potter and Martin Weigert, Institute for Cancer Research, Philadelphia, Pa. 19111 and National Cancer Institute, National Institutes of Health, Bethesda, Md. 20205

Variable (Vk) and constant(Ck) region genes of the mouse kappa light chain have been compared in inbred strains and wild mice by restriction endonuclease analysis. In all cases, the Ck gene is found on a single fragment and the Vk genes of the Vk-19 and Vk-21 groups are each found on several (7-10) fragments. Differences in the context of Vk19 and Vk21 genes are linked to each other and to the Ly-3 locus. SJL has unique Vk21 and Ck contexts but a Vk19 pattern identical to strains with the Ly 3.2 allele.

Patterns of Vk21 and Vk19 seen in inbred strains are also found in certain wild mice and indicate the origin of the kappa locus in inbred strains. M. musculus castaneus is unique in that a crossover has occurred mapping Vk19 closer to Ly-3 and Vk21 closer to Ck on chromosome 6. Other species show patterns somewhat similar and less related species show no apparent similarity in context to inbred strains.

These diverse patterns suggest that this group of genes may have been amplified in various ways. Among all the wild species studied thus far, the number of hybridizing bands is relatively constant suggesting in this instance, gene expansion to a set number of genes.

056 SEROLOGIC AND STRUCTURAL ANALYSES OF ALLOTYPE-DEFINED RABBIT IMMUNOGLOBULIN CHAINS SECRETED BY RABBIT-MOUSE HYBRID CELL LINES, Kevin L. Dreher, Martin L. Yarmush, Frederick T. Gates,III, and Thomas J. Kindt, Laboratory of Immunogenetics, NIH, Bethesda, MD 20205

Standard cell-fusion techniques using rabbit spleen cells and non-secreting mouse myeloma cell lines have generated hybrid cell lines secreting rabbit Ig chains. After repeated cloning, a number of stable rabbit-mouse hybrid cell lines which have maintained the ability to secrete Ig chains for a period of a year or longer were obtained. Large amounts of monoclonal rabbit Ig chains have been obtained by growing these hybridoma cell lines in spinner cultures and as ascites tumors propagated in both athymic BALB/c mice or in normal BALB/c mice after appropriate adaptation. Two cell lines (7D2, 7D6) secrete rabbit heavy (H) chains having a molecular weight of 55,000. Serological analyses performed on these H chains have shown them to contain a full complement of rabbit variable and constant region allotypes (a3, d11, e15). These H chains are secreted in a complex with a molecular weight of 130,000 and serologic studies indicate that they occur in association with a mouse Kappa light (L) chain. Two cell lines (4C1, 12F2) produce L chains of b4 allotype, while a third cell line (1D4P5) produces a L chain expressing allotype b5. Serologic, SDS-PAGE and amino acid sequence analyses performed on secreted L chain products have confirmed their rabbit origin. Results indicate that these rabbit-mouse hybrid cell lines can provide a stable source of homogeneous rabbit Ig chains and mRNA of a defined allotype.

Structural Studies of C Region Gene Products

057 THE ROLE OF GENERALIZED RECOMBINATION IN IMMUNOGLOBULIN GENE EXPRESSION. Amy Kenter and Barbara K. Birshtein. Department of Cell Biology. Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, N.Y. 10461.

We have isolated a number of variants of the MPC 11 mouse myeloma cell line which synthesize altered immunoglobulin heavy chains.[1] In contrast to the parent γ2b heavy chain, several variant chains have serological characteristics of the γ2a subclass, indicative of the expression of previously unexpressed genetic information. Although some "γ2a" variant chains may have the complete γ2a constant region, others are actually γ2b-γ2a hybrid chains. For one such variant, the junction between γ2b and γ2a sequences has been shown to lie within the C_H2 domain.[2] This junction comprises 24 amino acids which are identical both at the protein and nucleic acid level[5,6] between γ2b and γ2a subclasses and therefore provides a large region of sequence identity which can serve as a target for recombination events. We have noted that there exists within the γ2b gene a DNA sequence, Chi,[7] which in E. coli, facilitates generalized recombination.[8] This same sequence occurs in V_HIII of mouse and human heavy chains, in the 3' mouse κ constant region, and was generated during the heavy chain class switch which gave rise to MC 101. Since the frequency of Chi is higher in immunoglobulin genes than in bulk DNA, we, therefore, ponder: 1) whether the Chi sequence that works in E. coli plays a similar role in eukaryotic cells 2) whether Chi could play a particular role in immunoglobulins and 3) how homologous recombination may be related to unique features of immunoglobulin gene expression.

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058 THE STRUCTURE AND GENETICS OF MOUSE IMMUNOGLOBULIN HEAVY CHAIN CONSTANT REGIONS DEFINED BY MONOCLONAL ANTI-ALLOTYPE ANTIBODIES, Leonard A. Herzenberg, Marilyn Parsons, Chun-Ming Huang and Vernon T. Oi, Genetics, Stanford University School of Medicine, Stanford, California, 94305

Upon entry into the hybridoma era we decided that genetic studies of murine IgC_H allotypes, started more than 15 years ago, could be fruitfully restarted. A series of 25 different monoclonal anti-allotype antibodies directed against the heavy chains coded for by Igh-1, Igh-3 and/or Igh-4 loci were used. The antigenic determinants recognized by each of these antibodies was localized to the hinge, CH_2 or CH_3 domains employing proteolytic fragments of the respective Ig antigens in solid-phase radioimmunoassays. The methods used are similar to those previously published (Oi and Herzenberg, 1979, Molecular Immunol. 16: 1005).

Expression of the determinants that some of these monoclonal antibodies detect is dependent on the tertiary and quaternary structure of the antigen molecule. For example, one anti-Igh-4b antibody preferentially binds to intact Igh-4b molecules but not with the Fc fragment of the same molecule. Further, different Igh-4b immunoglobulins (with different variable regions) are not bound equally by certain anti-Igh-4b antibodies. Further evidence that quaternary structure is important was provided by studying a hybrid Igh-1a/4a molecule from a cell hybrid (made by somatic cell genetic techniques) between 1a and 4a producing hybridomas any determining which allotypic determinants were expressed. Only two of eight Igh-1a determinants and one Igh-4a determinant were found on this molecule.

When these monoclonal antibodies were used to examine the immunoglobulins of inbred and wild mice, several new haplotypes were found. These new haplotypes result from loss of single determinants and from unusual combinations of determinants found in the immunoglobulin allotype "type strains."

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059 STRUCTURAL STUDIES OF MURINE CH ALLOTYPES : MULTIPLE SUBSTITUTIONS BETWEEN γ2a Ig-1a AND Ig-1b HEAVY CHAINS. By J.M. Dognin, M. Lauwereys+ and A.D. Strosberg - Groupe d'Immunologie Moléculaire - I.R.B.M. - C.N.R.S. - Université Paris 7 2, Place Jussieu - 75221 PARIS Cédex 05 (France) and + Biochemical Pathology, Free University of Brussels, V.U.B., Brussels (Belgium).

The amino acid sequence of the Fc region of myeloma protein CBC-101, an IgG2a of allotype Ig-1b, was established by automated and manual Edman degradation of peptides obtained by enzymatic and chemical cleavage. Extensive use was made of High Pressure Liquid Chromatography for the separation and purification of the peptides. The comparison with the heavy chain constant region of protein MOPC-173, an IgG2a of allotype Ig-1a reveals a total of twenty four amino acid differences out of 171 positions examined : six substitutions are located in the CH2 and eighteen substitutions are located in the CH3 domains (1,2). It is likely that several of these differences correlate with the four distinct antigenic determinants identified by the use of monoclonal anti-Ig-1b allotype antibodies (3).

The comparison was also made with the sequence of protein MPC-11, an IgG2b of allotype Ig-3a and of protein MOPC-21, an IgG1 of allotype Ig-4a. At 22 out of the 24 positions where possibly allotype related substitutions are found in the IgG2a constant regions, different residues are found in the IgG2b and IgG1 chains. It is likely that many of the residues at these "variable" positions are located at the surface of the molecules, where they contribute appreciably to the antigenicity of the isotypic and allotypic forms of the murine γ chains.

From the evolutionary point of view, the γ1 chain is clearly the most different from the other chains. The strikingly large number of differences between the allotypic forms of the γ2a chains suggests also an early divergence, difficult to reconcile with current models for the origin of allelic forms. The prolonged coexistence of duplicated γ2a genes and inherited control mechanisms for their expression (4) could constitute an attractive alternative.

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060 NEW Igh-HAPLOTYPES FOUND IN WILD MICE WITH MONOCLONAL ANTI-ALLOTYPIC ANTIBODIES, Chun-Ming Huang, Marilyn Parsons, Edward K. Wakeland*, and Leonard A. Herzenberg, Dept. of Genetics, Stanford University Sch. of Med., Stanford, CA, 94305. (*Dept. of Pathology, University of Florida, Gainesville, FL, 32610)

Sera from 122 wild mice (Mus musculus) collected in several parts of Europe, Egypt, Chile, and Taiwan were tested in solid-phase radioimmunoassays for reactivity with monoclonal antibodies directed against gene products of the Igh-1, Igh-3, and Igh-4 loci. New alleles were found at each of the loci, thus defining new haplotypes. In fact, almost half of the mice show unusual combinations of allotypic determinants not seen in inbred strains. For example, two Taiwanese mice and all of the Polish mice tested possess Igh-1b determinants but lack the Igh-4b determinant. Sera from three Egyptian mice reacted with anti-Igh^a reagents, and also reacted with three of the eight anti-Igh-1b antibodies. The determinants detected by these three antibodies are in the CH3 domain; CH2 Igh-1b allotypic determinants were not found in these same sera. The immunoglobulin which bears the Igh-1b CH3 determinants also has a determinant common to Igh-1a and Igh-3a proteins (detected by a monoclonal antibody). Surprisingly, in most populations Igh^b-related alleles were found, although Igh^a-related alleles were more common. With mutation creating new alleles as indicated by the loss of isolated allotypic determinants and recombination assorting new haplotypes, the Igh gene complex shows an extensive polymorphism in wild mice, similar to the Gm system in man.

This work supported, in part, by NIH grants AI-08917 and CA-04681.

061 MOLECULAR HETEROGENEITY OF THE HUMAN μ-CHAIN-C-PART. Heinz U.Barnikol, Edith Mihaesco*), Constantin Mihaesco*), Shitsu Barnikol-Watanabe and N.Hilschmann, Max-Planck-Institut für experimentelle Medizin, Göttingen, Germany, *) Inserm U108, Hôpital Saint-Louis, Paris, France.

The primary structure of the C-part of the human μ-chain-disease protein BOT has been determined. The C-part of protein BOT corresponds to the entire CH_2-CH_4 stretch, starting with pos.223 (GAL numbering), ending with pos.571, the C-terminus of secreted human μ-chains. 2 amino acid exchanges in comparison to protein GAL have been found. At pos.309 Ser of GAL is substituted by Gly in BOT, at pos.333 Val by Gly. This finding was remarkable because the two previous established μ-chains GAL and OU also differed at pos.309 by a Ser/Gly exchange. From 2 additional μ-chains, SCO and CO, these positions have been further analyzed. In SCO pos.309 has Ser, pos.333 Gly, in CO Gly was found in both positions, as in BOT.
The presence of exchange pairs at pos.309 (Ser/Gly) and at pos.333 (Val/Gly) is understood as a molecular polymorphism of the human μ-chain-C-part. We distinguish 4 μ-chain phenotypes which may be termed as 1. GAL-type: Ser(309), Val(333); 2. SCO-type: Ser,Gly; 3. BOT/CO-type: Gly,Gly and 4. OU-type: Gly,Val. The genetic background of the 4 phenotypes of human μ-chain-C-parts will be discussed. The amino acid sequence of the V-part fragment and the determination of the deleted stretch of the μ-chain-disease protein BOT is currently under study.

062 IDIOTYPE SHARING BY ANTI-CARBOHYDRATE ANTIBODIES FROM RAT STRAINS DIFFERING IN IgG2c ALLOTYPES, Gerrie A. Leslie, Dept. of Microbiology and Immunology, University of Oregon Health Sciences Center, Portland, Oregon 97201. We have previously shown that rat antibodies to Group A Streptococcal carbohydrate (anti-SACHO) are primarily of the IgM and IgG classes. Furthermore, the IgG antibodies are predominantly of the IgG2c subclass and Id-1, a public idiotype, is associated with IgG2c. In our current studies we have shown that anti-dextran, anti-phosphocholine and anti-pneumococcal polysaccharide antibodies are also mainly IgG2c but lack Id-1. In order to study the linkage between anti-carbohydrate V-region genes (V_H) and genes coding for C_H regions we prepared anti-IgG2c allotype antisera. At this time we have defined at least 2 γ2c allotypes. Preliminary data has shown that Id-1 (V_H) can be associated with either of the allotypes (C_H).

063 DIFFERENTIATION FROM IgM TO IgA EXPRESSION IN A MURINE B LYMPHOMA (I.29), Roberto Sitia, Anna Rubartelli and Ulrich Hämmerling, Dept. of Immunology, P.C.P.C.-XIII USL, Genoa, Italy and The Sloan-Kettering Institute for Cancer Research, New York, N.Y. 10021
I.29 is a monoclonal B lymphoma ($Lyb.2^+$, $I\text{-}E^+$, FcR^+, mIg^+) originated spontaneously in the I mouse in 1961. When growing in vivo (ascites) I.29 cells are distinguishable as three subpopulations, according to their membrane immunoglobulins (mIg): IgM^+ IgA^-, IgM^- IgA^+ and IgM^+ IgA^+. IgM and IgA of I.29 have the same L chain (λ), and share the same idiotype. Both heteroantisera and monoclonal antibodies to I.29 idiotypic determinants precipitate μ and α chains from I.29 cell lysates. Although the relative proportions of μ^+, α^+ and $\mu^+\alpha^+$ cells vary in the serial passages of the tumor, the three subpopulations are constantly observed. When I.29 cells are cultured in vitro the percentage of IgM^+ cells decreases rapidly, whereas IgA^+ cells become the predominant subset. This conversion from IgM to IgA also occurs when highly purified IgM^+ IgA^- cells are cultured, suggesting differentiation (μ→α switch) rather than selective outgrowth as the cause of the change. Cloning of I.29 by limiting dilution yielded several cell lines of the IgM^- IgA^+ phenotype. Out of over 200 clones tested so far none expressed IgM. Implantation of one of these cell lines, BFO.3, in I or nude mice, produced a tumor which retained the IgM^- IgA^+ phenotype in successive passages. BFO.3 cells were found to actively secrete IgA in the tissue culture supernatants. Biochemical comparison of membrane associated, secreted and intracellular α chains synthesized by BFO.3 cells showed differences in size, charge, rate of synthesis and cellular localization. Membrane associated α chains were found to be heavier and more acidic than their secreted counterparts.

064 REGULATION OF MEMBRANE AND SECRETED IgM SYNTHESIS IN B CELL LYMPHOMAS. Carol H. Sibley, Holly A. Mar, Paul E. Mains, Department of Genetics, University of Washington, Seattle, Washington 98195

It has recently been established that heavy (μ) chains of membrane and secreted IgM are identical throughout their four constant region domains, but differ at their extreme carboxyterminus. The μ_m has a 41 amino acid segment containing a hydrophobic region which presumably interacts with the membrane, while μ_s has a 20 residue hydrophilic "tail" which is required for J chain interaction and assembly of pentamers for secretion. These two μ chains are encoded in a single μ gene. Separate exons specify the μ_s and μ_m specific segments, suggesting that the mRNA for μ_m and μ_s is constructed by alternative splicing of the primary μ transcript. We have examined four mouse tumors derived from the B lymphocyte lineage whose phenotypes resemble pre-B cells (internal μ only,70Z/3), small B lymphocytes (high levels of surface IgM, WEHI 231), lymphoblasts (both membrane and secreted IgM, WEHI 279.1), and plasma cells (copious IgM secretion, MOPC 104E). Despite the fact that 70Z/3 and WEHI 231 secrete no detectable IgM, all of the tumors synthesize at least intracellular forms of both μ_m and μ_s. The proportion of μ_m is stable and characteristic of each tumor, but is different in each tumor. The 70Z/3 cells and WEHI 231 cells synthesize about 65% of their total μ as μ_m, while WEHI 279.1 cells synthesize about 30% and MOPC 104E cells about 5% of their μ as μ_m. LPS-stimulated B lymphocytes show a similar progression during their differentiation. The proportion of μ_m correlates with other developmentally regulated parameters(Fc receptor, Ia and plasma cell antigen levels, and J chain) and can be used as a simple metric for comparison with developing B lymphocytes and determination of the developmental stage of a B cell tumor.

065 STRUCTURALLY DISTINCT MEMBRANE AND SECRETORY HUMAN GAMMA HEAVY CHAIN POLYPEPTIDES ENCODED BY SEPARATE mRNAs, W. Cushley, C.A. Mickelson and A.R. Williamson, Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ, Scotland.

The human B-lymphoblastoid cell line Bec-11 secretes two forms of IgG and inserts IgG into the cell membrane. Upon treatment of Bec-11 cells with tunicamycin, an inhibitor of N-glycosylation of proteins, two non-glycosylated gamma chains can be detected. Our data suggests that one of these chains is the non-glycosylated precursor of the membrane γ-heavy chain, while the other is the non-glycosylated precursor of the two secreted γ-chains. This implies that the two secreted γ-chains differ only in the amount of carbohydrate they possess, and are not distinct polypeptides. Translation of Bec-11 poly-A^+ mRNA in vitro followed by immunoprecipitation of the translation products and analysis by SDS-PAGE shows two γ-chain bands. This evidence is consistent with the hypothesis that the membrane and secretory γ-chain polypeptides are encoded by distinct mRNA molecules. The difference in apparent molecular weight between the membrane and secretory γ chains is much greater than that measured for the two forms of μ chain and is almost equivalent to a complete domain. This suggests different evolutionary pathways. Studies are proceeding to compare surface and secreted IgA.

The Definition of Public and Private Idiotypes

066 IDIOTYPES OF ANTI-MHC ANTIBODIES, David H. Sachs, Marian Knode, Patricia Kiszkiss, Jeffrey A. Bluestone, Suzanne L. Epstein, and Keiko Ozato, Immunology Branch, National Cancer Institute, NIH, Bethesda, MD 20205

Heterologous anti-idiotypic reagents directed against several anti-H-2 and anti-Ia monoclonal antibodies have been prepared and used as probes of anti-MHC receptors. By hemagglutination inhibition analysis, all four anti-H-2 anti-idiotypic reagents examined appeared to detect private idiotypes in that they reacted specifically only with the hybridoma monoclonal antibody against which they were prepared and not with other monoclonal antibodies of similar H-2 specificity nor with conventional antisera directed against the same H-2 antigens. In contrast, one of the anti-Ia anti-idiotypic reagents examined appeared to detect a public idiotype expressed in the immune sera of mice immunized against the same Ia antigen. Studies of the genetics of idiotype expression for this anti-Ia idiotype suggest that background genes (perhaps Igh-linked) as well as appropriate H-2 type are required for the production of the idiotype upon immunization. The idiotype could be removed from immune sera by in vivo absorp-

tion in strains expressing the Ia antigen to which the monoclonal antibody was directed, but not in strains lacking this antigen, consistent with its presence on specific anti-Ia antibodies in the alloantisera.

Despite failure to detect the anti-H-2 idiotypes in immune sera of appropriate specificity, all of these idiotypes could be induced in appropriate mice by treatment with purified anti-idiotypic antibodies. Production of idiotype in response to such treatment appeared to be Igh-linked, both by strain distribution and by a formal backcross analysis. In some treated mice, the induced idiotype has been shown to be present on anti-H-2 antibodies of similar specificity as the original monoclonal idiotype, while in others the idiotype has been found in the absence of detectable anti-H-2 activity.

Thus, if one defines "private" and "public" idiotypes as those which appear or do not appear during the normal immune response to a particular antigen, respectively, there is evidence for both kinds of idiotypes among the hybridoma anti-MHC repertoire we have studied. However, these definitions are not absolute. Even in the case of the "private" idiotypes detected on anti-H-2 monoclonal antibodies, the genetic information needed for idiotype expression is clearly present in mice of the appropriate strain, since idiotype can be induced by treatment with anti-idiotypic reagents. Such induction of anti-H-2 reactivity is consistent with the existence of a network of cellular interactions in the immune response to MHC antigens, and suggests that anti-idiotypic reagents may be useful in dissecting and manipulating this immune response.

067 CHARACTERISATION AND DISTRIBUTION OF INDIVIDUAL IDIOTOPES, M. Reth, T. Takemori, G. Kelsoe and K. Rajewsky, Institute for Genetics, University of Cologne, Cologne, F.R.G.

The cell hybridisation technique of Köhler and Milstein enabled us to isolate monoclonal antibodies, which bind to idiotypic determinants on the variable region of an antibody. We generated a set of such anti-idiotope antibodies against the primary anti-NP antibody B1-8, a λ1-bearing IgM protein, which is the secreted product of a hybrid cell line. The V_H gene, which is expressed on the B1-8 molecule, is found in the germ line of C57BL/6 (c.f. A. Bothwell et al., this volume). Anti-idiotope antibodies define on the B1-8 variable region different idiotopes, which were characterized by their proximity to the NP binding site and their distribution on a series of monoclonal anti-NP antibodies. One group of idiotopes is binding site related as their reaction with the corresponding anti-idiotope antibodies is inhibited by the hapten NP. In the case of the other group of idiotopes, only hapten-carrier conjugates, not the free hapten, function as inhibitors. All mouse anti-idiotope antibodies inhibit each other in their binding to the B1-8 molecule. All idiotopes so far defined are not found on isolated chains of B1-8 nor on a combination of the B1-8 heavy chain with a κ light chain. Expression of the idiotopes therefore seems to require the presence of a λ light chain. B1-8 idiotopes are found independently from each other in different combinations on λ-bearing anti-NP hybrid cell and serum antibodies. In the primary immune sera of C57BL/6 mice these idiotopes are regularly expressed on 1-10% of the anti-NP antibodies, with a lower frequency for the binding site related idiotopes than for idiotopes of the other groups. Generation of idiotope bearing antibodies in the NP response could be enhanced or reduced by preinjection of anti-idiotope antibodies in phosphate buffered saline (c.f. K. Rajewsky et al., this volume). Absorption of anti-NP sera on NP-Sepharose eliminated also all idiotope-bearing molecules. Idiotope positive, λ1-bearing antibodies, which appeared in the sera of C57BL/6 mice after an immunization with conjugates of anti-idiotope antibody and keyhole limpet hemocyanine in CFA, are in their majority not NP specific. Only 10% of these molecules exhibit NP binding activity. At least some B1-8 idiotopes can therefore be expressed on antibody molecules with a different and yet unknown specificity. B1-8 idiotopes show not strict linkage to the Igh-b allotype of C57BL/6. An analysis of the strain distribution of idiotope expression after NP immunisation reveals a characteristic pattern for each idiotope.

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068 THE REGULATION OF IDIOTYPES ASSOCIATED WITH THE ANTI-ARS RESPONSE OF THE A/J MOUSE, Malcolm L. Gefter[1], Ann Marshak Rothstein[1], James D. Benedetto[1], and Michael N. Margolies[2], Massachusetts Institute of Technology[1], Department of Biology, Cambridge, MA 02139 and Massachusetts General Hospital[2], Department of Surgery, Boston, MA 02139.

Hybridomas isolated from A/J mice responding to the hapten azophenylarsonate (Ars) have been characterized by chemical and serological means. This analysis at the molecular level has allowed us to draw several conclusions about the qualatative nature of the response and to permit a detailed quantitative study of its control.

The dominant idiotype associated with this response is composed of antibodies containing approximately 30-100 unique heavy and light chain sequences. Serological analysis reveals that these individual species appear reproducibly in responding A/J mice and constitute "private idiotypes" which are genetically controlled. Strains unable to produce the dominant idiotype (public idiotype) are unable to produce the private idiotypes. Suppression of the dominant idiotype with conventional or monoclonal anti-idiotypic antibody leads to an immune response in which the private idiotypes do not appear.

Analysis of the hybridomas synthesizing antibodies that are not members of the dominant idiotype family revealed a second idiotype family present in A/J mice responding to the Ars hapten. This second idiotype is regulated independently from the first one, has a different strain distribution and by chemical analysis is structurally distinct.

Conventional anti-idiotypic antisera prepared in rabbits can be shown to contain reactivities against both of these distinct families.

069 ALLELIC FORMS OF ANTI-PHOSPHORYLCHOLINE ANTIBODIES: THEIR EXPRESSION IN INBRED AND WILD MICE, Rose Lieberman, Michael Potter, Stuart Rudikoff, William Humphrey, Jr. and Lawrence A. Hoostelaere, National Institutes of Health, Bethesda, MD 20205, and Litton Bionetics, Rockville, MD 20854

IdI or private idiotypic markers, C3 and T15, specific for two phosphorylcholine (Pc) binding myeloma proteins, have been previously described (1). Four phenotypes have been found in inbred mice - $C3^-T15^+$, $C3^+T15^-$, $C3^+T15^+$, $C3^-T15^-$. Genetic studies with these two markers were done with BALB/c ($C3^-T15^+$) and C57BL/6 ($C3^+T15^-$), and with associated backcross progeny, Ig-congenic, and recombinant inbred mice, derived from these two parental strains. These studies indicated that C3 and T15 IdI behaved as single allelic gene products except in the BAB-14 congenic strain, where both markers were found. These results suggest that BAB-14 which carries the Igh-C of C57BL has V_H genes of both BALB/c and C57BL. The location of the recombination is not yet determined. In a survey of inbred strains, $C3^+T15^-$ IdI was found to be associated with the Igh-C^b and Igh-C^j, and $C3^-T15^+$ IdI with the Igh-C^a, and not the Igh-$C^{c,d,e,f,g}$ haplotypes. The C3 and T15 IdI and allotypic determinants have been examined in 19 stocks of mice from the genus Mus, that have been recently isolated from Asia, Europe and the United States. These include a number of species and subspecies that probably have been separated by millions of years. These mice were immunized with R36 pneumococci to produce phosphorylcholine antibodies. All strains responded except one stock of M. m. domesticus from the eastern shore of Maryland. Many of the common allotypic markers, as well as the C3 and T15 IdI, were found in these mice, including M. cervicolor, M. caroli and M. spretus. Three of the phenotypes, $C3^-T15^+$, $C3^+T15^+$ and $C3^-T15^-$ were found. The combination of $C3^+T15^+$ has been found in Mus m_e castaneus. All of these mice carry only the IgG_{2a}.4 allotypic determinant of the Igh-C^e haplotype, suggesting the lack of Igh-C polymorphism in this stock. Two stocks of M. m. musculus from two different regions of Czechoslovakia also carry these two markers. Appropriate matings are being made to establish whether the C3 and T15 markers have recombined. It is possible that the $C3^+$ and $T15^+$ were together in the primordial gene and may have evolved independently in the continuing polymorphism of the Ig genes.

1. Lieberman et al. J. Immunol. in press, 1981.

070 EXPRESSION OF NP^b AND NP^a IDIOTYPE IN DIFFERENT STRAINS OF MICE, Thereza Imanishi-Kari and Mary White-Scharf, Institute for Genetics, University of Cologne, Weyertal 121, D-5000 Köln 41, F.R.G.

Hybrid cell lines secreting anti-4-hydroxy-3-nitrophenylacetyl hapten (NP) antibodies were randomly selected following fusions of C57BL/6, BALB/c and SJL splenic lymphocytes with the myeloma cell line X63-Ag8.6.5.3. Comparative analysis of monoclonal anti-NP antibodies with serum antibodies suggests that the isolated monoclonal antibodies represent products which are normally present in the serum of mice immunized with NP-protein conjugates. We have characterized the NP^b (C57BL/6, SJL) and NP^a (BALB/c) idiotype positive responses according to fine specificity, idiotype and IEF patterns through the analysis of monoclonal anti-NP antibodies. Idiotype positive antibodies (as identified by polyspecific anti-idiotypic sera) always possess λ light chains. In C57BL/6 mice 90% of the anti-NP antibodies bear λ light chains, whereas 50% of BALB/c and 10% of SJL antibodies have λ light chains. Idiotype positive antibodies from C57BL/6 origin could be classified into 6 different groups according to their idiotypic specificities. Representative antibodies from each group can be pooled to make up the complete NP^b idiotype. Within a group, however, no idiotypically identical molecules were found. The NP^a idiotype is composed of λ-bearing antibodies which are not identical but which seem to fall into a single group. The NP^a positive antibodies demonstrate a strong cross-reaction with one of the NP^b groups of C57BL/6 origin. Analysis of idiotype positive monoclonal antibodies of SJL origin indicates that NP^b determinants are present and that these monoclonals can be classified as a single group which corresponds to one of the C57BL/6 groups. However, the λ light chains which SJL mice express are not λ1 light chains as is the case with C57BL/6 and BALB/c monoclonal antibodies. The results of the analysis of monoclonal anti-NP antibodies suggest that the NP^b and NP^a idiotype positive responses are very heterogeneous, composed of many molecular species sharing cross-reactive idiotypic determinants.

T. Imanishi-Kari, M. Reth, G.J. Hämmerling and K. Rajewsky: in Current Topics in Microbiology and Immunology, Vol. 81 Lymphocyte Hybridomas. F. Melchers, M. Potter and N. Warner (Eds.), Springer-Verlag, Berlin, Heidelberg, 1978, p. 20.
M. Reth, T. Imanishi-Kari and K. Rajewsky, Eur. J. Immunol. 9, 1004 (1979).

071 THE NATURE OF IDIOTYPES AMONG THE MONOCLONAL HUMAN RHEUMATOID FACTORS, David W. Andrews and J. Donald Capra, University of Texas Health Science Center at Dallas, Dallas, Texas 75235

The complete amino acid sequences of the variable regions of two IgM anti-gamma globulins are reported. The two proteins, Sie and Wol, share idiotypic determinants that are distinct from those shared by the two previously sequenced IgM anti- gamma globulins, Lay and Pom.

In contrast to Lay and Pom, Sie and Wol have very little homology in the complementarity-determining regions of their heavy or light chains. However, the J segments of both their heavy and light chains are homologous, and these data suggest that the predominant idiotypic determinant in this system may be related to the amino acid sequence in the J segments of these molecules.

072 *REGULATION OF A SHARED IDIOTYPE IN SJL AND B6 ANTI-NP ANTIBODIES*, Shyr-Te Ju, Baruj Benacerraf, and Martin Dorf, Harvard Medical School, Boston, MA 02115

Hybridoma cell lines secreting antibodies specific to (4-hydroxy-3-nitrophenyl) acetyl (NP) were generated by fusion of NP-immunized SJL spleen cells with SP2/0 cell line. One hybridoma anti-NP antibody (μ,λ) was found to partially inhibit NP^b idiotype binding (30-35%). Iodinated hybridoma antibody could be completely bound with either anti-NP^b antiserum or anti-idiotypic antiserum made against specifically purified SJL anti-NP antibodies. The latter idiotypic interaction was used to define the NP-1 idiotype. The NP-1 idiotypic interaction could be completely and specifically blocked to a similar extent with comparable concentrations of NP-caproate or NIP-caproate between pH 6.5-7.9, indicating a close association of NP-1 idiotypic determinants with NP combining sites and weak heteroclicity of NP-1 idiotypic hybridoma antibody. Strain distribution studies indicate that gene(s) controlling the production of NP-1 idiotype is closely associated with genes linked to the $Igh\text{-}1^b$ and $Igh\text{-}1^e$ alleles. In addition, SJL mice which produce low or no NP^b idiotype due to a defect in their λ chain production also express NP-1 idiotype. Specifically purified B6 and SJL anti-NP antibodies fully express NP-1 idiotype. Adsorption experiments using Sepharose conjugated with anti-λ reagent indicate that both λ- and K-bearing anti-NP antibodies exhibited NP-1 idiotype, suggesting NP-1 idiotype is an H-chain controlled idiotype, independent of L-chain class. Furthermore, the concentration of NP-1 idiotype is markedly reduced in late immune sera of both B6 and SJL mice. (Supported by Grants AI-16980 and AI-14732.)

073 MONOCLONAL ANTIBODY SPECIFIC FOR A CROSS REACTIVE IDIOTOPE ON ANTI-p-AZOPHENYL-ARSONATE ANTIBODIES FROM A/J MICE, Peter V. Hornbeck, George K. Lewis and Joel W. Goodman, University of California, San Francisco, Ca 94122

Cloned rat hybridomas secreting anti-idiotype antibodies were obtained from cross-reactive-idiotype (CRI)-immunized Lewis rat spleen cells fused with a rat tumor line. Antibody from hybridoma AD8 is exceptionally reactive with CRI, and has been shown, with ELISA's, to be idiotype specific by the following criteria: (1) the binding of AD8 to CRI is completely inhibited by CRI-Fab or CRI, but is not inhibited at all by non-immune A/J IgG-Fab, and (2) specifically purified rabbit anti-CRI completely inhibits the binding of AD8 to CRI-Fab. Hapten inhibition studies revealed that monovalent ABA compounds and multivalent haptenated proteins up to 14,000 molecular weight do not compete with AD8 for binding to CRI-Fab,whereas ABA-derivatized proteins with molecular weights of 69,000 or greater specifically inhibit at least 92% of the binding of AD8 to CRI-Fab. AD8 antibodies displace 10-25% of the CRI-Fab bound by the one purified, polyvalent rabbit anti-CRI antiserum so far examined. Monoclonal anti-CRI reagents are useful in elucidating the ontogeny of idiotype expression in individual strain A mice, the regulatory effects of anti-CRI on the immune response, and the cellular expression of idiotype. This research is supported by N.I.H. grants NOI CB-74178 and AI 05664

074 ANTI ABPC48 ANTIIDIOTYPIC MONOCLONAL ANTIBODIES : ANTIBODY AND IDIOTYPIC SPECIFICITIES Pierre Legrain, Gérard Buttin and Pierre André Cazenave, Institut de Recherche en Biologie Moléculaire, 75221 Paris Cedex 05, France

Seventeen monoclonal syngeneic antiidiotypic anti ABPC48 antibodies (= IDAs) have been obtained. The monoclonal antibodies were purified from ascitic fluids and analyzed for their specificities. Some of them recognize idiotopes present on both antilevan ABPC48 and UPC10 myeloma proteins. By competition assays between IDAs for the binding to ABPC48, we identify 6 groups of antibodies corresponding to - at least - three families of idiotopes on the ABPC48 idiotype. Syngeneic antiidiotypic antisera obtained against several IDAs (= anti-antiidiotypic antibodies) show that IDAs belonging to the same group share idiotopes. Monoclonal anti IDA10 antibodies (anti-antiidiotypic antibodies) have been recently isolated from one mouse. A fine analysis of the specificity of these antibodies is in progress : some recognize IDAs of different groups, showing that antiidiotypic antibodies with different combining sites share idiotopes. This material is exploited to analyze idiotype - antiidiotype interactions at the level of defined idiotopes and to estimate the influence of these interactions in the expression of ABPC48 idiotype during the antilevan response.

075 MONOCLONAL ANTI-IDIOTYPES AS PROBES FOR IDIOTOPES ON PHOSPHORYLCHOLINE SPECIFIC B AND T CELL RECEPTORS, R. Wallich and G.J. Hämmerling, Institut für Immunologie und Genetik, Deutsches Krebsforschungszentrum, 69 Heidelberg, West Germany

Anti-PC antibodies of most strains of mice appear to be restricted in their diversity as indicated by the predominant occurence of idiotypic determinants shared with the PC binding myeloma proteins T15 and HOPC8. Furthermore, PC specific B and T cells appear to share the T15 idiotype. In this study the expression of idiotopes on serum antibodies, B and T cells was compared utilizing monoclonal HOPC8 specific anti-idiotype hybridomas.

The results show that a polyspecific rabbit anti-T15 anti-id serum reacts with anti-PC antibodies from most strains of mice. In contrast, the monoclonal anti-id could be divided into 3 groups: I. Some monoclonal anti-id react with anti-PC antibodies of all strains, thus defining public idiotopes. II. Some anti-id react only with anti-PC sera from some but not all strains. III. Other anti-id do not significantly react with immune sera from any strain. These results show (1) that the immune response to PC is more heterogeneous than expected (2) that only some of the idiotopes found on the HOPC8 molecule are present on serum anti-PC antibodies and (3) that strains of mice differ with regard to which idiotopes are expressed. Similarly, analysis of various monoclonal anti-PC antibodies allowed also characterization of idiotopes as public and private ones. One of the private idiotopes could be assigned to the D segment of the HOPC8 v-region. In functional studies designed to compare the idiotopes on PC specific B and T lymphocytes, it was found that only some anti-id (the public ones) would react with PC specific precursor B cells and with PFC, and that only some anti-id would either stimulate PC specific DTH or react with PC specific suppressor cells.

076 UNIQUE IDIOTYPIC DETERMINANTS ASSOCIATED WITH PC-BINDING ANTIBODIES OF THE M511 IDIOTYPE FAMILY. Jacqueline Wolfe, Catherine M. Andres and J. Latham Claflin, Dept. of Microbiology/Immunology, A^2, MI 48109

In mice, the immune response to phosphocholine (PC) consists of antibodies bearing idiotypic determinants of the PC-binding myelomas T15, M511 or M603. Recent studies have revealed a significant level of heterogeneity among serum antibodies bearing M511 idiotypic determinants. These comprise the M511 idiotype family. In order to examine the nature of this idiotypic diversity we have isolated PC-binding hybridoma proteins that bear M511 idiotypic determinants and have prepared anti-idiotypic antisera to these individual members of the M511 family. These antisera identify unique or private idiotypic determinants on M511-positive hybridoma proteins and antibodies. We have examined the frequency of hybridoma specific determinants in various PC-immune sera in order to investigate possible idiotypic regulation of individual members of the M511 idiotype family.

077 DISSECTION OF AN IDIOTYPE, Claudia Berek, Basel Institute for Immunology, 4005 Basel, Switzerland

The immune response of Balb/c mice to the antigen phosphorylcholine (PC) seems to very restricted, as the majority of the antibodies bear the idiotype of the myeloma protein TEPC15 (T15). PC-binding hybridoma proteins were screened with monoclonal antibodies specific for idiotopes of the myeloma protein T15. The data indicate that antibodies classified by anti-idiotypic serum as $T15^+$ can differ in at least some of their idiotopes from the myeloma protein T15.

Injection of monoclonal anti-idiotypic antibodies in newborn, but also in adult, mice leads to a suppression of the immune response to PC. The remaining antibodies are negative for the idotope which is detected by the monoclonal antibody used for suppression. In general, the expression of idiotope and idiotype seems to parallel. However, in individual mice plasma cells secrete T15-like antibodies which are negative for the specific idiotope.

Together these results indicate that the T15 idiotype is a family of very closely related antibodies and that using antibodies specific for a single idiotope, subsets of this family can be regulated.

078 THE IDIOTYPE OF AN ANTI-Ia MONOCLONAL ANTIBODY: EXPRESSION IN HUMORAL IMMUNE RESPONSES, Suzanne L. Epstein, Keiko Ozato, Jeffrey A. Bluestone, and David H. Sachs, Immunology Branch, National Cancer Institute, NIH, Bethesda, MD 20205.

The idiotype of a monoclonal anti-Ia antibody has been detected using a heterologous anti-idiotype, and its expression as a component of humoral responses to the corresponding Ia antigen has been studied. The monoclonal antibody, 14-4-4S, was derived from a C3H.SW anti-C3H ($H\text{-}2^b$ anti-$H\text{-}2^k$) immunization, and is an IgG2a, k protein recognizing Ia-7, a public specificity of I-E/C antigens. A pig was immunized with Protein A-purified antibody from 14-4-4S culture supernatant. The resulting antiserum was absorbed with LPC-1, an IgG2a, k myeloma protein with no known binding activity, to remove reactivity with constant region determinants. The anti-idiotypic antibodies were then purified by adsorption and elution from 14-4-4S coupled to Sepharose. In inhibition enzyme-linked immunosorbent assays, these anti-idiotypic antibodies reacted with 14-4-4S but not with other IgG2a, k monoclonal proteins. The idiotype was readily detectable in individual C3H.SW anti-C3H immune sera, but not in C3H.SW normal serum. The idiotype could be removed from C3H.SW anti-C3H serum by in vivo absorption in B10.A(5R) but not in B10.A(4R) mice, consistent with its presence on specific anti-Ia-7 antibodies in the antiserum. Thus this idiotype appears to represent a major shared idiotype of anti-I-E/C antibodies occurring in the response of C3H.SW mice. Little or no idiotype was detected in alloantisera containing anti-Ia-7 activity made in B10, A.BY, A.SW, and D2.GD mice. Immunization and idiotype testing of allotype congenic and backcross mice are in progress. The expression of idiotype by alloreactive T cells is also under investigation.
S.L.E. is a fellow of the Jane Coffin Childs Memorial Fund.

079 PUBLIC AND PRIVATE IDIOTYPIC DETERMINANTS OF CRI^+ A/J ANTI-p-AZOPHENYLARSONATE HYBRIDOMA PROTEINS AND ANTIBODIES, Edmundo Lamoyi, Lorraine A. Gill-Pazaris and Alfred Nisonoff, Department of Biology, Rosenstiel Research Center, Brandeis University, Waltham, MA 02254

It has recently been shown that anti-p-azophenylarsonate (Ar) antibodies of A/J mice, which share a cross-reactive idiotype (CRI), are not homogeneous but actually comprise a family of idiotypically related molecules. Earlier studies, using antiidiotypic antibodies prepared against individual CRI^+ hybridoma products (HP), revealed that they possess unique or private idiotypic determinants (IdI). In addition, we were able to demonstrate the presence of highly conserved, binding-site associated "public" determinant(s) by using an assay system in which anti-id against one HP reacted with a different HP as a ligand. Nearly all HP's tested were equal in inhibitory capacity in this assay. We have now quantified private determinants with antiidiotypic antisera made specific for the IdI by adsorption with A/J normal immunoglobulins and CRI^+ HP's lacking the private determinant. One such IdI was found in 0.1 to 3.0% of anti-Ar antibodies of every mouse tested. However, another IdI was present in only about one-half of the individual sera assayed, the remainder showing negligible quantities of the IdI. The data reflect the existence of a very large repertoire of CRI^+ anti-Ar antibodies, requiring a large number of germ line genes or, more probably, somatic diversification from a limited number of germ line genes.

080 FINE ANALYSIS OF IDIOTYPIC DETERMINANTS EXPRESSED BY MONOCLONAL ANTI-GAT ANTIBODIES. L. Leclercq, G. Sommé, J.C. Mazié, J. Thèze. Institut Pasteur, Paris, France.

A major idiotype, GAT-715, was defined by a rabbit anti-idiotypic serum raised against BALB/c anti-GAT antibodies. Idiotypic determinants cross-reacting with GAT-715 have been found on anti-GAT antibodies from 14 strains of mice and from rats and guinea pigs. Idiotype GAT-715 is composed of 3 types of idiotypic specificities. AKR, C57Bl/6, C57Bl/10, CBA and SJL mice express only part of the GAT-715 idiotype after immunization with GAT. The idiotypic determinants expressed by these strains are similar and represent a public idiotypic specificity, p.GAT. Strains like BALB/b, DBA/2, ATL, BUB and DBA/1 express, in addition, a strain restricted idiotypic specificity, s.r.GAT. Using recombinant strains of mice, it was shown that the expression of s.r.GAT follows the genetic distribution of V_H genes of BALB/c origin. Rat and guinea pig anti-GAT antibodies bear a third idiotypic specificity, called highly conserved idiotypic specificity, h.c.GAT.

14 BALB/c anti-GAT hybridomas have been obtained in 4 independent fusion experiments. The 14 monoclonal anti-GAT antibodies exhibit a higher affinity for GT and GAT than for GA. They all bear h.c.GAT and p.GAT, but not s.r.GAT. With sera of BALB/c mice immunized with one of the monoclonal antibodies, we have identified an individual idiotypic specificity called i.GAT. This specificity is present on two additional monoclonal antibodies and is normally expressed on BALB/c anti-GAT antibodies although in small amounts.

More recently these results have been confirmed with a BALB/c anti-idiotypic serum raised against BALB/c anti-GAT antibodies. The model relative to GAT-715 idiotype is presently challenged with monoclonal anti-idiotypic antibodies.

081 PRIMARY STRUCTURAL ANALYSES OF MONOCLONAL A/J ANTI-ARSONATE ANTIBODIES BEARING A CROSS REACTIVE IDIOTYPE. Mark Siegelman, Clive Slaughter, Larry McCumber, Pila Estess and J. Donald Capra. University of Texas Health Sciences Center Dallas, Texas 75235. The induced antibody response to the hapten p-azophenylarsonate in the A-strain mouse has a predominant homogeneous component bearing a serologically defined cross reactive idiotype (CRI). This homgeneity in the serum response has lent itself particularly well to primary structural analysis. While early efforts centered on the serum molecules, more detailed analysis of the structural basis of the CRI has directed attention to a comparison of monoclonal arsonate (Ar) specific antibodies bearing or lacking the CRI. An examination of the variable region sequences of heavy and light chains from several CRI positive molecules has revealed a degree of heterogeneity in the framework and hypervariable regions which contrasts markedly with the homogeneity of the serum antibody sequences. While earlier analyses supported the notion that the hypervariable regions of both heavy and light chains contributed to the cross idiotypic determinant(s), the numerous differences found in the first and second hypervariable regions of these monoclonal antibodies forces a re-evaluation of this idea. Rather, the data implicate the third hypervariable regions and/or J-segments of both chains as comprising these entities. The variable region sequences of several idiotype positive and idiotype negative hybridoma molecules will be presented.

082 INDIVIDUAL AND CROSS-REACTIVE IDIOTYPES CAN BE DEFINED BY A SINGLE MONOCLONAL ANTIIDIOTYPIC ANTIBODY, Michael C. Pawlita, Elizabeth B. Mushinski and Michael Potter, National Cancer Institute, National Institutes of Health, Bethesda, MD 20205 Spleen cells from A/J mice immunized with XRPC 24(X24) Galactan binding myeloma protein (GalBMP) were fused with Sp2/0 cells. Hybrids producing antibodies that bound XRPC 44(X44), a related GalBMP were selected and cloned. Hy_{X24}-14 is a monoclonal antiidiotypic antibody specific for some but not all of the closely related GalBMP. Relative binding affinity as measured in competitive solid phase RIA is highest for X24, intermediate for X44, low for T601 and nondetectable for J539. With ^{125}I-X24 as target, Hy_{X24}-14 defines an individual idiotype (X24IdI) inhibitable to 50% with only 7.2 ng cold X24, whereas 2800 ng of X44 are needed to give the same inhibition. When ^{125}I-X44 and ^{125}I-T601 were used as targets X24, X44, T601 and a few other GalBMP were able to inhibit. The idiotype defined by Hy_{X24}-14 is hapten inhibitable, related more to the heavy than to the light chain variable region and not identical to any of the idiotypes defined by conventional homologous antisera. Using the amino acid sequences of 4 GalBMP variable regions and a hypothetical three dimensional model of J539 the relevant amino acids forming the idiotype recognized by Hy_{X24}-14 are tentatively localized in and around VH_2.

Relationship of Idiotype to Antibody Specificity

083 THE CONTRIBUTION OF IDIOTYPE-BEARING NON-ANTIGEN-SPECIFIC Ig TO THE POOL OF ANTIGEN-INDUCED, NON-SPECIFIC Ig, Michael H. Julius, C. Heusser and J.W. Johnson, Basel Institute for Immunology, 4005 Basel 5, Switzerland

The concurrent production of specific antibodies and immunoglobulin (Ig) with no apparent specificity during the course of thymus-dependent humoral responses has been well documented. Considering a set of Ig specific for a given antigen, Jerne has postulated the existence of non-antigen-specific Ig related to the former as a consequence of bearing common idiotopes. This "non-specific parallel set" would conceptually be subject to similar pressure mediated by anti-idiotypic antibody as would the antigen-specific, idiotype-bearing Ig. The object of the following study was to assess the contribution of non-specific parallel Ig to the non-specific Ig produced upon antigen or mitogen stimulation.

The idiotypically homogeneous humoral immune response to phosphorylcholine (PC) in BALB/c mice was analyzed in three plaque-forming cell (PFC) assays designed to detect cells secreting (i) idiotype-bearing anti-PC Ig, (ii) idiotype-bearing Ig, and (iii) Ig. The analysis revealed that the ratio of cells secreting idiotype/cells secreting antigen-specific idiotype was >1, suggesting some contribution to total Ig by non-specific parallel Ig. However, neither of these PFC assays developed more than 10-20% of the total Ig-secreting cells, indicating that at best the contribution of non-specific parallel Ig to the total increase in Ig would be small. Nonetheless, a measurable number of antigen-induced, idiotype-bearing, non-antigen-specific PFC would provide direct evidence for idiotype-focused interactions among Ig's.

By definition the obligatory requirement prior to designating Ig as part of the non-specific parallel set is the demonstration of its lack of antigen specificity. Further experiments employing hapten inhibition of PFC demonstrated that all idiotype-secreting PFC were antigen-specific. Hence, in these circumstances the idiotype PFC assay was simply demonstrated as being a more sensitive assay for antibody than the conventional PFC assay employing antigen-coated indicator cells.

While these experiments do not exclude the antigen induction of idiotype-bearing, non-antigen-specific Ig at some frequency beyond the resolution of techniques employed, it is clear this species of Ig is in even greater minority than antibody relative to the total Ig produced.

084 ANTIGEN-DEPENDENT INDUCTION OF A CROSS-REACTIVE IDIOTYPE

M. Bosma and E. Enghofer, Institute for Cancer Research, Fox Chase Cancer Center, Phila., PA 19111

U10-173 is an antigenic marker in mice that identifies a small family of heavy chain variable regions found on Igs of different classes (IgM, IgG, IgA) and on Igs known to bind different carbohydrate antigens (e.g. 2-6 levan, 1-6-D-galactan); it is detected in most mouse strains and shows autosomal dominant inheritance (1,2). With respect to the latter, two strains of allotype-congenic mice are of particular interest: $C_3H{\cdot}SW{\cdot}Ig^a$ (CWA) and $C_3H{\cdot}SW{\cdot}Ig^b$ (CWB). U10-173 is detectable in CWB but not in CWA. Immunization of these two strains against bacterial levan showed the following: 1) both CWA and CWB mice made large quantities of anti-levan antibody (900-1500 μg/ml serum) but whereas none of the antibody was U10-173$^+$ in CWA virtually all of the antibody in CWB was U10-173$^+$; 2) unexpectedly, about half of the CWB mice produced ∿2x more U10-173 than anti-levan; and 3) mild x-irradiation (200 R) of CWA mice prior to their immunization with levan caused some mice (25-30%) to produce anti-levan antibody that was U10-173$^+$. X-irradiation alone did not result in detectable U10-173 production. These results demonstrate two interesting phenomena: antigen-dependent induction of a specific population of Ig (U10-173$^+$) that does not bind the immunizing antigen and antigen-dependent induction of a specific Ig marker in mice previously thought to lack the corresponding Ig gene(s).

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085 IgG ANTIBODIES TO PHOSPHORYLCHOLINE EXHIBIT MORE DIVERSITY THAN THEIR IgM COUNTERPARTS

Patricia J. Gearhart, Nelson D. Johnson, Richard Douglas, and Leroy Hood, Department of Embryology, Carnegie Institution of Washington, Baltimore, MD 21210, and Division of Biology, California Institute of Technology, Pasadena, CA 91125.

One goal in molecular immunology is to understand the mechanisms responsible for antibody gene diversification. We have analyzed the diversity in anti-phosphorylcholine antibodies from BALB/c mice by determining the amino acid sequences of the variable regions of heavy and light chains from IgM and IgG hybridoma proteins and comparing them to the sequences of IgA myeloma proteins. The data reveal two striking findings.

(1) The variable region segments of the heavy chain encoding amino acids 1-95 demonstrate sufficient variability to raise the possibility of somatic diversification of germline genes. The number of different protein segments is greater than the number of DNA restriction fragments that cross-hybridize with a cDNA copy of a variable region in this group. The protein sequence data show that amino acid variation occurs as frequently outside as within the hypervariable region, suggesting that the presumptive mechanism for somatic variation is not confined to the hypervariable regions.

(2) The IgG antibodies exhibit significantly more variability than their IgM counterparts. The IgA antibodies are also more diverse than the IgM antibodies. These data suggest that the selection of infrequent germline or somatically-generated clones gives rise to greater heterogeneity in the IgG and IgA pools than in the IgM pool. The mechanism of selection is not clear since the IgM, IgG and IgA antibodies have the same range of affinities for phosphorylcholine.

086 IDIOTYPE AND SPECIFICITY OF LEWIS RAT ANTIBODIES AGAINST ENCEPHALITOGENIC PEPTIDES

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Lewis rat antibodies to encephalitogenic peptides 68-85 and 68-88 of guinea pig myelin basic protein were purified by affinity chromatography and used to immunize rabbits. After exhaustive absorption of the rabbit antisera to remove anti-rat immunoglobulin activity, the antisera retained activity directed against the immunogen as shown by their ability to block reaction of radioiodinated peptide with the active site of the rat anti-peptide antibodies. Intrastrain idiotypic cross-reactivity was assessed by testing the rabbit antisera against a panel of Lewis anti-peptide 68-88 antibodies of similar, but non-identical specificities. The results of this experiment showed that multiple idiotopes were present on the anti-peptide antibodies, that each rabbit antiserum recognized a particular set of idiotopes, and that the degree of cross-reactivity was a function of each individual rabbit antiserum. Lewis rat antibodies against the closely-related peptides 68-88 and 68-85 were found to contain no common idiotopes when tested with rabbit anti-idiotypic antisera. These results showed a strong positive correlation of anti-peptide antibody fine specificity and idiotype. Anti-peptide 68-88 antibodies raised in F344 rats were found to carry interstrain cross-reacting idiotopes when tested with rabbit anti-(Lewis anti-peptide 68-88) antibodies. (Supported by USPHS research grant NS-10721.)

087 IDIOTYPIC DETERMINANTS OF AUTOANTIBODIES AND LYMPHOCYTES IN SPONTANEOUS AND EXPERIMENTALLY INDUCED AUTOIMMUNE THYROIDITIS, Maurizio Zanetti, Randall W. Barton and Pierluigi E. Bigazzi, University of Connecticut Health Center, Farmington, CT 06032

We have recently shown that autoantibodies to thyroglobulin found in sera of Buffalo (BUF) rats with spontaneous autoimmune thyroiditis share idiotypic (Id) determinants and that their in vivo production can be partially suppressed by passive anti-Id immunity (Eur. J. Immunol., in press). In the present study, we have compared idiotypic determinants of spontaneous autoantibodies to rat thyroglobulin (SART) with those of autoantibodies experimentally induced (EART) in BUF rats. Anti-Id antibodies reacted with radiolabeled SART and EART (42% vs 22%) and inhibited the binding of both SART and EART to radiolabeled rat thyroglobulin (51% vs 29%), suggesting that cross-reacting Id determinants are located on the antigen-binding sites of both autoantibodies. Spleen lymphocytes from BUF rats with circulating SART or EART were studied by indirect immunofluorescence using anti-Id antibodies. We found that both groups of animals had significant numbers of Id-bearing cells when compared to normal animals. In addition, anti-Id antibodies inhibited the binding of radiolabeled rat thyroglobulin to lymphocytes from rats of both groups (49% vs 23%). Finally, cross-reacting Id determinants were found on EART from Fischer rats, rabbits and guinea pigs, as well as SART of human origin. These results demonstrate that common idiotypic determinants are present on both spontaneous and experimentally induced autoantibodies to thyroglobulin, even across species barriers.

088 CHARACTERIZATION OF THE M511 FAMILY OF MOUSE ANTI-PHOSPHOCHOLINE HYBRIDOMA PROTEINS, Catherine M. Andres, N. Martin Young and J. Latham Claflin, University of Michigan, A^2MI, 48109, Nat. Res. Council of Canada, Ottawa, Canada K1A OR6

The M511 family of mouse anti-phosphocholine (anti-PC) hybridoma proteins is one of four known families that constitute the restricted response to PC. This family was characterized immunologically using anti-idiotypic (Id) rabbit serum, and biochemically using competitive antigen binding studies. Hybridoma proteins, (HPs) were tested for their ability to inhibit the binding of ^{125}I-HP to specific anti-Id antiserum. Two systems were run in parallel in these radioimmunoassays: C70 anti-M511 with ^{125}I-M511 (the myeloma protein), and C77 anti-101.6G6.2 with ^{125}I-101.6G6.2 (an M511 HP). In the first system M511 was the best inhibitor with 15 ng/ml giving 50% inhibition; the other HPs required from 60-300 ng/ml. Using C77, M511 and one HP, 101.3C2.2, showed very little inhibition while two other HPs inhibited 50% with 35 to 300 ng/ml. C70 thus appears to recognize shared Id determinants present on all HP structurally related to M511 whereas C77 recognizes private Id on a subpopulation of M511 HP. Competitive antigen binding studies using radioimmunoassays in which PC analogues compete with ^{125}I-PC-protein for binding to purified myeloma protein or HP confirmed differences seen in the anti-Id analysis. For example, 5-phenyl-pentyl trimethyl ammonium iodide inhibited binding of PC to M511 completely at 10^{-3} M but showed only 20% inhibition for 101.3C2.2 and 101.6G6.2. Complete inhibition by 4-carboxybutyl trimethyl ammonium iodide at 10^{-3} M occured for 101.3C2.2 but this compound showed 68% inhibition for M511 and 56% for 101.6G6.2. Thus, among M511 antibodies structural differences (idiotypes) translate into antibody combining site diversity.

089 SHARED IDIOTOPES AMONG MONOCLONAL ANTIBODIES TO DISTINCT DETERMINANTS OF SPERM WHALE MYOGLOBIN, Yoichi Kohno, Ira Berkower, Gail Buckenmeyer, John Minna and Jay A. Berzofsky, NCI, NIH, Bethesda, MD 20205

We have prepared anti-idiotypic antibodies against mouse monoclonal antimyoglobin antibodies by immunizing guinea pigs, tolerized to mouse Ig, with affinity-purified monoclonal anti-myoglobins and then absorbing the resulting antisera to remove antibodies to normal mouse Ig. The monoclonal antibodies (all IgG_1) bind to different sites on myoglobin as judged by their different fine specificities for myoglobin from a variety of species with known sequence differences (Berzofsky, Hicks, Fedorko and Minna, J. Biol. Chem., Dec., 1980, and unpublished observations). Moreover, antibodies from clone 4 and clone 5 can bind simultaneously to monomeric myoglobin, and must therefore bind to distant sites on the antigen molecule. Therefore, these antibodies would be expected to have distinct idiotypes. Surprisingly, by competitive binding studies, we found that anti-idiotypic antiserum #1382 to clone 5 inhibited myoglobin binding by antibodies of clones 1 and 4 as well as clone 5 (but not clone 6). Similarly, anti-idiotypic antiserum #1004 raised against clone 4 also inhibited clone 1 but not clones 5 or 6. Therefore, despite having different combining sites specific for different antigenic determinents, antibodies from clone 4 share some idiotope(s) with clone 1 antibodies; likewise, antibodies from clone 5 share idiotope(s) with clone 1 and clone 4 antibodies. Why should antibodies share idiotypes if they bind to structurally unrelated antigenic determinants which have in common only the property that they happen to be linked on the same antigen molecule? The explanation might lie in some idiotypic network regulation of the overall response to myoglobin.

090 COMPARATIVE ANALYSIS OF MONOCLONAL AND HETEROLOGOUS ANTI-IDIOTYPIC REAGENTS, Brian L. Clevinger and John F. Kearney, Washington University School of Dental Medicine, St. Louis, MO 63110 and University of Alabama in Birmingham, Birmingham, AL 35294

We have compared the fine specificity of heterologous anti-dextran idiotype reagents with hybridoma-derived monoclonal reagents. The heterologous reagents were produced by appropriate absorption of rabbit and goat antisera raised against the α1,3 dextran binding myeloma proteins M104 and J558. The monoclonal reagents were derived from hybridization of J558 or M104 immune mouse lymphocytes. These reagents were tested for their ability to bind to a panel of 20 dextran binding hybridoma proteins whose V_H amino acid sequence had been determined. No differences were found between monoclonal and heterologous reagents specific for a cross-reactive idiotype (IdX) associated with the second hypervariable regions of dextran binding proteins. However, unexpected discordance was found comparing reagents to D-dependent idiotypes. The only differences in the V_H regions of M104 and J558 are found in the two amino acids comprising their D segments, positions 100 and 101. Heterologous reagents clearly react only with proteins sharing D segments. However, monoclonal reagents selected to be D-specific reagents showed specificities for dextran binding proteins that were inexplicable based on existing structural data. These data demonstrate that the specificity of monoclonal proteins must be carefully analyzed and that analysis of specificity based on a small number of ligands may be misleading. It is also clear that in some cases conventional antisera can be better reagents for following a particular structure.

091 IDIOTYPIC HETEROGENEITY OF AUTOIMMUNE ANTI-DNA ANTIBODIES, Tony N. Marion, Alexander R. Lawton, III and David E. Briles, University of Alabama in Birmingham, Birmingham, Ala. 35294 and Vanderbilt University, Nashville, Tennessee, 37232

In order to assess the clonal diversity of spontaneous anti-DNA antibodies in autoimmune mice, we have produced 13 different, cloned anti-DNA antibody-secreting hybridomas by fusing spleen cells from an unimmunized (NZBxNZW)F_1 mouse. As previously reported, the hybridoma antibodies are heterogeneous by isoelectric focusing and antigen-binding analyses. We have subsequently studied the binding characteristics of the hybridoma antibodies with several different types of nucleic acids. While also indicating that the hybridoma antibodies are heterogeneous, these results have allowed us to group the antibodies into four antigen-binding categories according to their relative avidities for double- and single-stranded DNA, ribosomal RNA, poly I-poly C, poly A-dT, and transfer RNA.

Anti-idiotype (anti-Id) sera were prepared in rabbits against three of the hybridoma antibodies. After adsorption with normal NZB and NZW serum, which lacked anti-DNA activity, all three anti-Id sera reacted very strongly with the respective immunizing antibody. One anti-Id serum (anti-DNA-2) cross-reacted weakly while another (anti-DNA-3) cross-reacted strongly. Anti-DNA-3 reacted most strongly with those hybridoma antibodies in the same antigen-binding group as the immunizing antibody. These anti-Id sera are now being used to screen sera from individual autoimmune mice for the presence of the respective idiotypic determinants recognized by the anti-Id sera. The results of these studies will not only provide information as to the clonal nature of autoimmune responses in mice but also help to determine the feasibility of using idiotype suppression to control autoimmunity.

092 H-L COMBINATORIAL DIVERSITY AMONG ANTI-GAC HYBRIDOMAS. Moon H. Nahm and R. Jerrold Fulton. Washington University School of Medicine, St. Louis, MO 63110.

Despite our improved understanding of intrachain diversity, the rules regulating heavy (H) and light (L) chain pairing (combinatorial diversity) are largely unknown. Previously we demonstrated that the charge diversity of the IgG3 anti-GAC antibodies in A/J mice results from the combination of a few types of L and H chains. To analyze this combinatorial diversity further, we generated hybridomas to GAC in A/J mice and produced rabbit anti-idiotype antibodies to these hybridoma proteins. One anti-idiotypic antiserum recognizes L chains. The idiotype + IgG3 proteins, are molecules with an identical L chain IEF pattern, but a different isoelectric focusing point, indicating that their L chains are the same and the same L chain can combine with many different H chains. Furthermore, the idiotypic determinant is expressed when the L chain of the idiotype + protein is recombined with another IgG3 H chain of idiotype - proteins. This idiotype is not inhibited by hapten. The idiotype is found in large amounts in anti-GAC sera from A/J, BALB/c and C57BL/6 mice but in low amounts in normal sera. It appears that the idiotype is not always associated with anti-GAC antibodies. A direct plaque assay was developed based on the facilitation of protein-A plaques which showed that ~1/1000 immunoglobulin secreting cells possessed the idiotype. Furthermore, we were able to find two non-GAC-specific hybridomas bearing this idiotype from fusions of spleen cells from LPS stimulated BALB/c and NZB mice. Further characterization of their L chains is in progress. Expression of the L chain idiotype in diverse immunoglobulins suggests that L chains have a degree of freedom to associate with H chains. Supported by NIH grants AI-11635 and AI-15926.

093 THE ANTI-p-AZOPHENYLARSONATE CROSS-REACTIVE IDIOTYPIC FAMILY OF STRAIN A MICE INCLUDES MEMBERS WITH NO REACTIVITY TOWARD p-ASOPHENYLARSONATE, Lawrence J. Wysocki, Vicki L. Sato, The Biological Laboratories, Harvard University, 16 Divinity Avenue, Cambridge, MA 02138.

The sera of unimmunized A/J mice contain low but detectable levels of immunoglobulin, bearing cross-reactive idiotype (CRI) determinants previously described as diagnostic of the strain A anti-p-azophenylarsonate (Ars) response. Such molecules cannot be adsorbed onto high density Ars-coupled affinity columns. After extensive immunization with Ars-coupled proteins, this CRI^+ Ars^- component shows no detectable increase even though a large CRI^+ Ars^+ population appears. Immunization with a hapten-inhibitable monoclonal rat anti-CRI, which was originally raised against an Ars-binding, CRI^+ component elicits high concentrations of Ars^+ as well as Ars^- CRI^+ immunoglobulin. Three hybridoma proteins produced from such an immunized animal react with all tested anti-idiotypic sera from three species of animals, but show no reactivity toward Ars in several different assays. One hybridoma protein from the same fusion demonstrates Ars binding capacity.

094 IDIOTYPIC COMPARISON OF ANTI-PHOSPHOCHOLINE, T15 ID POSITIVE HYBRIDOMA AND SERUM ANTIBODIES, Anne Maddalena and J. Latham Claflin, University of Michigan, Ann Arbor, MI. 48109

Hybridoma and serum antibodies binding phosphocholine are being compared between BALB/c (Igh^a) and CBA (Igh^j) mice. We aim to describe the relatedness of the T15 Id+ variable regions expressed by mice which carry different heavy chain alleles, and which exhibit differences in heterogeneity of T15 Id+ antibodies. Among T15 Id+ hybridoma proteins (HP) from BALB/c and CBA as well as other strains, we have found no differences in fine specificity of binding with 15 choline analogs. However, these antibodies vary in their reactivity with both heterologous and homologous anti-idiotypic sera. Heterologous sera contain separable reactivities for heavy chain allotype-associated and -nonassociated idiotypic determinants, the latter of which are more likely to be hapten-inhibitable. Expression of idiotypic determinants may also be affected by heavy chain subclass and light chain pairing. Initial data on homologous anti-HP idiotypic sera show the ability to distinguish between T15 Id+ antibodies within an allotype and within a C_H subclass. Moreover, individual mouse serum antibodies contain HP-defined idiotype-positive and -negative subgroups, separable by isoelectric focusing. Thus in CBA, the T15 Id identifies a family of antibodies which are distinguishable by isoelectric point and idiotypy, but interestingly demonstrate the same binding specificity for phosphocholine.

095 IDIOTYPES AND PARATOPES OF ANTI-INFLUENZA HYBRIDOMAS, Louis M. Staudt and Walter U. Gerhard, The Wistar Institute, Philadelphia, PA 19104.

The relationship between idiotype and combining site specificity was assessed in hybridoma antibodies(Abs) specific for the hemagglutinin(HA), a glycoprotein of the Influenza A virus. The advantage of this system is that monoclonal anti-HA Abs can be used to select antigenic variants of the virus which have single point mutations in the HA. A panel of 39 distinct mutant viruses(defining 4 antigenic sites on the HA) was used to determine the fine specificities of 118 anti-HA Abs derived from 13 individual Balb/c mice. 118 different paratopes were distinguished. Rabbit anti-idiotypic sera, raised against 6 of these Abs, showed 2 types of idiotype-paratope relationships. Firstly, 4 idiotypic systems showed a strict idiotype-paratope correlation. No idiotypic crossreactions were seen unless the Abs had identical fine specificities. The crossreacting Abs, even though derived from different individual mice, were idiotypically identical and thus probably represented the same clonotype. Secondly, 2 other idiotypic systems showed partial crossreactions(50-80% inhibition) between Abs of very similar yet slightly different fine specificities. However, other Abs derived from the same individual mouse as the idiotypically crossreactive Abs and also having similar fine specificities were idiotypically unrelated. One of these idiotypic systems showed partial crossreactions with Abs of completely different fine specificities derived from different individual mice. This panel of Abs was also tested in a serological assay for the Vk21 light chain and 13 positive Abs were found. These Abs, derived from 4 individual mice, were all specific for the same antigenic site on the HA and represented 43% of all Abs specific for this site.
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096 IDIOTYPIC ANALYSIS OF ANTIBODIES AGAINST GROUP A STREPTOCOCCAL CARBOHYDRATE SECRETED BY TWO VARIANTS OF A HUMAN B CELL LINE, C.Polke,B.Greger and K.Eichmann, Institut für Immunologie und Genetik, Deutsches Krebsforschungszentrum, Heidelberg, Fed.Rep.Germany

The B17 cell line is an Epstein-Barr virus transformed human lymphoblastoid cell producing anti Group A streptococcal carbohydrate (A-CHO) antibodies of the IgM-kappa type which appear to be moleculary homogeneous. A subclone of this cell line, termed F6, secretes antibodies of the same class and specificity. While the isoelectric points of the u-chains of the two antibodies are identical, the pI of the two kappa chains are different in a way which is compatible with a single amino acid exchange. Anti-idiotypic antibodies against the purified anti-A-CHO antibodies of B17 or F6 cells were prepared in guinea-pigs that had been made tolerant to human Ig. The anti B17 anti-idiotypic antiserum binds F6 antibodies 200 fold less efficiently than the B17 antibody. The reverse is true for the binding-pattern of anti-idiotypic antiserum anti F6. There are two altertnative interpretaions of these results:

1. F6 cell clone represents a minor population of the B17 cell line.
2. F6 cells have arisen through a somatic mutation in the kappa chain of B17 cells.

To decide between the alternatives we are presently investigating the relationship between structural and idiotypic differences of these two antibody molecules.

097 Characterization of the antibody response to a chemically defined, naturally occurring antigen. Michael S. Weaver and Julia G. Levy. University of British Columbia, Vancouver, Canada V6T 1W5.

Ferredoxin is a low molecular weight protein (6200d) isolated from Clostridium pasteurianum. This bacterial antigen possesses two non-cross reactive antigenic determinants and engenders a restricted antibody response in selected strains of mice. Immunochemical studies of ferredoxin have shown that antibody responses in several species are confined to two sequences of between five and seven amino acids in extent. Serum antibodies from immune mice bind these epitopes in proportions which are regulated by genes mapping in the I-region of the H-2 complex. Hybrid cell lines secreting monoclonal ferredoxin binding antibody have been isolated from immune mice through fusion with the SP2/0 myeloma cell line. The resulting antibodies bind to proteolytic enzyme derived fragments of the native antigen as well as peptides synthesized by the Merrifield technique which correspond in amino acid sequence to these two distal regions of the molecule. A correlation between the determinant specificity of the individual hybridoma protein and a particular idiotype has been observed using rabbit anti-idiotype sera. Analysis of the distribution of the hybridoma idiotype in serum antibodies from congenic mouse strains indicates that it is a major idiotype expressed in different inbred strains.

098 EVIDENCE FOR A SUBSET OF RHEUMATOID FACTORS THAT CROSS-REACT WITH DNA-HISTONE AND HAVE A DISTINCT CROSS-IDIOTYPE, Vincent Agnello, Anne Arbetter, Graciela Ibanez de Kasep, Richard Powell, Eng M. Tan, and Fenneke Joslin, Tufts-New England Medical Center, Boston, MA 02111

Cross-reactivity of a monoclonal rheumatoid factor with an antigen present on IgG and DNA-nucleoprotein was demonstrated, and evidence presented that the combining site of the antibody was involved in the reaction. The antigen on the DNA-nucleoprotein was shown to involve both DNA and histone fraction H2A+H2B and was trypsin sensitive. The relative binding affinity of the antibody appeared to be greater for IgG than the DNA-histone antigen. Similar polyclonal cross-reactive rheumatoid factors were found in a variety of diseases. A high incidence was found among patients with rheumatoid arthritis and mixed connective tissue disease. None were detected in patients with systemic lupus erythematosus and idiopathic cryoglobulinemia. Studies on one representative isolated polyclonal rheumatoid factor demonstrated the same reactivity with DNA-histone H2A+H2B as the monoclonal antibody. Cross-idiotype studies using antigen-binding inhibition methods demonstrated the same cross-idiotype on the polyclonal and the monoclonal rheumatoid factor which reacted with DNA-histone. This cross-idiotype was shown to be distinct from the cross-idiotypes previously demonstrated on monoclonal IgM proteins with anti-γ-globulin activity.

099 REGULATION OF ID-460: A PREDOMINANT IDIOTYPE IN THE ANTI-DNP RESPONSE, Elaine A. Dzierzak, Ethan A. Lerner and Charles A. Janeway, Jr., Yale University, New Haven, Ct. A predominant idiotype, Id-460 (related to the idiotype found on the BALB/c, DNP-binding myeloma protein, MOPC 460), is produced in BALB/c mice in an immune response to the antigen DNP-OVA. Id-460 expression is found to be highly regulated early after boosting immunization. It is a major idiotype expressed in Ig^a allotypic mice, as shown by genetic studies. Interestingly, Id-460 detected in pre-immune serum does not bind DNP, whereas that in immune serum does. Thus, the activation of Id-460 positive B cells by DNP may reflect the action of related helper T cells specific for this idiotype and may be dependent on this normally occurring Id-460. To isolate a cell producing the non-DNP-binding Id-460 detected in pre-immune serum, hybridomas were made from LPS-stimulated normal BALB/c spleen cells. A hybridoma yielding such a product has been isolated. The antigen binding specificity of this Id-460 positive, non-DNP-binding monoclonal immunoglobulin is being sought. The role of such idiotypic immunoglobulin in the Id-460 dominance of the anti-DNP response is also being investigated.

100 HUMAN HYBRIDOMA CELL LINES; A NOVEL METHOD OF PRODUCTION, Stuart A. Clark and William H. Stimson, Biochemistry Dept. Strathclyde University, Glasgow; Alan R. Williamson and Heather M. Dick, Depts. of Biochemistry and Bacteriology, Glasgow University, Glasgow.

Human hybridoma cell lines have been derived by fusing RPMI 8226 and PWM-stimulated peripheral blood mononuclear cells. Selection was achieved by pre-treatment of the myeloma cells with diethylpyrocarbonate (Wright, W.E. Experimental Cell Research 112 : 395-407 (1978)).

Successful fusions were detected by assaying for immunoglobulin using an enzyme immunoassay procedure. Fusion frequencies in the range $2\text{-}5 \times 10^5$ were obtained in the initial experiments. The specificity of the immunoglobulins secreted is under investigation by various techniques.

101 SELECTIVITY OF THE BACKGROUND AND PRIMARY MOUSE IMMUNE RESPONSE FOR POLYMORPHIC DETERMINANTS ON XENOGENEIC ERYTHROCYTES. *Tim R. Mosmann, R.G. Ritzel, and B. Michael Longenecker, University of Alberta, Edmonton, Alberta, Canada.* Background responses have been assessed by fusing LPS-stimulated spleen cells from unimmunized mice with MOPC 315.43 myeloma cells. The resulting hybrids have been screened for the production of antibody against chicken red blood cells (CRBC) and other antigens. CRBC-specific clones represented about 1% of total hybrid clones (5-10,000 hybrids were obtained per mouse). The majority of the anti CRBC clones (>90%) secreted antibody against polymorphic CRBC determinants (present on CRBC from some but not all chickens) rather than species-specific determinants present on all CRBC. Some of the polymorphic determinants were linked to the *B* locus (the MHC of the chicken) and some were non-B antigens. The relative amount of these two categories varied slightly according to the mouse strain. Similar specificities were obtained using mice primed three days previously with CRBC. Almost all anti-CRBC clones produced antibody directed against polymorphic histocompatibility or blood group antigens. Fusion of unimmunised mouse spleen cells also yielded a small number of anti SRBC clones, some of which were specific for polymorphic determinants. These results have considerable importance for the use of xenogeneic erythrocytes as "standard" antigens. We are currently exploring the specificity of the mouse anti CRBC response in more detail, both genetically and biochemically. By injection of mRNA into frog oocytes, we have obtained synthesis, assembly and secretion of functional antibody. We are using this system to explore the relative contributions of heavy and light chains to antibody fine specificity and idiotype, by assembling different combinations of heavy and light chains in oocytes.

102 IDIOTYPIC CHARACTERIZATION OF ANTI-DNP RESPONSIVE B-CELLS IN NEONATAL MICE, Kathleen A. Denis and Norman R. Klinman, University of Pennsylvania, Philadelphia, PA 19104, and Scripps Clinic and Research Foundation, La Jolla, CA 92037.

With the advent of hybridoma technology, it became possible to derive an immunoglobulin producing cell line from any B-lymphocyte and thus create new idiotype systems of carefully chosen design. Previous studies in this laboratory have shown the neonatal anti-dinitrophenyl response to be sufficiently restricted to enable individual clonotype identification, yet diverse enough to represent a normal antibody response. In addition, these clonotypes present have been subject to a minimum of genetic and environmental effects but exist at a time where the chance to observe generative and regulatory events is high. We have derived hybridomas from neonatal B-lymphocytes which have been stimulated with DNP either in vitro in a splenic fragment system or in vivo in an adoptive transfer system. Anti-idiotype reagents have been made against four of these neonatal antibodies and used to examine the anti-DNP response in both neonatal and adult Balb/c, B10.D2 and (Balb/c X B10.D2)F1 mice as well as study the crossreactivities among the hybridomas. Using this set of reagents, we are able to characterize a significant proportion of the anti-DNP responsive B-lymphocytes present during the first week of life in Balb/c mice. We find this response to be of a restricted nature, temporally patterned, reproducable among individuals of a strain and influenced in its expression by the heavy chain allotype of the responding cell.

Soluble Specific Products from T Cells

103 IMMUNOGENETICS OF MONOCLONAL SUPPRESSOR T CELL PRODUCTS, Judith A. Kapp and Barbara A. Araneo, The Jewish Hospital of St. Louis, St. Louis, MO 63110 and Shyr-te Ju and Martin E. Dorf, Harvard Medical School, Boston, MA 02110.

The synthetic polymers L-glutamic acid^{60}-L-alanine^{30}-L-tyrosine^{10} (GAT) and L-glutamic acid^{50}-L-tyrosine^{50} (GT) stimulate specific suppressor T cells in certain strains of mice (1,2). Extracts from these T cells contain factors that specifically bind antigen and inhibit development of both plaque forming cell (PFC) responses and proliferative responses to GAT or GT by T cells from mice primed with GAT or GT complexed to methylated bovine serum albumin (MBSA) (3,4). To obtain sufficient specific factors for biochemical analysis, we have produced several T cell hybridoma cell lines by fusion of GAT- or GT-primed splenic T cells from various strains with the HGPRT$^-$ AKR thymoma, BW5147. These clones constitutively produce GAT-specific or GT-specific T cell-derived suppressor factors (TsF) that inhibit both antibody and proliferative responses by lymphocytes from mice syngeneic with the spleen cell donor used for fusion (5). Suppression of immune responses by this hybridoma factor,like suppression by extracts (6),is mediated by a new round of specific suppressor T (Ts2) cells. Serological and functional characterization of this panel of monoclonal TsF should provide insight into the molecular biology of one class of antigen-specific T cell products that show subtle differences in fine specificity. All of the TsF examined bear determinants encoded by the I-J subregion of the suppressor T cell donor's haplotype. Nevertheless, the monoclonal TsF can inhibit responses by allogeneic lymphoid cells by causing the induction of Ts2 cells. We have previously shown that rabbit anti-mouse anti-GAT antibodies bound GAT-TsF in lymphoid cell extracts (7). Preliminary experiments indicate that monoclonal TsF also bear idiotypic determinants some of which are shared with murine antibodies specific for the same antigen.

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104 ANTIGEN SPECIFIC T CELL HYBRIDS, Nancy H. Ruddle, Belinda B. Beezley and Wendy B. Barry, Department of Epidemiology, Yale University Medical School, New Haven, CT 06510

The aim of our work has been to develop T cell lines which recognize specific antigen and/or produce lymphokines. We have prepared somatic cell hybrids between BW 5147 and azobenzene-arsonate (ABA) specific suppressor cells (Hyb 51 series) and BW 5147 and suppressor cells for the IgE anti OVA response (Hyb 49 series) and have identified antigen specific activity. Some hybrid lines form rosettes with antigen coupled red cells, and this activity is inhibited by preincubation of the hybrid cells with soluble specific antigen (1,2). Antigen also influences growth rate of the hybrids when included in the culture medium. Hybrid clones have been identified which produce ABA specific suppressor factors (3). Because we determined that most of the activities peaked in late logarathmic phase of growth, regulation of their expression in the course of culture phase and cell cycle has been analyzed.

We have developed an azaguanine resistant variant of YAC-1 (Ly $1^{\pm}$ Ly 2^{+}) on the assumption that its more highly differentiated phenotype would permit expression of a wider variety of T cell activities after hybridization than the Ly negative BW 5147. Hybrids have been made between YAC-1 (azg^{r}) and OVA specific delayed hypersensitivity effector cells and are being analyzed for lymphotoxin production.

Hybrids have been made between BW 5147 and cells from C57BL/6 mice sensitized to NP-CGG in order to study cross reactive delayed hypersensitivity to NIP at the single cell level.

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105 DETECTION AND ANALYSIS OF HAPTEN SPECIFIC MOLECULES RELEASED BY SELECTIVELY IMMUNIZED T CELLS. George K. Lewis, Peter V. Hornbeck, Craig T. Morita, Diane Scott, and Joel W. Goodman. Dept. of Microbiology and Immunology, University of California at San Francisco, San Francisco, California, 94143.

Sensitive in vitro radiochemical and kinetically read hapten binding protocols were developed which readily permit the detection of hapten binding molecules released during a two day culture by as few as 10^{7} unfractionated, activated lymphocytes. Using well established procedures which selectively activate T cells to factor production, usually with the production of demonstrable B cell tolerance, we have found marked contamination of these preparations with hapten specific IgM. Depletion experiments suggest that this IgM is B cell derived. Additionally, it was found that those preparations containing spleen cells also produced the most IgM. In addition to IgM, these preparations also contain molecules which appear not to be conventional immunoglobulin. In labelling experiments, these molecules do not enter native SDS gradient gels (mw 300Kd). By contrast, reduction and alkylation reveals molecules of 92Kd, 68Kd, and 45Kd nominal molecular weight. Preliminary binding studies on factors separated by Sepharose 6B suggest that a peak of activity is seen at about 400Kd and 80Kd. Taken together, these data point to the existence of T cell derived products which associate with an unknown stoichiometry to form a biologically active complex. This research is supported by NIH grants NOI CB-74178 and AI 05664

106 AN ANTIGEN-SPECIFIC SUPPRESSOR T CELL FACTOR - IT'S FUNCTIONAL AND MOLECULAR ORGANIZATION - Masaru Taniguchi, Izumi Takei, Takashi Saito and Takeshi Tokuhisa, Department of Immunology, School of Medicine, Chiba University, Chiba, Japan.

The functional and molecular organizations of an antigen-specific suppressor T cell factor were investigated by using the products from T cell hybridomas. The hybrids were made by the fusion of AKR-derived BW5147 thymoma cell line with antigen-specific suppressor T cells of C57BL/6 mice primed with keyhole limpet hemocyanin enriched by antigen(KLH)-coated Petri dishes. The antigen-specific suppressor activity was obtained in the extracted materials of the hybridoma cells(clone 34S-18) and in ascites from hybridoma-bearing mice. From the studies on the immunochemical and molecular analysis of the active materials, the antigen-specific suppressor factor was found to be composed of two distinct molecules: One is the molecule having antigen-binding site probably encoded for by genes linked to those coding for the variable region of the immunoglobulin heavy chain, the other the molecule encoded for by genes in the I-J subregion of the H-2 complex. These two molecules are independently synthesized in cytoplasm and secreted as the suppressor factor after their association with disulfide(S-S) bond. The association of the two molecules are necessary for acting as the active suppressor factor. The molecular weight of the factor was between 45,000 and 70,000 daltons on the gel filturation analysis using Sephadex G-200 column.

Furthermore, the antigen-specific suppressor factor(TsF_1) derived from the clone 34S-18 hybridoma was found to stimulate and activate another I-J positive T cell hybridoma(34S-281) with acceptor site for TsF_1, which did not have any functional activities by itself. The acceptor T cell hybridoma(34S-281) after the treatment with TsF_1 in the absence of antigen turned to produce the active I-J positive suppressor factor(TsF_2), suppressing the responses of syngeneic or semisyngeneic mice, but not allogeneic recipients in an antigen-specific fashion. In this sence, there is no differences between TsF_1 and TsF_2. However, despite its antigen-specific suppressor activity, TsF_2 has not been shown to have antigen-binding capacity. Therefore, it is quite possible that TsF_2 has the complementary structure for TsF_1 like anti-idiotypic receptor since specific-antigen binding site composed of V_H structure of immunoglobulin has been shown to be expressed on the TsF_1.

The results clearly indicate that the cooperation between two subsets of suppressor T cells with different functional specificities are necessary for the initial step of suppressor pathway, in which the interactions via V_H and I-J determinants on the factor should be involved.

107 ISOLATION OF ANTIGEN BINDING MOLECULES FROM T-CELLS, Robert W. Rosenstein, James Murray, Robert Cone and Richard Gershon, Yale University, New Haven, CT 06510

Hapten specific, T-cell antigen binding molecules (ABM's) were isolated from the supernatants of cultured, immune spleen and LN cells and from their cell membranes. The supernatant material is associated with the specific suppression of delayed-type hypersensitivity skin reactions induced by reactive forms of the haptens dinitrophenyl, trinitrophenyl and oxazolone. Radioactive amino acids included in the cell cultures are incorporated into the ABM's. The molecules are isolated from culture supernatants by a combination of hapten affinity chromatography and molecular sizing using sephacryl gels. Cell surface ABM's are isolated by immunoprecipitation from detergent lysates of radioiodinated cell membranes using antisera raised against supernatant derived material.

SDS-PAGE analysis ABM's isolated either from culture supernatants or from cell surfaces yields chains of 68-72Kd size, with occasional smaller sized chains at 45Kd and 25Kd.

Competitive binding radio-immunoassays suggest that ABM's do not bear conventional Ig determinants, nor antigens encoded in the H2 region, including the I region. Antisera raised against ABM can be shown to bind to T-cells but not B-cells.

108 ANTIGEN BINDING MOLECULES IN DTH T-SUPPRESSOR SUPERNATANTS, Craig T. Morita, Joel W. Goodman and George K. Lewis, University of California, San Francisco, San Francisco, CA

Culture supernatants (CS) were derived from spleen and lymph node cells of mice immunized to induce DNP-specific, DTH supressor T cells (Zembala and Asherson, Eur. J. Immunol. 4:799). The CS contained DNP-specific binding activity as measured by haptenated phage inactivation (HPI) and as directly visualized on SDS-PAGE gels of affinity purified, biosynthetically labelled factors (APF*). These CS neutralized only DNP-phage and not ABA, NP or native phage. Analysis of APF* with anti-mouse IgM, anti-mouse IgG and anti-mouse H and L chain immunoabsorbents revealed molecules of H and L chain molecular weights upon reduction and alkylation. Consistent with these results was the reduction of HPI titers when the CS was passed through an anti-mouse Ig column (reactive vs. H and L chains). HPI titers, however, were not totally removed even upon sequential absorption. An equivalent titer of anti-DNP activity in mouse antiserum was completely removed on a single column passage. Additional evidence for a non-Ig component was derived from a biphasic kinetic pattern of HPI. In contrast, monophasic patterns were seen with MOPC 315, MOPC 460, mouse anti-DNP and the "Ig" component of CS eluted from the anti-Ig column. Estimates of k_r calculated indirectly for the fast component of HPI and directly by hapten reactivation of neutralized phage gave $k_r \geq .06$ sec^{-1} and $k_r \geq .02$ sec^{-1}, respectively. MOPC 315 gave estimates of 3×10^{-4} sec^{-1} indirectly and 8×10^{-3} directly. Also, hapten inhibition of HPI gave a 50% inhibition point at 10^{-3} M for CS versus $10^{-5.6}$ M and 10^{-5} M for MOPC 315 and MOPC 460, respectively. These data suggest that a low affinity/avidity, non-Ig molecule exists in CS in addition to conventional antibody. (NOI CB-74178 and AI 05664)

109 THE PRESENCE OF Ia ANTIGENS AND IDIOTYPES IN A T-CELL FACTOR WHICH COMPLEXES Ig AND ANTIGEN. K.C. Wang and F. Paraskevas, Dept. of Medicine, Univ. of Manitoba, Winnipeg, Canada R3E OV9

Antigen (Ag) complexed with Ig is detected in the serum of animals 6 hours after immunization. The complexes which act as potent amplifier factors in antibody formation are formed through the mediation of a factor released from T-cells. We demonstrate here that the T-cell factor contains both Ia antigens and idiotypic determinants. The presence of the factor is demonstrated by its ability to generate cytophilic Ig for T-cells in the presence of 7S Ig and Ag. Supernates from purified T-cells were passed through anti-Ia immunoadsorbents and were shown to lose the ability to generate cytophilic Ig for all antigens tested. An anti-idiotypic antiserum was made by injecting rabbits with a highly purified antibody to the hapten azobenzenarsanilic acid (ABA). T-cell supernate which was passed through an anti-idiotypic immunoadsorbent was tested using ABA-MSA (mouse serum albumin) as well as a conjugate of ABA with a synthetic peptide of glutamine and tyrosine. In addition other foreign proteins, such as fibrinogen and egg albumin were used. In contrast to the absorption with anti-Ia the anti-idiotypic immunoabsorbent removed the ability of the supernate to generate cytophilic complexes after the addition only of the ABA conjugates, but not after addition of fibrinogen or egg albumin. In conclusion these results demonstrate that the complexing factor contains Ia antigens and idiotypic determinants. The former correspond to a constant region while the latter form the variable region of the factor.
Supported by grants from NCI and MRC of Canada.

110 RECEPTOR SPECIFICITY OF H-2 ANTIGEN-REACTIVE SUPPRESSOR T CELLS. S. RICH, D. DENNISON AND R. RICH, BAYLOR COLLEGE OF MEDICINE, HOUSTON, TX. 77030.

The H-2 antigens required for triggering MLR-TsF production were studied to clarify the receptor specificity of allo-reactive Ts and to investigate antigen binding properties of the MLR-TsF molecule. MLR-Ts were primed against full H-2 differences and restimulated with cells sharing K and D, D only or I region haplotypes with the priming stimulator cell. Each of the region-specific stimuli triggered partial but significant MLR-TsF activity, while third party stimuli were ineffective. Thus, MLR-Ts with specificity for each H-2 region were demonstrable. In addition, we determined whether MLR-TsF expresses bound stimulator cell antigenic determinants. MLR-TsF was prepared in H-2 congenic combinations, and passed over immunoabsorbents specific for stimulator or third party H-2 haplotypes. Suppressive activity was partially or completely removed only from stimulator-specific column effluents and was recovered in acid eluates. The H-2 region origin of bound stimulator determinants was established through adsorbent analysis of MLR-TsF from Ts restimulated with H-2 region restricted stimulators. TsF from K/D specific restimulation was adsorbed by sequential application to anti-K and anti-D columns. Similar studies have also demonstrated TsF-associated stimulator I region molecules. Thus a major portion of MLR-TsF is complexed with stimulator H-2 molecules and discrete TsF species binding K, I or D determinants are generated. Such complexed MLR-TsF is however functionally antigen-nonspecific. In contrast, TsF which is not removed by anti-stimulator H-2 adsorbents, and presumably is not bound to alloantigen molecules, suppresses MLR in an antigen specific fashion. Supported by USPHS Grants AI 13810 and AI 17048

111 Ovalbumin Binding Activity In Supernatants of Immune T Cells Expanded In Conditioned Media, T.M. Folks, R. Champer, K.W. Sell. Immunobiology Section, Lab. of Immunogenetics, NIAID, NIH, Bethesda, MD. 20205

Evidence has been obtained that an ovalbumin binding factor is produced by murine T cells maintained in long term culture. BALB/c mice were given a footpad injection of ovalbumin (ova) in CFA and after 2 to 4 weeks spleens or thioglycolate-induced peritoneal exudate cells were removed. T cells were purified on nylon-wool columns and restimulated in vitro with ova. T blast cells were harvested and continuously proliferated in the presence of supernatants from Con-A activated normal BALB/c spleen cells. Some T blast cells were first expanded in soft agar containing ova only and then clones were picked and expanded in liquid culture with Con-A supernatants. Culture supernatant fluid from 5 ova primed T cell lines maintained from 1 to 8 weeks were tested for ova binding activity by a radiobinding assay. Two of the lines tested positive. Supernatants taken from similarly treated T cells primed to pork insulin or from EL-4 or BW 5147 cultures contained background levels of ova binding activity. Studies in progress aim to characterize the ova binding activity from the T cell preparation.

112 MHC RESTRICTION OF SOLUBLE HELPER MOLECULES IN T CELL RESPONSES TO TNP ALTERED SELF. Janet Plate, Rush Presbyterian St. Lukes Medical Center, Chicago, IL. 60612.

T lymphocytes that serve regulatory functions in the generation of effector T cells to allo-antigen and to altered syngeneic cells express a distinguishing H-2 associated cell surface marker that maps within the I-A region. The removal of these I-A positive T cells has allowed us to detect MHC restriction of soluble helper molecules in the generation of syngeneic killer T cells to TNP-altered self. Ia bearing T cells obscure the observation of such interactions, thus, must be removed in order to detect MHC restriction of "non-specific" soluble helper factor supernatants. Genetic mapping studies demonstrate that the strain producing HFS must be compatible in the H-2IA, H-2IB region of the MHC with the strain utilizing the helper molecules in order for optimal helper signals to be delivered. MHC restriction of soluble helper molecules in the absence of I-A region bearing Ia^+ T cells has been observed only in syngeneic responses and not in allogeneic responses. We have recently obtained evidence for H-2 antigen specific helper molecules. Possible MHC restriction of the antigen specific factor in helping allo-responses is under study.

113 CHARACTERIZATION OF AN Ia BEARING T HELPER CELL SUBSET, James E. Swierkosz, St. Louis University School of Medicine, St. Louis, MO 63104

Using an anti-Ia serum produced against Con A stimulated T cell blasts, we previously showed that a subset of T helper cells bears I region encoded determinants. Deletion of these Ia^+ T cells by anti-Ia + C treatment inhibited secondary in vitro anti-protein PFC responses which could be restored by the addition of a non-specific helper factor. This suggested that Ia^+ T cells function in the delivery of non-specific activating signals to B lymphocytes. We have strengthened this argument in current studies using a "bystander" helper T cell assay. T cells primed in vivo to KLH will help B cells respond to sheep erythrocytes in vitro in the presence of the priming antigen (KLH). We can directly demonstrate that these T cells, also thought to act by elaboration of a non-specific mediator(s), express Ia antigens. In addition, they can be enriched for by binding to KLH pulsed macrophages indicating specificity for cell-associated antigen. Preliminary experiments indicate that we are not merely detecting Mø Ia bound to the T cell receptor. Thus, an Ia^+ T helper subset (TH_2) known previously to interact with B cells, also recognizes Mø bound antigen. Ia may serve as a marker or as a regulatory site on these cells during their interactions in the helper circuit.

114 SUBSET OF CON A INDUCED SUPPRESSOR T CELLS EXPRESS I REGION DETERMINANTS
Rashmi Dixit and James E. Swierkosz, St. Louis Univ.Sch.Med.,St. Louis, Mo. 63104.

B cells respond in culture (by direct PFC) to sheep erythrocytes in the presence of a T cell-derived helper factor. This response can be highly suppressed by the addition of spleen cells previously cultured for 48 hr with Con A. Such Con A induced suppressor cells are T lymphocytes (non-adherent to nylon wool) and completely sensitive to elimination by monoclonal anti-Thy 1 antibody plus complement treatment. In addition, they are partially sensitive to similar treatment with an anti-Ia serum (A.TH anti-A.TL) prepared against mitogen stimulated T blasts. Suppression is reduced on an average of 50%. We have previously shown that this antiserum readily detects Ia antigens on functional helper T cell subpopulations. The partial abrogation of suppression suggests that Con A suppressor T cells are also heterogeneous in their expression of I-region antigens. In preliminary studies we have mapped at least one determinant coded for by genes to the right of the I-J subregion. We also can find no evidence that the Ia positive T suppressors in this system act via the release of soluble suppressor substances in a manner analogous to the release of helper factors by Ia positive T helpers.

115 H-2 LINKED FINE SPECIFICITY OF T CELLS,
Ira Berkower and Jay A. Berzofsky, NCI, NIH, Bethesda, Md. 20205

We have studied the fine specificity of the mouse T cell proliferative response to myoglobin from a variety of mammalian species. As shown previously, the response to sperm whale myoglobin is controlled by H-2 linked Ir genes. However, one nonresponder strain to sperm whale myoglobin is a responder to horse myoglobin. When these animals were immunized with horse myoglobin, the T cells proliferated to horse but not sperm whale myoglobin. Comparison of T cell responses to a series of myoglobins with amino acid sequences intermediate between horse and sperm whale has identified four amino acids in horse myoglobin, one of which is a necessary part of the antigenic site recognized by T cells. When animals which are high responders to both myoglobins were immunized with horse myoglobin, the T cells proliferated to horse but not sperm whale myoglobin. In addition, one myoglobin variant with a sequence intermediate between horse and sperm whale also caused T cell proliferation. Comparison of amino acid sequences identified one amino acid which is a necessary part of the antigenic site recognized by this strain. This amino acid substitution is not sufficient for antigen recognition by the first strain. Thus, two high responder strains for horse myoglobin, after priming to horse myoglobin, respond to different sequences on the myoglobin molecule. When the second strain was immunized with sperm whale myoglobin, T cells proliferated to sperm whale but not horse myoglobin. The pattern of response to intermediate myoglobin variants was not reciprocal to the pattern after horse myoglobin priming. Reciprocal patterns of response would be expected if the antigenic determinant of both myoglobins were different amino acids substituted at the same position.

116 CHARACTERIZATION OF MONOCLONAL SUPPRESSOR FACTOR SPECIFIC FOR POLY(GLU^{50}TYR^{50})(GT),
Carl Waltenbaugh and Huan-Yao Lei, Department of Microbiology-Immunology, Northwestern University Medical School, Chicago, Illinois 60611.

T lymphocyte hybridomas were generated by fusing GT-primed B10.BR spleen cells with the AKR thymoma, BW5147. Five stable antigen-specific suppressor factor (GT-TsF)-secreting hybrid cell lines were established. Monoclonal suppressor factor (mGT-TsF) acts both in vivo and in vitro by generating a second set of suppressor T cells, but only in the presence of a very small quantitiy (<50 nanograms) of GT. The mGT-TsF preferentially binds to and can be eluted from a GT-Sepharose immunoadsorbent column. Furthermore, mGT-TsF bears both I-J^{k} and idiotypic determinants as shown by its binding to a monoclonal anti-I-J^{k} (WF8.C12.8) immunoadsorbent and an anti-GAT idiotype (anti-CGAT) immunoadsorbent column. The mGT-TsF can be sequentially adsorbed onto and eluted from the WF8.c12.8 and anti-CGAT columns. Treatment of the sequentially purified mGT-TsF with either 25 mM 2-mercaptoethanol or 5 mM dithiothreitol for 30 min at 22^{o}C causes no detectable reduction in suppressive activity. Neither the effluent nor the eluate of the reduced, sequentially purified mGT-TsF adsorbed on a monoclonal anti-I-J^{k}-Sepharose column under mild reducing conditions shows suppressive activity. If, however, equal portions of effluent and eluate are admixed, suppressive activity is restored - indicating that mGT-TsF is comprised of at least two polypeptide chains linked by a disulfide bond.

117 T CELL DERIVED HAPLOTYPE SPECIFIC SUPPRESSOR FACTOR FROM NEONATALLY TREATED MICE, Craig M. Sorensen and Carl W. Pierce, Jewish Hospital of St. Louis, St. Louis, MO 63110

Culture supernatants of spleen cells, nylon wool purified splenic T cell or lymph node cells from C57BL/10 (B10) or BALB/c mice neonatally treated with semi-allogeneic (B10.D2 X B10)F_1 cells and restimulated with Mø syngeneic at I-A with that same allogeneic haplotype contain a soluble factor capable of suppressing primary in vitro antibody responses of normal spleen cells. Suppression is antigen non-specific and is restricted to primary in vitro antibody responses; the factor has no effect on MLR or CTL responses. The suppressor factor (TsF-H) is inactivated by trypsin, low pH, and 30' at 50°C, and has a molecular weight in the range of 45,000-68,000 daltons as determined by gel filtration. Studies with specific immunoabsorbents show the presence of determinants encoded by the I-A subregion of H-2 but fail to detect I-J subregion determinants, or immunoglobulin constant region determinants. Suppression is restricted to primary in vitro antibody responses of spleen cells syngeneic at the I-A subregion of H-2 with the cell producing the factor. Suppressive activity may be absorbed by MLR blasts of the appropriate specificity. For example, TsF-H from B10 mice is absorbed by B10 anti-B10.D2, but not by B10 anti-B10.A MLR blasts. B10.A(4R) anti-B10.D2 MLR blasts also fail to absorb TsF-H demonstrating a requirement for I-A syngenicity in the target cell population and suggesting an anti-idiotypic component to TsF-H. The TsF-H producing cell has been cloned and these same results have been obtained using the monoclonal product.

118 PURIFICATION AND PARTIAL CHARACTERIZATION OF A MONOCLONAL SUPPRESSOR T CELL FACTOR, Karen Krupen, Barbara Araneo, Judith Kapp,, Stanley Stein, Kenneth Wieder and David Webb, Roche Inst. of Mol. Biol., Nutley, NJ 07110; Jewish Hosp. of St. Louis, MO 63110

The immune response to the synthetic terpolymer L-glutamic acid^{60}-L-alanine^{30}-L-tyrosine^{10} (GAT) by inbred strains of mice is controlled by an immune response (Ir) gene(s) that map(s) to the I-A subregion of the H-2 gene complex. Immunization with GAT stimulates antibody formation in vivo and in vitro and primes for T cell proliferation to GAT in vitro in mice bearing the responder H-$2^{a,b,d,f,k}$ haplotypes. In non-responder (H-$2^{b,q,s}$) mice immunization with GAT does not stimulate GAT-specific PFC or proliferation responses, unless GAT is complexed with methylated bovine serum albumin (GAT-MBSA). Extracts of GAT-specific suppressor T cells from non-responder mice contain a soluble suppressor factor(s), called GAT-TsF, which mediates the effects obtained with non-responder suppressor T cells. Recently, a hybrid T cell line has been obtained which produces a GAT-specific suppressor factor. The biological activity and serological characteristics of the T cell product(s) from specific clones are the same as GAT-TsF found in tissue extracts. That is, the inhibitor binds to GAT, bears no detectable Ig heavy or light constant region determinants, contains determinants encoded by the I subregion of the H-2 gene complex and inhibits both GAT-specific PFC and T cell proliferative responses. The isolation of the monoclonal suppressor factor specific for GAT has been accomplished by the combination of affinity chromatography and high pressure liquid chromatography. The purified factor has been characterized by polyacrylamide gel electrophoresis and peptide and amino acid analysis. A detailed report of the purification method and physical and biochemical characteristics of the GAT suppressor factor will be presented.

119 ANTIGEN-BINDING T CELLS FROM BURSECTOMIZED CHICKENS, Yong Sung Choi and Gloria C. Higgins, Sloan-Kettering Institute for Cancer Research, Rye, New York 10580

Antigen-binding cells (ABC) from spleens of human gammaglobulin(HGG)-immunized, bursectomized agammaglobulinemic chickens were studied by autoradiography with ^{125}I-HGG. Chicken T cells were capable of binding soluble protein antigen in the absence of macrophages: 1~3 ABC per 10^3 spleen cells. The specificity of antigen binding was demonstrated by competitive inhibition with HGG and a specific increase in ABC after immunization. The temperature-dependence and azide stabilization of the ABC were characteristic of T cells. The number of ABC was increased by neuraminidase treatment. Further, HGG-binding T cells could be enriched by adherence to HGG-gelatin plates and released by temperature shift. Enriched HGG-binding T cells showed at least a 20-fold enrichment of suppressors of the antibody response to TNP-HGG, as measured by adoptive transfer. Specific suppressor T cells appear to be negative for Fc receptors. HGG-binding T cells were biosynthetically labeled with ^{35}S-Methionine and the protein binding antigen, HGG, was isolated by affinity chromatography. The result showed a single peptide with a molecular weight of 67,000 daltons in SDS-PAGE.

120 MOLECULAR TRANSFER OF HUMAN Ia ANTIGENS, Dominique J.Charron and Edgar,G.Engleman, Department of Tumor Immunology INSERM U 152 Hopital Cochin Paris 75674 France and Department of Pathology Stanford Medical School,Stanford;CA 94305.

The Human Ia antigens,primarly expressed on B lymphocytes and monocytes have been recently detected on stimulated T cells.Using a monoclonal anti HLA-DR antibody and a two dimensional (2D) electrophoresis genotyping method(1) we previously demonstrated that alloreactive T cells synthesize Ia antigens of the responder type;Moreover the 2D gel electrophoretic pattern of T cells and B cells Ia antigens are similar(2).Comparaison of Ia fingerprints from ^{125}I lacto peroxydase surface labeled and ^{35}S methionine internally labeled alloreactive T cells shows that both Ia molecules of the responder and of the stimulator type are present on the T cells at day 4OF A one way MLR. No Ia antigens were detected at day 11. Our data present molecular evidence for the transfer of stimulator Ia antigens to the responder T cells and may represent a model to study the T cell receptor for allogeneic and/or syngeneic Ia antigens.

1) D.J. CHARRON and H.O. McDEVITT (1980) J. Exp. Med. 152, 2:18.

2) D.J. CHARRON, E.G. ENGELMAN, C. BENIKE and H.O. McDEVITT (1980) J. Exp. Med. 152, 2:127.

121 A HUMAN SUPPRESSOR MOLECULE SYNTHESIZED BY T LYMPHOCYTES, Philip S. Crosier, E. Jayne Watkins and Brian C. Broom, Department of Medicine, Christchurch Clinical School of Medicine, Christchurch, NEW ZEALAND.

The activation of human cytotoxic lymphocytes (T_c) has been shown in previous studies to be capable of being regulated by alloantigen-activated suppressor T lymphocytes. We have recently described a suppressor molecule (T_sF) elicited from primed human T lymphocytes which is capable of regulating T_c activation. The molecule is only elicited after alloantigen restimulation of primed lymphocytes. By adding the T_sF sequentially after the initiation of a T_c-generating MLC it was shown that the T_sF only acts in the early stages of the T_c differentiation pathway up to 24 hours after initial alloantigen contact. Beyond 24 hrs an MLC is refractory to the effects of the T_sF and maximal T_c responses are generated. The T_sF does not act at the T_c effector cell stage. Using preincubation and mixing experiments it was shown that the T_sF acts upon an adherent cell population. Absorption experiments show that purified T cells and mononuclear adherent cells express receptors for the T_sF. There is some indication that the molecule functions in initiating a 2-step regulatory pathway operating through an adherent cell and an activated suppressor effector cell. Biochemical evidence using reverse affinity chromatography has excluded the possibility that the molecule is IgG, IgM or albumin. Some purification is achieved using an anti-human β_2-Microglobulin Sepharose-4B affinity column. Utilising metabolic blocking experiments the T_sF is only synthesized by actively metabolising and dividing cells. Assaying each fraction of the T_sF is facilitated using 2 continuously growing TCGF-dependent human T_c cell lines. The above findings illustrate a mechanism whereby human T_c activity can be regulated.

122 PRODUCTION OF ANTIGEN-SPECIFIC SUPPRESSIVE T CELL FACTOR BY RADIATION LEUKEMIA VIRUS-TRANSFORMED SUPPRESSOR T CELLS, Luciano Adorini, Paola Ricciardi-Castagnoli° and Gino Doria, CNEN-Euratom Immunogenetics Group, Laboratory of Radiopathology, CSN Casaccia, 00100 Roma, °CNR Center of Cytopharmacology, Dept. of Pharmacology, University of Milano, 20129 Milano, Italy

Hen egg-white lysozyme (HEL) specific suppressor T cells induced in C57BL/6 mice have been selected by sequential passage over anti-Ig and HEL-coated plates. These suppressor T cells, 80% $I\text{-}J^+$, were infected in vitro with Radiation Leukemia Virus and injected i.v. into sublethally irradiated syngeneic recipients. After 4-6 months, 6 out of 20 injected mice developed thymic lymphomas which were mantained by transplantation into histocompatible hosts and subsequently estabilished as permanent cell lines. Cells of these 6 thymomas were screened for the presence of Thy 1.2, Lyt 1, Lyt 2, $I\text{-}J^b$ and sIg by direct or indirect immunofluorescence. One tumor (thymoma L4) was found to express the expected phenotype of suppressor T cells (Thy 1.2^+, Lyt 2^+, $I\text{-}J^+$). Extracts obtained from L4 cells were able to induce HEL-specific suppression in a T cell dependent proliferative assay, demonstrating the presence of antigen-specific suppressive T cell factor(s). This procedure should represent a powerful approach to the production of stable antigen-specific T cell lines and permit biochemical analysis of antigen-specific T cell products.

Antibodies to T Cell Receptors and Factors

123 Serological and Biochemical Studies of a T-cell Protein Related to Defined Human Serum Immunoglobulins and Fragments. John J. Marchalonis, Gregory W. Warr, Jeffrey C. Hunt and An-Chuan Wang, Department of Biochemistry and Department of Basic and Clinical Immunology and Microbiology, Medical University of South Carolina.

Current concepts of the antigen-specific receptor of thymus-derived (T) lymphocytes suggest that this molecule bears immunoglobulin (Ig) variable (V) regions, but does not represent a classical serum antibody. We immunized rabbits with a globulin fraction obtained from an in vitro cultured marmoset T cell lymphoma line which expressed surface components related to immunoglobulins. Quantitative immunoprecipitation and competition radioimmunoassay studies carried out using characterized monoclonal human Igs and their chains and fragments show that this rabbit antiserum, raised against a T cell product, reacts with a particular, conformational V_H-determinant which is formed by interaction with λ light chains and with two distinct μ chain (IgM) related determinants. Neither μ chain determinant is a major isotype specific marker, and each occurs only on certain distinct subsets of serum μ chains. One is located in the Fc fragment; the location of the other μ related determinant is not known. These results show that this rabbit antiserum recognizes both variable and constant region determinants on the T cell receptor heavy chain. Rabbit antibody to the T cell product which cross-reacts with serum μ chain was isolated by immune affinity chromatography on Sepharose-derivatized covalently with one particular reactive IgM protein, the Waldenstrom macroglobulin Can ($\kappa\mu$). This antibody precipitated a biosynthetically labeled component of approximate mass 70,000 as assessed by polyacrylamide gel electrophoresis in Na Dod SO_4-containing buffers. This molecule also reacts with antibodies directed against V_H fragments and monomeric-Fab fragments derived from IgM myeloma proteins. Two low molecular weight peptides, of 10-12 produced by CNBr degradation of the T cell molecule, reacted with anti-$(Fab)_1$. Structural studies of these fragments are in progress. These data support the conclusion that certain T cells express and synthesize a molecule related to Ig V_H-regions which also has a constant region sharing antigenic markers with IgM subpopulations. Functional studies using some of these sera suggest that these molecules are involved in primary recognition of antigen. (Supported in part by American Cancer Society grant RD-101 to John J. Marchalonis and National Science Foundation grant PCM 79-24043 to An-Chuan Wang).

124 SEROLOGICAL ANALYSIS OF T CELL FACTORS/RECEPTORS, Marc Feldmann, Roger James, Ian Todd, Eric Culbert, Reiko Makidono, Michael Cecka, ICRF Tumour Immunology Unit, Department of Zoology, University College London, Gower St., London WC1E 6BT, England and Sirkka Kontiainen, Department of Serology & Bacteriology, University of Helsinki, Helsinki, Finland.

Several types of antisera have been raised against mouse (and human) antigen specific helper or suppressor factors: 1) Heterologous anti constant region, (analogous to anti Ig isotype) in rabbits or rats 2) Syngeneic anti-variable region (analogous to anti idiotype 3) Alloantisera, which may be analogous to antiallotype.

These antisera have been used to characterize factors serologically, define membrane molecules, analyze responses in vivo, and attempts are being made to convert the vascous sera to monoclonals.

We have used antifactor sera, anti V_H and antigen binding to characterize factors from T cell hybrids and normal cells and receptors from hybrids, normal T cells and T cell tumours. The structure of these molecules and the effects of monoclonal antifactor reagents on the immune response in vitro and in vivo will be discussed.

125 SEROLOGICAL EVIDENCE FOR A Ts CELL RECEPTOR CONSTANT REGION DETERMINANT LINKED TO Igh-1, Frances L. Owen, Tufts Medical School, Cancer Research Center, 136 Harrison Ave., Boston, MA 02111.

The molecular nature of the T cell receptor for antigen and its relationship to immunoglobulin is as yet unknown. It is generally accepted that variable regions of some immunoglobulins are expressed on T cells. The existence of a distinct "constant" region for T cells, analogous to immunoglobulin heavy chain class specific markers, is hypothesized in this work. As reported previously, an Igh-1 linked cellular antigen, closely associated with a Ts cell receptor for idiotype, has been characterized with alloantisera. Suggestive evidence for a constant region marker is based upon the activity of the antiserum. This anti-allotypic reagent is T cell specific and reacts preferentially with an Lyt $1^{-}2^{+}$ T cell in the spleen, mature thymocytes, and Con A induced lymphoblasts. The antiserum acts as an in vivo polyclonal inducer for T suppressor cells for SRBC's in an Igh-1 restricted system. Suppression of an anti-hapten response (on T dependent carriers) is achieved under similar conditions and is not restricted to any isotype of the immune response, nor is there a restriction in the affinity of antibody nor the clonotype suppressed. Recent studies have shown the gene(s) which codes for this determinant(s) is on chromosome 12 adjacent to the immunoglobulin constant region complex. Recombination events between the Ts marker and Igh-1, assayed in several panels of recombinant inbred strains and recombinant inbred lines, have shown Tsu^{d} to clearly be outside the Igh-1 complex, and map it between Ig-1 and prealbumin. Blocking of binding of Ts cells in A/J hyperimmune suppressed mice with this antibody suggests that the antigen has at least a stearic relationship at the cell surface with a T cell receptor. New data to be presented here show that the antisera can precipitate a radiolabeled (^{35}-S-methionine) antigen from cells activated in vitro with concanavalin A. The molecular weight of the precipitated antigen is 68,000 D with no apparent association with a second chain. A serological relationship to the immunoglobulin isotype markers has been explored by immunoprecipitation. The antigen is allotype restricted, as evaluated in Igh-1 congenic mice. C.AL-20 ($H\text{-}2^{d}$, $Igh\text{-}1^{d}$) mice express this antigen while BALB/c ($H\text{-}2^{d}$, $Igh\text{-}1^{d}$) and C.B-20 ($H\text{-}2^{d}$, $Igh\text{-}1^{b}$) mice do not. The contribution of genes encoded by the H-2 locus to antigen expression was examined in CAL.B mice, and through immunoprecipitation using antisera directed against molecules coded for by H-2 linked genes.

126 ALLOANTISERA WHICH BLOCK ANTIGEN RECOGNITION BY T CELLS. Scott K. Durum, Douglas R. Green, Richard K. Gershon, and Ralph T. Kubo. Yale University Department of Pathology New Haven, CT 06510, and The Howard Hughes Medical Institute at Yale University.

Mouse alloantisera were examine for the ability to inhibit interactions of T cells with antigen. Antisera were raised against several different preparations of T cells or their products. Two types of T cells were studied, cytotoxic T cells specific for histocompatibility antigens (assayed by chromium release), and "educated" T cells specific for sheep erythrocytes (assayed by rosette-formation). Preincubation of either type of T cell with appropriate alloantisera, in the absence of complement, profoundly inhibited their ability to interact with antigen. Strain distribution of the relevant determinants recognized by these sera indicate that the genes controlling them are not closely linked to the H-2 locus, the Ig-1 locus, nor to the genes encoding any previously described alloantigens. Inbred strains with the C57BL/6 or C57BL/10 backgrounds have one allele, whereas all other strains examined thus far have the other.

127 SEROLOGICAL AND BIOCHEMICAL CHARACTERISATION OF A T-T HYBRIDOMA-DERIVED HELPER FACTOR Eric J. Culbert, J. Michael Cecka, Sirkka Kontiaienen* and Marc Feldmann, University College London, London WC1E 6BT, England and *University of Helsinki, Finland.

Fusion of KLH-activated CBA T-cells with an AKR thymoma (BW5147) produced a number of clones which secreted a factor capable of replacing helper T cells in an in vitro response to KLH. These clones expressed both Thy1.1 and Thy1.2 by immunofluorescence and so were regarded as true T-T hybrids.

Functional clones were tested in parallel by rosetting and immunofluorescence with various antisera previously shown to react with antigen-specific helper factors. All expressed V_H, but not V_L, determinants, and bound antisera, raised in rabbits, shown to recognise constant region determinants of secreted helper factors. Rabbit antisera similarly reactive to suppressor factors failed to bind to any of the clones. Additionally, an anti-idiotypic serum to helper factor (CBA anti-CBA HF_{KLH}) bound to a number of the clones. Supernatants from hybrids separated by rosetting with rabbit and mouse anti-factor sera were shown to help an in vitro anti-KLH response, and thus rosetting is a useful alternative to continual re-cloning in maintaining lines with stable helper function. SDS-PAGE analysis of the products precipitated by anti-V_H and CBA anti-CBA HF_{KLH} from clone E1.6c12 showed 2 chains of M.W. 90,000 and 70,000. In contrast to many previous reports of T-cell derived factors, no "light chain" was precipitated in this system.

128 Ly-19, A NEW Igh-1 LINKED DIFFERENTIATION ALLOANTIGEN, Alison Finnegan and Frances L. Owen, Tufts University Medical School, Department of Pathology and Cancer Research Center, Boston, MA 02111.

Ly-19, a new Igh-1 linked differentiation alloantigen has been identified serologically. An antiserum was produced in (CB.20 x CBA/Tu Igh-1^b)F_1 mice against BSA density gradient selected lymphoblasts from BALB/c spleen cells primed in vivo and restimulated in vitro against CBA/J spleen cells. This same antiserum has been used to define a Igh-1 linked marker present on cytotoxic T cells, Ly-18. Visual complement dependent lysis of activated cells with the antiserum revealed a second antigenic specificity with a different strain distribution, Ly-19. C.AL-20, CBA/Tu, and CBA/Tu Igh-1^b are resistant to elimination of cytotoxic effectors but sensitive to antibody and complement when enumerated by visual dye exclusion. Therefore the antiserum recognizes at least two unique specificities. Ly-19 is detectable on Con A and LPS induced splenic blasts but not on resting cells of the thymus, spleen, lymph node, or bone marrow. T cells are not required for expression of Ly-19 since BALB/c nude spleen cells stimulated with LPS express the marker. CL18 (H-2^k) and P.3ul (H-2^d), both plasmocytomas of late differentiated B cells, are negative for the marker, suggesting the marker may have some biological role other than a cell cycle antigen. Expression of Ly-19 is cell cycle dependent, being present at 24 hrs after Con A stimulation but absent by 96 hrs. In a limited strain distribution BALB/c, C.AL-20, BAB.14, CBA/Tu, CBA/Tu Igh-1^b and C57L/J are positive while CB.20, C57BL/6, and C57BL/Ka are negative. This strain distribution is unique for an allotype-linked antigen.

129 ANTISERA TO TWO SEPARATE ANTIGEN SPECIFIC SUPPRESSOR FACTORS WHICH DEFINE DISTINCT T CELL SUBPOPULATIONS. Jerome A. Mattingly and Charles A. Janeway Jr., The Ohio State University, Columbus, Ohio 43210 and Yale University, New Haven, Connecticut 06510.

Sheep erythrocytes (SRBC) were fed daily to C57Bl/6 mice for 1 week. Spleen cell cultures from these mice produced two distinct antigen specific SRBC-binding suppressor factors (FrI and FrII) which were readily separated by passage through Sephadex G-100. The kinetics and activity of these factors have been described elsewhere (Mattingly, et al., J. Exp. Med. 152: 545, 1980). Antisera were made to each of these factors and were found to inactivate the suppressive activity of their corresponding factor with no detectible cross-reactivity. Antiserum against FrI, referred to as 2318, when preincubated with C57Bl/6 spleen cells, totally abolished the ability of these cells to respond to PHA and diminished their ability to act as suppressor cells in an in vitro plaque-forming cell (PFC) response, while having no effect on their ability to respond to Con A or to induce a graft versus host reaction (GVHR). Conversely, spleen cells after preincubation with 2537 (antibody to FrII) were significantly depressed in their ability to respond to Con A and to induce a GVHR, but their PHA response and their suppressor cell activity was not affected. Both antisera diminished a one-way mixed lymphocyte response (MLR) reaction equally, which was approximately a 60% reduction of controls. These results indicate that these antisera, 2318 and 2537, which were produced against antigen specific suppressor factors which are produced by different T-cell types, may detect cell surface markers on different T-cell populations which can be used to define functional differences.

130 ISOLATION OF ANTIGEN SPECIFIC T CELL RECEPTORS, Michael Cecka, Sirkka Kontiainen, Luciano Adorini, Paula Ricciardi, David Givol and Marc Feldmann, ICRF Tumour Immunology Unit, Department of Zoology, University College London, London WC1E 6BT, England

We have isolated byosynthetically radiolabelled antigens from a variety of T cell sources which serologically resemble T-cell receptors. Rabbit antisera produced against affinity purified antigen specific helper and suppressor factors precipitate four distinct polypeptide chains from murine splenic T-cells. By SDS-page, these have apparent molecular weights of 105K, 85K, 60K, and 30K daltons. These same polypeptides are precipitated by a rabbit αV_H antibody produced against the heavy chain variable region of MOPC 315. The largest and smallest of these polypeptides may be all or in part attributed to contaminating B-cells as shown by mixing experiments. The 85K and 60K polypeptides appear not to be of B-cell origin. We have also isolated an 85K dalton polypeptide chain from two T-cell tumors using the rabbit anti-V_H reagent. One of these tumors produces a factor which specifically inhibits the proliferative response to lysozyme. Lysozyme-immunadsorbents also bind this 85K dalton polypeptide. A T-cell line which has been grown in the presence of KLH and TCGF produces a polypeptide of 105K which is precipitated by RαV_H and binds to KLH immunoadsorbents. Further chemical characterization of these polypeptides, including CNBr peptide mapping and microsequence analysis is currently in progress to compare them with immunoglobulin heavy chains.

131 MONOCLONAL ANTIBODIES AGAINST ANTIGEN SPECIFIC FACTORS, Roger James, Sirkka Kontiainen, Ian Todd, John Maudsley and Marc Feldmann, ICRF Tumour Immunology Unit, Department of Zoology, University College London, London WC1E 6BT, England.

Antigen specific helper factors (HF) and suppressor factors (SF), which replace T cells in humoral responses in vitro, have been eluted from antigen colums and used to immunize Wistar rats.

Spleen cells from such immunized rats have been fused with NS-1 myeloma cells by the Kohler/Milstein hybridization technique to produce clones of antibody producing cells.

A technique has been devised to screen these clones for activity against T cell products - 'T cell plaque assay' - and products of these clones selected by this method have been shown to influence the induction of antibody producing cells in vitro.

For instance, supernatants derived from anti-HF clones will suppress both primary and secondary responses to TNP-KLH, while supernatants from anti-SF clones will enhance these responses.

These monoclonal antibodies have also been shown to affect the induction of anti-sheep red cell responses in vivo.

132 ALLOHELP TO K AND D IS NOT RESTRICTED TO I BUT IS BLOCKED BY AN ANTI Lyt2 REAGENT. Susan L. Swain, University of California, San Diego, California 92093

The requirements for the delivery of allogeneic help directed against Class 1 (K and D) mouse MHC antigens were studied. In spite of the fact that much allogeneic and all syngeneic help appears to involve Class 2 (I) region recognition two types of experiments indicated that this was not true for K and D stimulated help. First when cells were stimulated in primary MLC with either a K or a D difference, their ability to give allohelp in a secondary culture was dependent only upon the presence of the same allogeneic K or D region present during priming regardless of the I region present. Second, while a monoclonal anti Ia reagent blocked the production of T helper cell replacing factors (helper TRF) when the helper cells were primed and stimulated with allogeneic I region, no blocking was seen of factors produced in response to K or D. Additional experiments indicated that a monoclonal anti Lyt2 reagent which blocked cytotoxicity to K and D also blocked production of helper TRFs stimulated by K and D. The same reagent failed to block the killing of a cell line directed against I and the production of helper TRF stimulated by I. Thus it appears that Class 1; K and D antigens can perform all the functions of Class 2; I antigens, e.g., direct (not I-restricted) stimulation of helper function and production of helper TRFs, but that T cells responding to K and D are unique from those that respond to I in that they bear Lyt2 antigens and a reagent against that Lyt2 molecule blocks their activities. This may suggest that Lyt2 molecules play some role in recognition of Class 1 MHC antigens or in the subsequent events that follow that recognition.

133 PROPERTIES OF SYNGENEIC AND ALLOGENEIC ANTISERA RAISED TO TUMOR-SPECIFC SUPPRESSOR FACTOR FROM DBA/2J MICE. Julia G. Levy, Thomas Maier and Douglas G. Kilburn, University of British Columbia, Vancouver, B.C., Canada.

Tumor specific suppressor factor was prepared by injecting DBA/2J mice four days prior to sacrifice with soluble membrane extracts of the syngeneic mastocytoma, P815. The suppressor factor was partially purified by passage of spleen extracts over an immunoadsorbent containing P815 membrane components. Antisera raised in syngeneic and allogeneic (C57Bl/6) mice by repeated injections of suppressor factor were tested. It was found that these antisera, but not their controls, were capable of absorbing out the suppressor factor. The antisera were also capable, in the presence of complement, of eliminating suppressor cells from suppressive spleen cell populations. However, the antisera were not capable of eliminating syngeneic tumor specific in vitro generated killer cells, indicating that the receptor molecules on suppressor and effector cells in this system are distinct from each other.

Raising Functional T Cell Lines

134 Ir GENES, Ia ANTIGENS AND MLR DETERMINANTS ANALYZED USING MURINE T CELL CLONES, C.G. Fathman and Masao Kimoto, Department of Immunology, Mayo Clinic, Rochester, MN 55901

Studies in our laboratory utilizing clones of murine T cells have allowed us to examine the possible interrelationship among Ia antigens, Ir genes and MLR stimulating determinants. These studies made use of (1) T cells clones reactive either with GAT or (T,G)-A--L whose recognition of antigen was restricted by products of the I-A subregion and (2) certain A anti B6A alloreactive T cell clones which recognized unique I-A hybrid MLR stimulating determinants present on (B6A)F_1 which were absent on parental A and B6 cells. By deriving antigen [GAT or (T,G)-A--L] reactive cloned T cells from (B6A)F_1 immune mice, it was possible to demonstrate that certain clones recognized antigens presented uniquely by F_1 antigen presenting cells (1,2,). Genetic mapping studies suggested that the hybrid determinants recognized by alloreactive or soluble antigen reactive T cell clones might be identical. Studies using the I-A^b mutant B6.C-H-2^{bm12} (bm12) mouse whose defect in the normal expression of cell surface products of the I-A^b subregion can be partially circumvented through transcomplementation by deriving hybrids between bm12 and mice expressing normal I-A subregion products allowed us to further define such hybrid I-A subregion determinants. The defect in cell surface expression of the normal I-A^b products on cells of the mutant F_1 has allowed us to identify transcomplementing I-A products on H-2^a x H-2^b heterozygote mice consisting of $A^b_\alpha A^k_\beta$ and $A^k_\alpha A^b_\beta$. Additional studies allowed us to determine that such hybrid determinants which restrict antigen recognition by clones of T cells stimulate allo- reactive murine T cell clones and can react with monoclonal anti-I-A subregion antisera (3). Thus, these data support the concept that Ia antigens, Ir gene phenomena (defined as the ability of immune T cells to recognize soluble antigen) and I-A encoded MLR stimulating determinants are different manifestations associated with the same cell product. Preliminary data obtained in our laboratory have suggested that such antigen reactive T cell clones provide antigen-specific and MHC restricted B cell help. Studies currently in progress using T cell clones reactive with sperm whale myoglobin will allow us to determine whether cloned T helper cells recognize the same antigen epitopes as do immunoglobulin molecules. Thus, studies utilizing T cell clones have allowed us to dissect the interrelationship among several diverse phenomena controlled by genes within the I-A subregion.

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135 INTERACTING MOUSE T CELL CLONES. F.W. Fitch, A.L. Glasebrook, M. Sarmiento, C.T. Lutz, D.P. Dialynas, M.R. Loken, and D. Wilde. University of Chicago, Chicago, IL 60637

Cloned cytolytic T cell (CTL) and helper T cell (HTL) lines have been derived from murine MLC by limiting dilution (1,2). These clones, some of which have been carried more that 2 years in culture, have retained stable patterns of cytolytic activity, cell surface antigens, and normal chromosome number. Both CTL and HTL clones have been maintained by weekly passage with stimulating alloantigen and IL-2. HTL clones stimulated by alloantigen (Mls or MHC) secrete soluble factors (TCGF or IL 2) which in turn induce the proliferation of CTL. The IL-2 produced by HTL clones is neither antigen specific nor H-2 restricted. Although exposure to IL-2 induced significant thymidine incorporation by these cloned cells within 24 hours, proliferation of these cells over several days required the presence of splenic adherent, non-T "filler" cells. Although cloned CTL are not stimulated to proliferate by alloantigen alone, greater proliferation is observed if alloantigen is present in addition to suboptimal amounts of IL-2. Some cloned CTL react with H-2K or H-2D alloantigens; others have more complicated patterns of reactivity. These cloned CTL and HTL have proved to be useful for determining the biochemical and antigenic characteristics associated with particular cell functions. For example, cytolytic clones have cell surface polypeptide profiles which are very similar within a mouse strain but differ from those from another strain. Cell surface polypeptide profiles of HTL are different from each other (3). Some but not all functional HTL bear Lyt-1 surface antigen, and at least one HTL clone expresses Lyt-2 but not Lyt-3. Variants of one cloned CTL line which have been derived by mutagenesis with ethyl methanesulfonate specifically lack either Thy-1 or Lyt-2 and Lyt-3 (4). Analyses of these variants indicate that neither Lyt-2 nor Lyt-3 is responsible for the lethal hit and suggest that Lyt-2 and/or Lyt-3 are required for an antigen receptor functional in cytolysis. Thy-1 plays no role in cytolysis.

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136 T-LYMPHOCYTE CLONES SPECIFIC FOR SOLUBLE PROTEIN AND SYNTHETIC ANTIGENS, Ronald H. Schwartz, Chuan Chen, Louis A. Matis, Ethan A. Lerner, and Benjamin Sredni, Laboratory of Immunology, NIAID, NIH, Bethesda, MD 20205; Departments of Pathology and Cell Biology, Yale University School of Medicine, New Haven, CT 06510

A major goal of cellular immunologists has been to obtain cloned populations of antigen-specific T lymphocytes. During the past two years, this goal has been realized by several laboratories including our own. Using this new research tool, we have been able to demonstrate the following points: 1) For hapten-carrier conjugates, the phenomenon of carrier specificity results from the recognition by individual T cells of predominantly carrier determinants. Although the affinity of the receptor is greater for the antigen if the hapten is also attached, the presence of the hapten alone is not sufficient to stimulate the T cell. 2) The phenomenon of major histocompatibility complex (MHC)-restriction is the consequence of individual T-cell clones having specificity for both MHC-gene products and the antigen, rather than a secondary consequence of T-cell suppression. For clones specific for GAT and DNP-OVA, the restriction element maps solely in I-A. 3) The involvement of two Ir genes in the immune response to GLϕ results from the requirement of T-cell clones to recognize GLϕ in association with the β_{AE}-α_E-restriction element. In order to assemble this Ia-molecule, two separate gene products, one coded for in I-A and the other in I-E, must be expressed in the same antigen-presenting cell. 4) The concept of T cells specific for antigen in association with unique F_1-restriction elements has been generalized to other antigens by isolating such clones from (B10.A x B10)F_1 mice immunized with GAT. In this population of T cells, the F_1-specific clones represent only a minor subpopulation (20%). 5) The monoclonal antibody, Y-17, specific for the β_{AE}-α_E Ia-molecule, inhibited the proliferative response of the GLϕ-specific clone, which utilizes this Ia-molecule as its restriction element, but not the response of the GAT- or PPD-specific clones, both of which utilize β_A-α_A Ia-molecules as their restriction element. This result demonstrated that anti-Ia antibodies inhibit by masking selective Ia-molecules on the surface of the antigen-presenting cell rather than by inducing suppressor T cells. 6) T-cell clones selected originally on the basis of their specificity for a soluble protein antigen have been shown to also respond to specific alloantigens. In one case, the cells were rigorously recloned at high plating efficiency to establish that a single cell expressed both specificities. Genetic mapping showed that this B10.A clone recognized either $\underline{I\text{-}A}^5$ or DNP-OVA in association with $\underline{I\text{-}A}^k$. This overlap in allo- and nominal antigen T-cell repertoires has important implications for theories of T-cell recognition.

137 THE SELECTION AND GROWTH OF PHOSPHORYLCHOLINE-SPECIFIC T CELL LINES, James Watson, Barbara Fagg, and Stephen Hedrick, Department of Microbiology, University of California, Irvine, CA 92717

A procedure for the selection and growth of murine T cell lines specific for phosphorylcholine (PC) has been developed. PC-specific T cells are maintained by growth in the presence of PC-conjugated to irradiated filler cells, or stimulation by purified Interleukin 2 (IL-2). All PC-specific T cell lines are H-2 restricted in the expression of antigen-responsiveness. The cell surface and functional phenotype of these cells will be described as well as the screening for the presence of the TEPC-15 idiotype on the cell surface.

138 TRANSCOMPLEMENTING HYBRID ANTIGEN PRESENTING DETERMINANTS DETECTED BY ANTIGEN REACTIVE T CELL CLONES. Masao Kimoto and C.Garrison Fathman, Mayo Clinic, Rochester, MN 55901.

It has been possible to isolate antigen-specific T cell clones from lymph node cells of mice immunized with specific antigens. Such antigen-reactive T cell clones could be restimulated by antigen in the presence of antigen-presenting cells sharing certain portions of the I region of the major histocompatibility complex with the antigen-reactive T cell clone. By studying the reactivity of T cell clones derived from [(C57BL/6 x A/J)F_1 (B6A)F_1] mice immunized with GAT, we could identify clones of T cells which recognized antigen in association only with the F_1 antigen-presenting cells and not with antigen-presenting cells from either parental A/J or C57BL/6 mice. Genetic mapping studies using congenic recombinant mice and F_1 mice derived from them showed that the antigen-presenting determinants expressed on (B6A)F_1 cells which are not expressed on cells of either of the parents are trans-complementing hybrid molecules derived from genes residing within the I-A subregion. By studying T cell clones from (B6A)F_1 mice immunized with (T,G)-A--L we were able to isolate T cell clones which could be presented antigen by hybrid antigen-presenting determinants composed of products of both low responder and high responder parental genomes. These data suggest that neither the A_α nor the A_β chain of the low responder haplotype confer phenotypic low responsiveness to (T,G)-A--L. Furthermore by using cells from (B6.C-H-2^{bm12} x B10A)F_1 mice as antigen presenting cells it was possible to show that both trans-complementing hybrid determinants (i.e., $A^b_\alpha A^k_\beta$ and $A^k_\alpha A^b_\beta$) could be used as antigen-presenting determinants to such (T,G)-A--L reactive clone.

139 SPECIFICITY AND HELPER FUNCTION OF CLONED T-CELL LINES. Barry Jones and Charles A. Janeway, Jr., Department of Pathology, Yale University School of Medicine, New Haven, Connecticut 06510.

T-cell lines have been isolated from a population of BALB/c T-cells primed against ovalbumin (OVA), and propagated continuously in tissue culture. The cloned T-cell lines exhibited the Lyt-1$^+$, Lyt-2$^-$ phenotype, and were antigen specific and H-2 restricted in their proliferative interaction with antigen presenting cells (APCs). In addition, the cloned T-cell lines have been used to address the question of whether T_H cells are genetically restricted by the H-2 complex in cooperative interactions with B-cells. After Mitomycin C treatment the T-cell clones induced proliferation in non immune B-cells in a 48h assay. The T-B-interaction was OVA specific and H-2 restricted. The genetic restriction was not due to an inhibitory effect of mis-matching at the H-2 locus since collaboration could be induced between the same cells across the H-2 barrier using rabbit anti-mouse brain antiserum as a stimulus. The addition of BALB/c APCs to the cultures induced very little trans-stimulation of H-2 mis-matched B-cells by the BALB/c T-cell clones. The role of B-cell priming, and B-cell sub-class in determining the susceptibility of B-cells to trans-stimulation will be discussed.

140 CLONAL ANALYSIS OF A MURINE T-CELL RESPONSE TO BEEF APO-CYTOCHROME c, Giampietro Corradin, Rudolf Zubler and Howard Engers, Institute of Biochemistry, University of Lausanne and Department of Immunology, Swiss Institute for Experimental Cancer Research, Chemin des Boveresses, CH-1066 Epalinges.

The T-cell response to beef apo-cytochrome c (APO-B) in Balb/c mice was analysed. Lymph node cells from primed mice were restimulated in vitro and 5 days later the resulting T-cell blasts were cloned by limiting dilution in the presence of irradiated syngeneic spleen cells, APO-B and MLC supernatant as a source of TCGF. After 10-20 days, growth in individual wells was assessed either visually or by ^{3}H-thymidine incorporation. Positive cultures were then expanded and tested for APO-B specific T cell proliferation in the absence of added TCGF. Two independent experiments gave a similar frequency ($\sim$1/100) for APO-B specific T cells present after 5 days of in vitro culture of primed lymph node cells. Two of these clones were further expanded in vitro and tested for the capacity to respond to different peptides prepared from cytochrome c. While both clones responded to APO-B and apo-peptides 1-38 and 1-65, only one clone responded to the heme-containing peptide 1-65. In addition, both clones failed to respond to the native intact cytochrome c. The same two clones exhibited helper activity using a protein-A reversed plaque assay system. Taken together, these data show that the two clones are independent with respect to antigen recognition and suggest that macrophages may process cytochrome c and peptides thereof in a different manner.

141 EVIDENCE FOR TWO MAJOR ALLOREACTIVE T KILLER CELL SUBPOUPULATIONS WITH DIFFERENT PRECURSOR FREQUENCIES AND DISTINCT RECEPTOR REPERTOIRE, G.J. Hämmerling and C. Weyand, Institut für Immunologie und Genetik, Deutsches Krebsforschungszentrum, 69 Heidelberg

The specificity repertoire of cloned alloreactive T killer cells was investigated by target inhibition studies using six different monoclonal BALB/c anti-H-2K^k antibodies. Clonal analysis by limiting dilution techniques of BALB/c derived cytotoxic T cells (CTL) with specificity for the H-2K^k molecule revealed the existence of two major CTL populations with different precursor frequencies. At low responder cell numbers a frequent CTL population with a precursor frequency of about 1/400 is found. At increasing responder cell doses this frequent population will be suppressed while simultaneously a second, rare, CTL population becomes apparent (precursor frequency about 1/2000).

Target inhibition of limiting dilution clones with monoclonal anti-H-2K^k antibodies revealed the surprising finding that the anti-H-2K^k antibodies can be divided into two groups. Group I blocks the targets for both the frequent as well as the rare CTL population while group II blocks only targets for the rare but not the frequent population. It is of interest to note that in previous studies the antibodies were divided into the same two groups on the basis of their selective reactivity with two spatially separated major antigenic clusters on the H-2K^k molecule. We conclude from all these data that the repertoire of the frequent CTL is limited and different in comparison to the rare population: The frequent CTL recognize only one antigenic cluster while the rare population recognizes both clusters on the H-2K^k molecule.

142 CONSTRUCTION OF T-CELL HYBRIDOMAS WHICH COMPLEMENT AND REGULATE THE IMMUNE SYSTEM OF NUDE MICE. Michael L. Misfeldt and Edgar E. Hanna, National Institutes of Health, Bethesda, Maryland 20205

Fusion of nylon wool fractionated spleen T-cells from NFR/N mice with cells of the thymic lymphoma BW5147 has provided us with a library of perpetual T-cell phenotypes. Nylon wool passed spleen cells eluting in a sharp peak (Pool 1) were highly enriched for T-cells with complementing (helper) activity for NFR/N nude mouse anti-TNP PFC responses in vitro. Spleen cell fractions eluting later in a more complex profile (Pool 2) consist of cells with diverse capacities to influence the anti-TNP PFC responses of nudes. Hybridoma lines were constructed within Pool 1 and Pool 2. Cloned Pool 1 hybrid lines are hyperploid, Thy 1.2^+, Lyt 1^+, and they express V_H and Fc markers. Pool 1 hybrid lines show different magnitudes in their capacity to complement the anti-TNP PFC responses of nudes. Cloned Pool 2 hybrid lines have similar characteristics in that they are also hyperploid, Thy 1.2^+, but are Lyt 1^-. Moreover, selected Pool 2 hybrids also display diverse capacities to suppress the PFC responses of nude splenocytes that have been precomplemented with Pool 1 cells or Pool 1 hybrid cells. Other lines express additional phenotypes. Thus, our library of T-cell lines should facilitate delineation of the cellular interactions involved in regulation of the immune system.

143 FUNCTIONAL HUMAN T CELL CLONES, James T. Kurnick, Anthony R. Hayward and Peter Altevogt, Massachusetts General Hospital, Boston, MA 02114

Clones of phenotypically distinct functional human T cell clones were isolated from mitogen-activated and soluble-antigen specific T cell blasts. The clones were evaluated for surface antigens by fluorescent staining with T cell subtype-specific monoclonal antibodies. Clones isolated from PPD or tetanus toxoid-stimulated T cells all have T4 antigen associated with helper-inducer T cells, and lack T5 and T8 antigens characteristic of suppressor/cytotoxic cells. Clones from PHA-stimulated T cells have alternatively T4 and lack T5 and T8, or lack T4 and have T5 and T8. Additional T cell markers seen on all of the clones isolated thus far include T3 and T11, (both pan T cell antigens), and T9 and T10 seen on some T cells and a few non-T cells. The clones also have Ia-like antigens. The PPD-specific clones were evaluated for functional activity for induction of B cell maturation. Purified B cell fractions could be helped to plasma cell differentiation by the cloned T4 cells with pokeweed mitogen. The same cloned T cells induced suppression in unseparated blood lymphocytes. This suppression depended on the presence of additional T cells, and could be reversed if the PBL T cells were irradiated. Functional activity of the T5/T8 positive clones is currently under investigation.

144 IMMUNOGLOBULIN ALLOTYPE SPECIFIC T CELLS, H. Ralph Snodgrass, Melvin J. Bosma and Darcy B. Wilson, Institute for Cancer Research, Philadelphia, PA 19111, and University of Pennsylvania, Philadelphia, PA 19104

We present two lines of evidence that BALB/c (Ig^a) mice generate Igh-1^b-specific T cells in response to repeated injections of purified Igh-1^b. Firstly, as shown earlier, BALB/c mice immune to Igh-1^b contain T cells which specifically suppress Igh-1^b production upon adoptive transfer into C.B-17 mice; we now demonstrate that such T cells can be isolated by rosetting with Igh-1^b (but not Igh-1^a)-coated sheep erythrocytes. Secondly, BALB/c mice immune to Igh-1^b contain cytotoxic T (T_c) cells that specifically lyse Igh-1^b-producing cells. From such cytotoxic populations long term T_c lines and clones were derived. The conclusion that these T_c clones are specific for Igh-1^b is based upon three observations. 1) The generation of cytotoxic T cells requires <u>in vivo</u> immunization that can be accomplished with purified Igh-1^b. 2) Only Igh-1^b producing targets are lysed. Cell lines producing Igh-4^b or Igh-1^a, as well as Ig non-producing variants, are not lysed. 3) The most interesting observation is that the cytotoxic activity of these cloned T_c cells can be inhibited by preincubation with soluble Igh-1^b but not with Igh-1^a or Igh-4^b. These results comprise the first definitive demonstration of allotype-specific T cells. These T cell clones will be useful for studying mechanisms of T cell functions, as well as being potentially useful material for biochemically characterizing the T cell receptor.

145 THE EFFECT OF NONSPECIFIC T CELL FACTORS ON THE EXPRESSION OF SURFACE ANTIGENS ON BALB/c B LYMPHOID TUMOR CELL LINES. K. Jin Kim, Susan O. Sharrow and Gerard Chaouat, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20205

Nonspecific T cell factors such as allogeneic effect factors (AEF) and T cell replacing factors (TRF) have been shown to be able to substitute for T lymphocytes in the in vitro PFC- response to RBC. Further, these factors have been shown to influence B cells at certain stages of differentiation. However, there are few studies concerning either the biochemical nature of the B-cell receptor(s) or the events occurring at the level of the B cell membrane subsequent to factor binding.

We studied changes in the membrane phenotypes on B lymphoid tumor cell lines, mainly X16C 8.5 cell line ($sIgM^+$, $sIgD^+$, Ia^+ and Fc receptor$^+$), after incubation with nonspecific T cell factors along with LPS. Preliminary studies showed that there was 2-3 fold increase in the Ia expression (detected by ATH anti-ATL antiserum) after treatment with AEF or TRF. Further there was a striking increase in H-2 antigen (detected by hybridoma monoclonal antibodies) but no significant effect on the sIgM (detected by F-Goat anti-mouse μ) nor viral antigen expression (detected by rabbit anti-xenotropic viral antibodies) after TRF treatment. LPS selectively affected on sIgM but not Ia nor H-2 antigens. Using these tumor cells as a model, we are studying the mechanism(s) of the effect of nonspecific T cell factors at the level of B cell membrane. Also, we are examining the effect of these T cell factors on the phenotypes of normal splenic B cells.

146 ANTIGEN RECOGNITION BY CLONED LINES OF VIRUS SPECIFIC CYTOTOXIC T CELLS by T. J. Braciale, Wash. Univ. Sch. Med., St. Louis, Mo. 63110

Recent technological advances in the culturing and long-term maintenance of T. lymphocytes in vitro offer the possibility of examining the process of antigen recognition by continuous lines of T lymphocytes derived from individual T lymphocytes precursors. We have applied this technology to an analysis of Cytotoxic T lymphocyte (CTL) response of mice to type A influenza virus and have produced a series (14 in number) of continuous lines of H-2 restricted influenza virus specific CTL generated under clonal conditions in vitro. Individual CTL precursor were isolated either by colony formation in soft agar or by limiting dilution of CTL precursor in liquid culture. Progeny of individual CTL precursors were expanded and maintained in the presence of Interlukin-2 and virus-infected stimulator spleen cells. Data to be presented will primarily emphasize the patterns of H-2 restricted recognition and the viral antigen specificity exhibited by these cloned CTL lines. Data on the expression of the Thy-1, Lyt-1, and Lyt-2 cell surface markers by these cloned CTL lines as well as available information on the antigenic requirements for proliferation of the lines will also be presented.

147 SEROLOGICAL AND BIOCHEMICAL ANALYSIS OF Ia MOLECULES IN THE I-A MUTANT B6.C-H-2^{bm12} William P. Lafuse, John F. McCormick, Roger W. Melvold, and Chella S. David Department of Immunology, Mayo Clinic and Medical School, Rochester, MN 55901.

Strain B6.C-H-2^{bm12} has a mutation in the I-A subregion of the mouse H-2 gene complex, which causes skin graft rejection, MLR and alterations in the expression of Ia antigens. The mutation affects the expression of Ia.3,8,9,15, and 20 on normal spleen cells. When the spleen cells were stimulated with LPS the expression of all Ia specificites were found except Ia.8. Ia molecules when internally labeled with ^{3}H-leucine can be precipitated with antisera directed against Ia.3,8,15, and 20, but not Ia.8. When F_1's are made between the mutant bm12 and unrelated haplotypes, Ia.3,9,15 and 20 can be detected by microcytotoxic assay on normal spleen cells, but not Ia.8. These studies suggest: 1) The mutation affects either the amount of Ia molecules expressed on normal spleen cell surfaces or the molecule is anchored improperly in the cell surface such that it is not accessible for cytotoxicity and radio-iodination. 2) Specificity Ia.8 which may be a combinatorial determinant, is absent in the mutant due to a structural alteration in one of the chains, probably the alpha chain. 3) The mutation does not involve the Ae chain.

148 DISTINCT PATTERNS OF GENE EXPRESSION IN SMALL CORTICAL THYMOCYTES AND BLAST CELLS. Ellen Rothenberg, The Salk Institute, La Jolla, California 92037.
Among the immunologically incompetent cells in the thymus are blast cells, which include progenitors of various functional lineages of T lymphocytes, and most of their descendants, which are small resting cells. The first is a rapidly dividing population while the second is largely arrested in G_0. However, it is not known when the potential of these cells for growth and development becomes limited. To probe for specific differentiative changes, we have separated these two "immature" populations and compared their respective rates of synthesis of three products specific for PNA$^+$, immature thymocytes as a whole: terminal transferase (TdT), Lyt2, and TL. Using centrifugal elutriation, a blast cell population with over 65% of the cells in S+G_2+M can be isolated in less than 30 min. These cells and the small cells (over 90% in G_0/G_1) are at least 90% viable. However, the rate of protein synthesis per cell is about 20-fold higher in the blasts than in the small cells. Relative to total protein synthesis, the rates of synthesis of TdT, TL, and Lyt2 are strikingly different in the two populations. TdT is synthesized at similar rates in all size fractions, but small and large cells make TL and Lyt2 at reciprocal rates. The largest 10% of cells make TL preferentially, and very low levels of Lyt2. The isolated small cells make Lyt2 at a much higher rate than unfractionated thymocytes, and make TL as a lower fraction of total synthesis, and a much lower absolute rate per cell, than the large cells. This suggests that active synthesis of TL is restricted to the subcapsular phase of thymic development, with selective maintenance of TdT and Lyt2 synthesis as the cells arrest in the inner cortex.

149 LIMITING DILUTION ANALYSIS OF CYTOLYTIC INFLUENZA-SPECIFIC T CELLS. Judith A. Owen and Peter C. Doherty. The Wistar Institute, Philadelphia, Pa. 19104
Virus-immune T cells specific for influenza-infected syngeneic cells were stimulated in limiting dilution culture. The mean frequency of T cells specific for the immunizing virus, A/PR8/34 (HON1), (A/PR8), was calculated from 4 experiments to be 1 in 6600 nucleated spleen cells. A smaller number, 1 in 9500 nucleated spleen cells lysed targets infected with the serologically-unrelated influenza A virus, A/HK/X31/68 (H3N2) (A/HK). Following in vitro stimulation of A/PR8-immune cells with A/HK, the frequency of cells capable of lysing A/PR8-infected targets was 1 in 9600 spleen cells. However, in the same experiments, the frequency of cells which could lyse A/HK-infected targets was lower, only 1 in 11,600 cells. This implies that some T cells specific for the priming virus have been preferentially recalled by in vitro stimulation with a serologically unrelated virus and can be detected by assay on the A/PR8, but on the A/HK infected target. A similar phenomenon has been previously defined for the antibody response to influenza A viruses. However, in the case of cytolytic T lymphocytes it operates across a much wider range of subtypes. Three possible explanations exist for differences in the specificity of influenza-reactive B and T cells. (1) Similar receptor units may be employed in virus recognition by both lymphocyte sets, but the frequency of particular idiotypes in the two cell compartments may differ; (2) differences in the arrangement of the same receptor molecules on B and T cell surfaces may alter the way in which the virus is perceived; (3) completely different recognition molecules may be employed in the two systems.

150 ANALYSIS OF THE ORIGIN OF THE ALLOREACTIVITY DETECTED IN SOME LONG TERM T CELL LINES REPEATEDLY STIMULATED WITH HAPTEN TREATED SYNGENEIC CELLS. A-M. Schmitt-Verhulst, A. Guimezanes and F. Albert. Centre d'Immunologie INSERM-CNRS de Marseille-Luminy, Case 906, 13288 Marseille Cedex 2, France.
When T cells from hapten-sensitized mice are restimulated in vitro with the same hapten on syngeneic spleen cells, T cell lines can be established which are dependent for their propagation on stimulation with that hapten on I region matched cells. Alloreactivity can generally not be detected in such T cell populations from the third restimulation on as measured by incorporation of (^{3}H) thymidine. One line however (C57BL/10 anti-C57BL/10-TNBS) after having shown such a classical pattern of specificity for restimulation up to stimulation 8 subsequently behaved differently : in addition to the previous pattern of stimulation, equal stimulation levels were detected for unmodified allogeneic cells presenting the s, q, f or p alleles in the I region which share the Ia5 specificity. Cells of the k or d haplotypes were not stimulating. Two possibilities could explain such behaviour : A/ alloreactive clones which were initially a minor component of the T cell line, maintained through transstimulation, could start to out grow the line, B/ among different T cell clones reactive to TNP + I-A^b, a selection could have occurred for clones reactive to both TNP + I-A^b and public allogeneic I-A specificities such that a given T cell can bear the two types of receptor, or that the same receptor specific for self I-A + TNP would also have affinity for foreign I-A antigens. To distinguish these possibilities, reactivities are being analysed under limiting dilution conditions. Direct antigen binding assays are being developed to study receptor specificity.

151 INHIBITION OF IMMUNOGLOBULIN PRODUCTION IN MAN BY A SOLUBLE SUPPRESSOR LECTIN SECRETED BY A CONTINUOUS HUMAN T CELL LINE, Warner C. Greene, Thomas A. Fleisher, Takashi Uchiyama and Thomas A. Waldmann, NCI, NIH, Bethesda, MD 20205

We have previously defined the production of a soluble immune suppressor of immunoglobulin (Ig) biosynthesis (SISS-B) by Con A-activated human peripheral blood lymphocytes (J. Immunol., in press). SISS-B is of molecular weight 60-80,000, produced by T cells, and possesses binding specificity for the sugar L-rhamnose. We now report the identification of a continuous T cell line established from the peripheral blood of a patient with mycosis fungoides which produces large quantities of SISS-B. This cell line requires interluekin-2 for growth and in in vitro assays of Ig biosynthesis functions as a potent source of suppressor cells. By FACS analyses, this cell line displays antigens recognized by OKT 3, 4, 9 and 10 and anti-Ia monoclonal antibodies but fails to express determinants recognized by OKT 5 or 8 monoclonal reagents. Properties of the secreted soluble suppressor (CTC-SISS-B) include: 1) marked inhibition (>90%) of Ig production by either pokeweed mitogen stimulated human lymphocytes or highly purified B cells stimulated with EBV, 2) no inhibition of T cell proliferation or T cell mediated lympholysis, 3) a molecular weight of 60-80,000 and 4) binding specificity for the monosaccharide L-rhamnose and related simple sugars. These data indicated that the humoral immune response in man is modulated at least in part through the elaboration of an endogenous "lectin like" soluble factor which exerts suppressive activity through interactions with surface glycoproteins and/or glycolipids present on the membrane of human B cells. The CTC line which we have characterized appears to represent expansion of a peripheral blood lymphocyte(s) producing this lectin-like soluble suppressor.

152 ALLOREACTIVE T CELL CLONES SPECIFIC FOR GUINEA PIG Ia ANTIGENS, Thomas R. Malek, Robert B. Clark and Ethan M. Shevach, Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20205

Alloreactive T cell clones reactive with Ia antigens from strain 2 and 13 guinea pigs were established by plating short-term in vitro primed alloreactive T cells in soft agar and expanding each of the resulting colonies in liquid culture by repeated stimulation with irradiated allogeneic cells in conditioned medium. Mapping studies using stimulator cells from inbred and outbred guinea pigs indicated that these T cell clones are specifically stimulated to proliferate by Ia antigens. The proliferative response of several strain 13 anti-2 alloreactive T cell clones was also significantly inhibited in cultures containing allogeneic stimulator cells and alloantisera to strain 2 Ia antigens. In contrast, xenogeneic monoclonal anti-Ia antibodies had selective and differential inhibitory effects on the proliferative response of these alloreactive clones. This result suggested that each T cell clone is specific for a distinct epitope expressed on Ia molecules. Furthermore, unlike bulk cultures of primary and secondary guinea pig alloreactive T cells, these alloreactive T cell clones expressed significant cytotoxic T lymphocyte (CTL) activity when tested on the appropriate blast cell targets. This CTL activity is also apparently specific for Ia antigens since strain 13 anti-2 T cell clones lysed the Ia^+ tumor target cell, EN-L_2C, but not its Ia^- variant, BZ-L_2C. Thus, these alloreactive T cell clones may permit a detailed analysis of the heterogeneity of the T cell response to Ia antigens and may provide a model system for the study of the differentiation of T cells into CTL.

153 RECOGNITION OF A UNIQUE F_1 HYBRID IA DERMININANT ON ANTIGEN PRESENTING CELLS BY A CLONED, ANTIGEN SPECIFIC T CELL LINE. J. G. Woodward and J.A. Frelinger, University of Southern California Medical School, Los Angeles, CA 90033.

Continuous T Cell lines have been established from lymph node cells of PPD-immunized (B10xB10.A)F_1 mice. Cell lines were initially propagated by continuous stimulation with antigen (PPD) and irradiated syngeneic spleen cells as the source of antigen presenting cells (APC). After 2 weeks the cells were transferred to media containing sephadex purified Con A induced interleukin-2 as well as antigen and APC. One month later, the cells were cloned by limiting dilution. One clone of particualar interest (clone2E8) was found to proliferate to PPD only in the presence of (B10xB10.A)F_1 APC and not in the presence of either parental cell type. Clone 2E8 was also unrsponsive, with or without antigen in the presence of B10.D2, B10.M, B10.P, B10.Q, B10.RIII, B10.A(3R) or B10.A(4R) APC. The proliferative response to Con A by 2E8 was also dependent on APC, although no genetic restriction was apparent. The lack of response to B10.A(3R) or B10.A(4R) antigen presenting cells plus antigen indicates that the determinant being recognized is not a product of A-E complementation. Thus, F_1 APC appear to express unique F_1 hybrid Ia determinants distinct from A-E complementation products. Appropriate F_1 strains are in production to determine the location of these complementing genes. Serologic typing of clone 2E8 revealed that this clone was $Thy\text{-}1^+$, $H\text{-}2^+$, Ia^-, $Ly\text{-}1^+$, $Ly\text{-}2^-$ thus resembling the classical T helper cell. Additional clones are being characterized in a similar fashion and should yield important new information concerning the repertoire of T cell recognition structures, as well as the nature of the stimulating determinants expressed on the antigen presenting cell.

154 CONTINUOUS MURINE CYTOTOXIC T-CELL LINES SPECIFIC FOR AN H-2 MUTANT. Elizabeth McLaughlin and Jeffrey A. Frelinger, University of Southern California Medical School Los Angeles, California 90033

Continuous cytotoxic T-cell lines have been isolated from murine secondary mixed leucocyte cultures between strain C57BL/6 (B6,H-$2K^b$) and its K-region mutant B6.C-H-2^{bm1}. These cell lines have been maintained in continuous culture for over 3 months by passage in partially purified interleukin-2 isolated from Concanavalin-A activated mouse spleen cells. The initial growth rate of these lines was slow but increased as the cells adapted to culture. Cloning efficiency, as determined by limiting dilution analysis, also increased at least 10-fold with time in culture. Each cell line lyses its stimulator with high efficiency and specific cytolytic activity was maintained throughout the culture period. Line 1-A kills B6.C-H-2^{bm1} targets giving detectable ^{51}Cr release at an effector to target ratio of 1:1. Line 2-A shows detectable cytotoxicity on B6 targets at a ratio of 2.5:1. Against a panel of seven independent, unrelated haplotypes (H-$2^{d,f,k,q,s,p,r,}$), line B6 anti B6.C-H-2^{bm1} showed no cytotoxic activity at an E:T ratio of 20:1. However, line B6.C-H-2^{bm1} anti-B6 showed a strong positive reaction against targets of the H-2^r haplotype. Several cloned lines have been derived and these will be discussed in terms of their haplotype specificity and their cell surface phenotype.

155 FUNCTIONAL T CELL HYBRIDS, Philippa Marrack, Barry Skidmore, Janice White, Sam Graham and John W. Kappler. National Jewish Hospital and Research Center, Denver, CO 80206

We have prepared T cell hybridomas of various functions and with various specificities for antigen. Our aims in this research are two-fold. First, to identify different lymphokines which are involved in B cell responses to antigen and secondly to investigate some properties of the T cell receptor. As far as the first aim is concerned we have so far prepared two T cell hybridomas with interesting properties. One of these, FS6-14.13, secretes interleukin-2 (IL-2) upon stimulation with Con A. This lymphokine is identified by its ability to stimulate proliferation in already activated B or T cells. The second hybridoma, DT-1.5, on incubation with both Con A and macrophages, causes the production of IL-2 and another, unknown, lymphokine (TRF?). This lymphokine causes differentiation of activated B cells to antibody secretion.

Our second aim is to investigate the T cell receptor(s) on cells which have dual specificity for antigen and H-2 products. By fusing antigen specific T cell blasts to our hybridoma, FS6-14.13, we have produced a number of cloned T cell hybridomas which secrete IL-2 upon stimulation with the appropriate antigen plus H-2 combination. Further experiments have suggested that antigen and H-2 products are not recognized by two independent receptors on these hybridomas, and have provoked some very interesting thoughts about the origins of allo-H-2 specificity.

156 T-CELL CLONES WITH SPECIFICITY FOR BOTH STRONG MLS AND H-2 DETERMINANTS, Susan R. Webb, Katherine L. Molnar-Kimber, Darcy B. Wilson, and Jonathan Sprent, University of Pennsylvania, Philadelphia, PA 19104

In mice, strong primary mixed lymphocyte reactions (MLR), including a large proportion of reactive T cells, can be demonstrated against the products of two different loci, the H-2 major histocompatibility complex (MHC) and a minor lymphocyte stimulating locus (Mls). To investigate whether these two responses involve overlapping specificity repertoires, B10.D2 (H-2^d, Mls^b, (d,b) T cells were stimulated repeatedly in bulk cultures over a period of five months with either H-2 incompatible C57BL/6 (b,b) cells or with Mls incompatible DBA/2 (d,a) stimulator cells and then T-cell clones were derived by limiting dilution. When these clones were tested for their capacity to proliferate in mixed lymphocyte cultures against a panel of spleen cells from mouse strains bearing different H-2 or Mls determinants, three major specificity patterns emerged. Certain of the clones responded well only to the stimulating H-2^b determinants, others proliferated well to Mls determinants and not to any H-2 determinants tested (H-$2^{b,k,s,r,f}$), while a portion of the T-cell clones responded well when stimulated with either $Mls^{a,d}$ or H-2^b determinants. These results indicate the existence of T-cell clones with dual specificity for both Mls and particular MHC determinants.

157 CYTOTOXIC T LYMPHOCYTE HYBRIDOMAS WHICH MEDIATE SPECIFIC TUMOR-CELL LYSIS IN VITRO
Yael Kaufmann, Gideon Berke and Zelig Eshhar, The Weizmann Institute of Science, Rehovot, Israel.

Cytotoxic hybridomas were generated by polyethyleneglycol-induced fusion of cytotoxic T lymphocytes (CTL) and BW5147 lymphoma cells. The CTL populations used for fusion were obtained from BALB/c ($H\text{-}2^d$) mice primed with leukemia EL4 of C57BL/6 ($H\text{-}2^b$) and restimulated either in vivo or in vitro. To circumvent possible CTL-mediated non-specific lysis of BW5147 cells during fusion, the CTL were transiently inactivated by trypsin prior to fusion. Four cytolytically-active hybridomas were obtained, cloned and subcloned. Hybrid clones lysed all $H\text{-}2^b$ leukemic target cells tested but not lipopolysaccharide or Concanavalin A stimulated C57BL/6 lymphblasts, or non $H\text{-}2^b$ target tumor cells. The mechanism of hybridoma-mediated killing of target cells in vitro appears similar to that of parental CTL although some differences have been observed. The hybridomas appear to possess neither natural killing nor antibody-dependent cytolytic activity. Clones of hybrids propagated in culture for over 6 months without the addition of known external stimulus (i.e. independent of cell growth factor and antigen), exhibit specific lytic activity against $H\text{-}2^b$ tumor cells. Such autonomous hybridomas will provide a new tool for studying the mechanism of CTL-mediated lysis and the nature of the CTL receptors.

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158 FUNCTIONAL SEPARATION OF PROLIFERATING AND HELPER T CELLS IN LONG-TERM CULTURE,
David I. Cohen and Ronald H. Schwartz, Laboratory of Immunology, National Institutes of Health, Bethesda, MD 20205.

Although many parallel properties exist between antigen-induced T cell proliferation and antigen-specific T cell help, including the phenomenon of MHC restriction, whether these functions are inseparable at the cellular level has never been clearly established. By utilizing various techniques of long-term T cell culture, we have now shown separation of these two functional properties in T cells specific for the soluble antigen ovalbumin. Some long-term T cell lines derived by a modification of method of Schreier have maintained antigen-mediated proliferation and expansion without showing T cell help; conversely, other lines continue to mediate antigen-specific T cell help even after losing the capacity to proliferate in response to antigen. The same behavior has been seen in cells enriched via soft agar cloning. Clones of cells reactive to antigen failed to give T cell help in vivo or in vitro, whereas other colonies selected in a similar manner display such help. These findings suggest that the proliferative and helper T cell populations are not entirely coextensive.

159 ALLOSPECIFIC T CELL LINES AND CLONES COOPERATE IN THE HUMORAL RESPONSE TO SRBC WITH B CELLS EXPRESSING PRIVATE Ia SPECIFICITIES IN THE H-2 IA SUBREGION.
John F. Warner, Susan L. Swain, Douglas Waterfield, Richard W. Dutton and Gunther Dennert*, Univ. of California, San Diego, La Jolla, CA 92093; * Salk Institute for Biological Studies, La Jolla, CA 92037

Two long-term allospecific T cell lines, C.C3.11.75 ($H\text{-}2^d$ anti-$H\text{-}2^k$) and B6.C.7.76 ($H\text{-}2^b$ anti-$H\text{-}2^d$) were tested for their ability to cooperate with B cells in a primary anti-SRBC antibody response. Both lines cooperated specifically with B cells expressing private specificities encoded within the IA subregion of the H-2 complex (i.e., IA^k, C.C3.11.75; IA^d, B6.C.7.76). The T cell lines also exhibited specific antigen-dependent cell proliferation and one cell line (C.C3.11.75) showed specific cytotoxicity directed against the same IA-controlled determinants. In preliminary experiments, a third cell line, C.S.4.77 ($H\text{-}2^d$ anti-$H\text{-}2^s$) displayed similar cooperative, proliferative and cytolytic activities in response to antigens controlled by the IA^s subregion. In subsequent studies we employed clones derived from the C.C3.11.75 cell line. The cloned sublines were indistinguishable from the parent line in that they exhibited specificity for IA subregion encoded antigens in all three functional assays. Our results suggest that T cell lines maintained by repeated allogeneic stimulation are selected for clonal specificities but are multifunctional. These multiple functions are expressed by one cell type that can be cloned.

160 SPECIFIC MYOGLOBIN-REACTIVE, MHC-RESTRICTED, MURINE T-CELL CLONES, Anthony J. Infante, M. Z. Atassi and C. G. Fathman, Department of Immunology, Mayo Clinic, Rochester, MN 55901.

We have obtained clones of murine T-cells which recognize sperm whale myoglobin (Mb) in the presence of appropriate antigen-presenting cells (APC). In a proliferative assay these T-cell clones show a pattern of reactivity which supports the concept that there exist (1) multiple T-cell stimulating determinants on the Mb molecule and (2) multiple restriction sites on APC controlled by different subregions of the MHC. One T-cell clone which is stimulated by the carboxyl-terminal CNBr fragment of Mb (residues 132-153) is not stimulated by a heptapeptide (residues 145-151) which has been shown to be the antibody-binding determinant in this fragment of Mb. Work in progress will probe (1) similarities and differences between determinants on Mb recognized by T-cells and antibody and (2) requirements for associative recognition of antigenic determinants on Mb and Ia determinants on APC. Specifically, we are addressing whether one T-cell stimulating site is recognized in association with one or several APC Ia determinants and conversely, whether a single APC Ia determinant facilitates recognition of one or several T-cell antigenic sites. Mb reactive T-cell clones appear to be powerful tools for investigating the nature of the T-cell receptor for antigen and the mechanism(s) involved in associative recognition of antigen and Ia by T-cells.

161 DO T CELLS BIND CONFORMATIONAL DETERMINANTS? Antonio Lanzavecchia, Vito Pistoia, Fabrizio Manca and Franco Celada, Cattedra di Immunologia, Università di Genova, Italy.

There is some evidence from different laboratories that the capacity of recognizing conformation-dependent antigenic determinants on proteins may be a characteristic of B cell, rather than T cell-receptors. This unequal use of the V-gene library as a result of differentiation could explain the division of labor implicit in specific cooperation and directional presentation of the antigen molecule. In order to test this hypothesis two requirements must be fullfilled:(a), the use of a protein antigen endowed with both sequential and conformational determinants, recognizable from each other and,(b),the isolation of clonal population of lymphocytes in conditions allowing a test of their surface markers,functional properties and binding specificities.

We think that E.coli β-galactosidase approaches the characteristics of an ideal antigen for is purpose since a number of determinants of either type have been identified,and the antibody binding of different determinants causes measurable, distinct effects. We are presently isolating and cultivating (by means of an agar colony technique)T cells from stimulated mice and from naturally immune humans. Results of enzyme binding to membrane receptors and to secreted "factors" and of specific stimulation of the certified clones will be discussed.

162 IDIOTYPE EXPRESSION BY GAT SPECIFIC T CELL LINES AND T CELLS CLONES. Jacques THEZE, M.L. GOUGEON and M. SEMAN, Institut Pasteur and IRBM, Paris, France.

GAT primed T cells were assayed for helper activity by titration into microcultures containing constant number of TNP primed spleen cells and 0.5 µg/ml of GAT-DNP. The optimal conditions required for *in vitro* GAT specific proliferation of lymph node T cells have been established in GAT responder mice. *In vitro* proliferation generates a highly enriched population of GAT specific helper T cells as compared with the initial activity of the lymph node primed T cells. The helper cells have been cultured either in the presence of macrophages and antigen or in presence of TCGF. The results obtained with the two types of technique have been compared. After 2 to 3 months of culture the helper activity is very much enriched and as few as 100 cells were able to generate an *in vitro* response. In parallel T helper cell lines specific for OVA and HGG have also been studied. More recently T helper cell clones have been obtained in agar from GAT primed lymph node cells enriched *in vitro* for helper activity. These clones keep their activity and specificity after freezing and thawing ; other parameters of their growth requirements are under investigation.

Anti-idiotypic serum raised in BALB/c against BALB/c anti-GAT antibodies can block the helper activity of GAT specific cell lines without affecting the helper function of OVA cell lines. Studies on the expression of GAT-idiotypes by these cells are in progress using anti-idiotypic monoclonal products.

163 PARALLEL PHYLOGENETIC CROSS-REACTIVITY PATTERNS OF THE IMMUNE RESPONSES TO THE NATIVE AND ACETIMIDYL-DERIVATIZED C-TERMINAL FRAGMENT OF PIGEON CYTOCHROME C. Daniel Hansburg, Charles Hannum, Ettore Apella, John K. Inman, Emanuel Margoliash and Ronald H. Schwartz, National Institutes of Health, Bethesda, MD 20205 and Northwestern University, Evanston, IL 60201.

The specificity of the T-cell immune response to the C-terminal CNBr cleavage peptide of pigeon cytochrome c (C) has been characterized by its cross-reactivity with fragments from cytochromes of various species, thereby defining two immunodominant residues (Gln-100 & Lys-104) and a one-residue deletion (Ala-103) associated with heteroclitic stimulation. T cells primed in vivo to the ε-NH_2 acetimidyl derivative of the pigeon C-peptide respond poorly to the native peptide and vice versa. However, the strength of cross-reactions with other derivatized peptides parallels that seen previously with the native fragments. For one stimulatory cytochrome c, tuna, Lys-104 is replaced by serine, which is not derivatized. Nonetheless, pigeon C-peptide-immunized animals distinguish native and derivatized tuna C-peptides. Further, the responses to the acetimidyl peptides utilize the same I-region restriction element as the native peptide. These results, the parallel phylogenetic pattern of cross-reactivity of two sets of poorly cross-reactive antigens, can be most simply explained by hypothesizing two independent antigen receptors: one recognizes a determinant common to both native and derivatized peptides and thus accounts for their parallel cross-reactivities, the second must distinguish the native and derivatized peptides at a site(s) other than residues 100 or 104. Both receptors are necessary for the T-cell response.

164 CHARACTERIZATION OF A HELPER T CELL REPLACING FACTOR DISTINCT FROM IL1 AND IL2 OBTAINED FROM A T CELL LINE. Richard W. Dutton, John Warner, Gunther Dennert,* James Watson,+ and Susan L. Swain. Univ. of California, San Diego, La Jolla, CA 92093; *Salk Institute, La Jolla, CA 92037; +Univ. of California, Irvine, CA 92717

A long term alloreactive T cell line (C.C3.11.75) stimulated with Ia on either spleen cells or an Ia-positive guinea pig-mouse hybridoma with B cell markers (surface and secreted Ig) produces a helper T cell replacing factor (TRF) with unique characteristics. This factor is separable both functionally and by molecular weight from the two best studied Interleukins: IL1 (LAF) and IL2 (TCGF). This factor synergizes with IL2 in the primary response of B cells to SRBCs. We propose that this factor is a B cell differentiation factor which is in part responsible for the T cell replacing activity of supernatants obtained by mitogen and alloantigen-stimulation. Functional and biochemical comparison of this factor with IL1, IL2, and mitogen and alloantigen-stimulated supernatants will be shown.

165 ESTABLISHMENT OF HYBRIDOMA PRODUCING INTERLEUKIN 2, K. Pfizenmaier, M. Röllinghoff and H. Wagner, Institute for Medical Microbiology, Hochhaus Augustusplatz, 6500 Mainz, W.-Germany.

Cell hybridisation of alloimmunized C3 H/He lymphocytes with the AKR derived thymoma BW 5147 has been used to experimentally approach the question, whether cell lines can be established which produce Interleukin 2 (Il-2), the soluble mediator of T helper cell function. Fourtyeight hybridomas were screened for release of Il-2 into culture supernatants. Of these, three hybrids spontaneously produced substantial amounts of Il-2 like activity when cultured for 24 hours in normal tissue culture medium. The activity is largely enhanced, provided the hybridomas are stimulated with either ConA, PHA or Phorbol myristic acetate (PMA). The producer lines have been cloned and were passaged either in vivo or in vitro without loss of functional activity for more than 6 months. The hybrid nature of these cell lines has been verified by both caryotype-and isozyme-analysis. The biological activity of the hybridoma derived factor (s) resides in a 34 000 WM protein and is comparable to that of conventionally produced Il-2 in as much as it stimulates proliferation and sustains growth of antigen primed T killer cells. Moreover, these hybridoma factor (s) largely enhance (s) the generation of primary allospecific CTL responses from thymocyte CTL precursors.

166 BIOCHEMICAL CHARACTERIZATION OF HUMAN INTERLEUKIN-2, Mark Barton Frank, University of California, Irvine; Irvine, CA 92717

Large quantities of interleukin-2 (IL-2) produced by the human leukemia cell line Jurkat-FHCRC following stimulation by mitogens permit the biochemical characterization of this T cell factor. Steps used to characterize human IL-2 include DEAE-Sephacel and AcA54 column chromatography, isoelectric focusing, and SDS-polyacrylamide gel electrophoresis. Material that is capable of stimulating DNA synthesis in IL-2 dependent cytotoxic T cell lines is eluted from a DEAE column with 0.05 M NaCl, has an isoelectric point of approximately seven, and a molecular weight of 15,000 daltons. With the exception of the slightly higher isoelectric point, this material appears to be very similar to the IL-2 produced by activated human peripheral blood lymphocytes (Gillis, et al (1980) J. Immunol. 124:1954). We are currently in the process of producing sufficient quantities of this factor which will be used in determining the amino acid sequence for comparative studies with mouse IL-2, as well as for use in future investigations of the physiological role of human interleukin-2.

167 LYT AND Ia PHENOTYPE INVOLVED IN LYMPHOCYTE PROLIFERATION RESPONSE TO ACETYLCHOLINE RECEPTOR. P. Christadoss, C. J. Krco, V. A. Lennon, and C. S. David
Department of Immunology, Mayo Clinic and Medical School, Rochester, MN 55901.

Gene(s) controlling lymphocyte proliferative responses to acetylcholine receptor (AChR) and susceptibility to experimental autoimmune myasthenia gravis (EAMG) has been mapped to the I-A subregion of the mouse H-2 complex. The lymphocyte responses correlated closely with autoantibody responses and the electrophysiological defect of EAMG. Lymphocyte proliferative response to AChR is dependent on Lyt $1^{+}23^{-}$ phenotype, and the presence of adherent cells. Lymphocyte responses were eliminated by blocking Ia antigens on lymph node cell surface with specific anti-Ia sera. A point mutation at the I-A subregion of the B6 strain, which resulted in structural alteration of the Ia molecule, converted high responsiveness to low responsiveness. These data indicate involvement of the Ia molecule in induction of immune responses to AChR, probably in presentation of AChR to helper T cells expressing the Lyt 1 phenotype.

168 ALLOREACTIVE T-CELL CULTURES SPECIFIC FOR COMBINATORIAL Ia DETERMINANTS. Christopher J. Krco and Chella S. David, Department of Immunology, Mayo Clinic and Medical School, Rochester, MN 55901.

T-cells alloreactive to the combinatorial specificity Ia.22 were prepared by culturing nylon wool column purified lymph node T-cells in bulk cultures in vitro with I-E subregion incompatible irradiated spleen cells. Memory T-cells specific for the Ia.22 generating configurations of $A^{k}_{e}E^{k}_{\alpha}$ [B10.A(4R) anti-B10.A(2R)], $A^{b}_{e}E^{k}_{\alpha}$ [B10.A(18R) anti-B10.A(5R)], and $A^{s}_{e}E^{k}_{\alpha}$ [B10.S(24R) anti-B10.HTT] were tested for specificity in panel tests utilizing spleen cells representing three Ia.22 generating configurations ($A^{k}_{e}E^{k}_{\alpha}$, $A^{b}_{e}E^{k}_{\alpha}$, $A^{s}_{e}E^{k}_{\alpha}$). Memory T-cells specific for $A^{k}_{e}E^{k}_{\alpha}$ will respond (Δ cpm 10,000) to B10.A(2R) and other $A^{k}_{e}E^{k}_{\alpha}$ configurations (B10.AM, B10.ASR-2, B10.AKM) but poorly to B10.A(5R) and B10.S(9R) comprising the $A^{b}_{e}E^{k}_{\alpha}$ and $A^{s}_{e}E^{k}_{\alpha}$ configurations (Δ cpm's of 5,000 and 4,000). Similarly, $A^{b}_{e}E^{k}_{\alpha}$ specific T-cells respond (Δ cpm 12,000) to B10.S(9R) and B10.HTT (both $A^{s}_{e}E^{k}_{\alpha}$) and weakly to $A^{b}_{e}E^{k}_{\alpha}$ [B10.A(5R); Δ cpm 1,000]. These results suggest that these T-cell cultures are capable of discrimination subtle structural differences in I-E subregion molecules not detectable with conventional allo-anti-Ia.22 sera. Studies are underway to clone T-cells from these cultures.

Idiotypic T Cell Receptors

169 IDIOTYPES ON RECEPTORS OF TNP-SPECIFIC, H-2 RESTRICTED CYTOTOXIC AKR T LYMPHOCYTES, Peter H. Krammer, Ute Hamann, Rainer Rehberger, and Klaus Eichmann, Institut für Immunologie und Genetik,Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 280, 6900 Heidelberg, F.R.G.
We developed a system to define serological determinants of receptors on MHC-restricted 2,4,6-trinitrophenyl (TNP)-specific CTL using these cells directly as immunogens in syngeneic AKR mice. Cell populations enriched for TNP-specific, H-$2K^K$ restricted AKR CTL obtained from a five day culture of cells from the draining lymph nodes of AKR mice five days previously sensitized on the abdominal skin by 2,4,6-trinitrophenylchloride were repeatedly injected into syngeneic AKR mice. After several injections these mice developed AKR anti-(AKR anti-AKR-TNP) antisera, abbreviated as AKRa(AKRaAKR-TNP). Pooled antisera from several mice and antisera from single mice were strongly reactive with a major fraction of AKR anti-AKR-TNP CTL.This activity was specific as the antisera did not react with alloreactive AKR CTL, H-$2K^K$-restricted AKR CTL activated by fluorescein-isothiocyanate (FITC), and H-2 restricted, TNP-specific CTL of strain C57Bl/6. As we were unable to demonstrate any reactivity with the antigen TNP as such we concluded that the antisera reacted with specificity-associated determinants (idiotypes) on AKR anti-AKR-TNP CTL (Krammer et al.(1)). Particularly, the fact that AKRa (AKRaAKR-TNP) did not react with AKR anti-AKR-FITC CTL indicated that the antisera recognized either a receptor for TNP (if the two receptor model is correct) or a receptor for TNP-self (if the one receptor model is correct). Our experiments excluded reactivity of the antisera with anti-self receptors as these would be identical on both types of CTL. This situation is suitable to design experiments which help to delineate the genes coding for the receptors on H-2 restricted CTL and make predictions to decide between the validity of the one or two receptor model.
1.Krammer,P.H., Rehberger,R., and Eichmann,K., 1980. J.Exp. Med. 151:1166.

170 IDIOTYPIC AND ANTIIDIOTYPIC T CELL RECEPTORS AND SUPPRESSOR FACTORS. Alfred Nisonoff and Mark I. Greene, Rosenstiel Research Center, Brandeis University, Waltham, MA 02254 and Department of Pathology, Harvard Medical School, Boston, MA 02115.

From studies with A/J anti-azobenzenearsonate (anti-ABA) antibodies evidence has been obtained for the existence of idiotype-suppressor T cells with idiotypic (Ts_1) or antiidiotypic (Ts_2) receptors. Such cells selectively suppress the appearance of the major idiotype in the humoral response with little effect on the total anti-ABA response. The suppressor cells also greatly diminish the DTH response to the ABA hapten. Ts_1, but not Ts_2, are killed by anti-id + C. A suppressor factor can be obtained from each type of suppressor cell whose specificity (idiotypic or antiidiotypic; TsF_1 or TsF_2), corresponds to that of the cell source. Each factor has a molecular weight of 35,000-70,000 and possesses H-2 determinants. TsF_1 has I-J determinants present on the same molecule or complex as the binding site. TsF_1 but not TsF_2 has specificity for the Ar hapten. In suppression of DTH TsF_1 acts only at the afferent limb of the DTH response whereas TsF_2 appears to be an effector, capable of acting late in the response. Inoculation of TsF_1 induces Ts_2. TsF_1 but not TsF_2 can act across an H-2 barrier. BALB/c mice, which are id^- can nonetheless produce Ts_2 which suppress DTH to ABA or id^+ Ab production in C.AL-20 congenic mice, which differ in allotype from BALB/c; such Ts_2 have no effect on the humoral or DTH response in BALB/c mice. The expression of id in A/J humoral antibodies has previously been shown to be under control of the IgK-Trp (kappa chain) locus as well as the Igh locus. In contrast, the production of id^+ TsF_1 is independent of IgK-Trp and is linked only to Igh. Anti-id Ab specific for the H chain, but not site-specific Ab, binds TsF_1.

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171 STRUCTURAL AND FUNCTIONAL ANALYSIS OF Ig AND Tcr MOLECULES BEARING 5936 ANTISERUM DEFINED IDIOTYPIC DETERMINANTS. Bent Rubin, Alain Bourgois, Brigitte Kahn-Perles Marie Suzan, Claude Boyer and Claudine Schiff, Centre d'Immunologie INSERM-CNRS de Marseille-Luminy, Case 906, 13288 Marseille cédex 9, France.

Antiserum 5936 defines idiotypes on Ig and Tcr induced in B6 but not in C3H.SW mice by immunization with CBA cells. Antiserum 6036 was produced against B6 anti-CBA Tcr, and it does not react against Ig. 5936 and 6036 antiserum reactive Tcr were found on Lyt 1^+ 2.3^- T cells in three systems : 1) B6 anti-CBA but not C3H.SW anti-CBA, 2) B10.BR anti-TNP : B10.BR but not CBA anti-TNP : CBA and 3) B6 anti-FCS : B6 but not C3H.SW anti-FCS : C3H.SW T cell populations. Three different forms of Tcr with molecular weight (MW) of 50,000, 62,000 or 75,000 were found by external or internal labelling of T cells. Such molecules could be isolated from culture supernatants also. It appears that the 50,000 and 62,000 MW components are degradation products of the 75,000 MW component. The 50,000 MW molecules are non-glycosylated, single-chain polypeptides with an isoelectric point of 5.1. The NH_2-terminal amino acid of these polypeptides was blocked, but the amino acid composition verified the low isoelectric point of the molecules. The number of cysteyl residues together with the degradation by loss of 12-13,000 dalton fragments is consistent with a structure in domains.

The specificity of 5936-Id^+ Tcr or Ig is still unknown. 5936-Id^+ Tcr are not found on MLC-responding cells, CTL or CBA-membrane vesicle-binding B6 anti-CBA T cells. Our Tcr molecules may be implicated in T-B cell collaboration : e.g. B6-T cells ($H\text{-}2^b$, $Ig\text{-}1^b$, 5936-Id^+) but not C3H.SW-T cells ($H\text{-}2^b$, $Ig\text{-}1^j$, 5936-Id^-) reconstitute the production of 5936-Id^+ Ig in B6 nude mice, which by themselves cannot produce 5936 idiotypes.

172 Characterization of nonimmunoglobulin molecules from immune serum that specifically binds SRBC and bears a dominant T cell idiotype. G.M. Iverson, D.D. Eardley, S. Durum, and S.H. Kaufmann. Howard Hughes Medical Inst. at Yale U. Sch of Med, Dept. Pathol., New Haven CT, Harvard Sch of Public Health, Boston, MA; and Freie Universitate, Berlin, West Germany.

An anti-idiotype antiserum (anti-Id) against mouse anti-SRBC antiserum was produced in sheep. This anti-Id detects a dominant idiotype present on specific antigen binding T cells. The specificity of the anti-Id was determined by its ability to inhibit SRBC educated T cells from forming specific rosettes with SRBC. It does not inhibit HRBC educated T cells from forming cross reactive rosettes with SRBC. Injection of the anti-Id into mice induces suppression of DTH to SRBC but not to HRBC. Spleen cells from mice given three daily injections of anti-Id prior to cell transfer were capable of adoptively suppressing DTH to SRBC but not HRBC. The adoptive transfer of suppression was sensitive to treatment with anti-Thy1, anti-Ly1 and anti-Id plus C' but not to anti-Ly2 plus C'. The anti-Id was used to isolate antigen binding molecules from hyperimmune serum. These antigen binding molecules can be divided into two main groups: immunoglobulin and nonimmunoglobulin. The Id^+ immunoglobulin represents 10-25% of the Id^+ protein and less than 1% of the SRBC specific antibody in the serum. The nonimmunoglobulin Id^+ protein is similar both serologically and physically to T cell derived TNP specific suppressor factor and the T cell derived, Id^+ material described by Binz and Wigzell. We conclude from these experiments, that the serum of hyperimmune mice contains T cell derived antigen specific molecules. It is possible that it is this T cell antigen binding molecules present in immune serum and not immunoglobulin that is responsible for the suppressive activity observed when immune serum is adoptively transferred.

173 CYTOTOXIC T LYMPHOCYTE TARGET STRUCTURES: MOLECULAR AND CELLULAR EVIDENCE FOR T CELL RECOGNITION OF INTERACTION ANTIGENS, *D. Bellgrau, U. Hämmerling, O. Kämpe and P. Peterson, Uppsala Univ. Wallenberg Laboratory, Uppsala, Sweden and *Basel Institute for Immunology, Basel, Switzerland

We continued the work of Kvist et al. (PNAS 75,5674,1978) who reported that there existed on the cell surface of the adenovirus 2 transformed rat fibroblast line A2T2C4 complexes of major histocompatibility complex coded (MHC) antigens and a viral genome coded glycoprotein. We found that A2T2C4, derived from the HL strain ($Rt\text{-}1^c$), can be used to generate, in vitro, syngeneic or allogeneic cytotoxic T lymphocytes (CTL) and in all cases tested the cytotoxicity is specific for A2T2C4 but not untransformed HL targets. Anti-HL CTL do not lyse A2T2C4. Six monoclonal antibodies cytotoxic for HL do not lyse A2T2C4. Studies with two other adenovirus 2 transformed lines, A2F4 and A2F19, derived from rats of the $Rt\text{-}1^l$ haplotype produced different results. CTL of specificity anti-$Rt\text{-}1^l$ lysed A2F4 and A2F19. These lines could also be lysed with a monoclonal anti-$Rt\text{-}1^l$ antibody. Immunoprecipitation analyses do not support the hypothesis that Class I molecules on A2T2C4 are different from those on untransformed HL cells and confirm that a viral genome coded protein, designated 19K, co-precipitates with MHC proteins. Conversely, A2F4 has little if any and A2F19 has no detectable cell surface 19K. Given the apparent correlation between the expression of the 19K protein on the cell surface, its capacity to form complexes with MHC molecules and the inability of allogeneic CTL specific for non-transformed HL cells to lyse A2T2C4, a likely conclusion is that the anti-A2T2C4 CTL target structures are unique determinants formed by the molecular interaction of MHC and 19K.

174 THE EXPRESSION AND FUNCTIONAL SIGNIFICANCE OF NUCLEASE IDIOTYPE ON HELPER T CELLS. Richard J. Hodes, Paul I. Nadler, Geraldine Miller, and David H. Sachs, National Institutes of Health, Bethesda, MD 20205.

It has previously been demonstrated that the murine antibody response to Staphylococcal nuclease (nuclease) is characterized by the expression of allotype-linked idiotypic markers on nuclease-specific antibody molecules. The expression and the functional significance of idiotypic determinants on antigen-specific T helper (T_H) cell populations has been evaluated in an in vitro antibody response system. TNP-specific plaque-forming cell responses to TNP-conjugated nuclease were found to require the cooperation of nuclease-primed T_H cells as well as unprimed B cells and accessory cells. The expression of nuclease idiotypes on T_H cells was demonstrated by the specific elimination of T_H cells specific for nuclease with affinity-purified anti-idiotypic antibodies + C'. Moreover, the susceptibility of nuclease-primed T_H to elimination by such treatment was idiotype-specific in that anti-idiotype affected only T_H from strains normally expressing the same idiotypes in the humoral response to immunization with nuclease. A functional role of the idiotypes expressed on T_H was suggested by the fact that anti-idiotype, in the absence of C', suppressed the response to TNP-nuclease in an antigen-specific manner; this inhibition occurred at the level of T_H cells and was independent of B cell idiotype as shown in cell mixing experiments. Thus, nuclease-primed and specific T_H cells for the response to TNP-nuclease express nuclease idiotype, and this cell surface idiotype appears to be functionally involved in the activity of these helper cells.

175 REACTIVITY OF T HELPER FACTORS AND T HELPER CELL LINES WITH XENOGENEIC ANTI-T CELL RECEPTOR ANTISERUM. Margaret A. Cooley, Bent Rubin, Annick Guimezanes and Anne-Marie Schmitt-Verhulst. Centre d'Immunologie INSERM-CNRS de Marseille-Luminy, Case 906, 13288 Marseille cedex 9, France.

The 6036 antiserum was raised in a mouse immunoglobulin tolerant rabbit against material contained in B6 anti-CBA MLC supernatants and retained on a column of Sepharose coupled 5936 antiserum (anti-(B6 anti-CBA Ig)) and seems to recognize determinants on a class of T cell receptors (Rubin et al., 1979 ; J. Exp. Med., 150, 307 ; Rubin and Bourgois, Scand. J. Immunol., in press).

We have described a model for generation of specific helper T cells (Th) for the induction of cytotoxic T lymphocytes (CTL) to trinitrophenyl modified syngeneic cells from thymocyte precursors. The helper effect can be replaced by soluble mediator(s) secreted by the Th population, which has a molecular weight of approximately 45,000 daltons. We also developed both long term in vitro maintained helper cell lines (Guimezanes and Schmitt-Verhulst, J. Immunol., in press) and cloned CTL from in vivo primed T cell populations.

Analysis of helper lines and CTL clones with the 6036 antiserum showed that the latter contained no 6036-positive cells. Helper lines contained varying proportions of 6036^+ cells, but treatment of lines with 6036 plus complement did not inhibit their helper capacity. However, the helper factor produced by Th cells was partially retained on a column of Sepharose-coupled 6036. Further functional and immunochemical characterization of these reactivities is underway.

176 FAILURE OF DETERMINATION OF "SELF" BY THYMUS EPITHELIAL REMNANT GRAFTS. Terry R. Beardsley, Susan L. Swain, and Richard W. Dutton. University of California, San Diego, La Jolla, California 92093.

Recent studies addressing the question of whether or not the thymus is the major determinant of the H-2 phenotypic expression of T cells have yielded conflicting results. The thymus is composed of at least three distinct component parts: lymphoid, macrophage, and epithelial, any of which may be participating in the selection process in the thymus. We have approached the problem by engrafting nude mice with epithelial remnants which are devoid of any demonstrable lymphoid elements. BALB/c nu/nu recipients of syngeneic remnant grafts are fully reconstituted for T cell reactivity as measured by killer and helper T cell function in vitro. Furthermore, spleen cells from such mice display restricted killing for TNP-modified $H\text{-}2^d$ targets. BALB/c nu/nu recipients of allogeneic C57BL/6 remnant grafts are also fully reconstituted for T cell function. They display T cell killing to third party alloantigen comparable to normal BALB/c control mice. Surprisingly, spleen cells from recipients of allogeneic remnants also display significant T cell killing against host and donor type targets, and T cell help for both host and donor type B cells, despite the absence of in vivo evidence of host versus graft, or graft versus host reactivity. Furthermore, in contrast to recipients of syngeneic grafts, the recipients of allogeneic grafts kill TNP-modified cells of both host and donor H-2 type. The results suggest that the thymic epithelial component by itself is sufficient for effecting maturation of T cells, but is not sufficient for selection of appropriate MHC restriction phenotype, or for establishment of self-tolerance.

177 A THEORY ON THE SOMATIC GENERATION OF THE SPECIFICITY REPERTOIRE OF T LYMPHOCYTES, Wulf Dröge, Institut für Immunologie und Genetik, Deutsches Krebsforschungszentrum, D-69 Heidelberg, West Germany

A theory on the generation of MHC-restriction and alloreactivity is described. The theory states that T cells recognize usually conventional antigens (CA) plus major histocompatibility antigens (MHA) through one receptor with one or more V regions. CA and MHA determinants contribute approximately additively to the total binding energy of the receptor. The mature T cell repertoire is mainly the result of a selection through two stochastic processes, i.e. a positive selection which commits a cell to a given T cell function and self-tolerance induction as a negative selection. The probability for these selection processes is in first approximation a function of the affinity to corresponding antigens on inducing or tolerogenic cells, respectively. It is critical for the theory that the antigens which drive the selection processes are mostly self-CA in combination with self-MHA. Probability distribution profiles illustrate how this set of assumptions explains the self-restricted responses to foreign antigens and also the high proportion of alloreactive cells in the T cell repertoire. The mature repertoire is predicted to contain "self-restricted" cells which recognize self-MHA with higher affinity than allogeneic MHA, and "allo-restricted" cells which recognize allogeneic MHA with higher affinity than self-MHA. The self-restrictedness of an immune response is determined by the ratio of these two types of cells in the responding T cell population, and is predicted to be different for different CA. Self-restrictedness is expected to be the lower the more the antigen crossreacts with self-antigens (or the longer the antigen persists in the organism). Allogeneic cells are recognized by their MHA in combination with nonpolymorphic cell surface CA.

178 GVH HOST Ia ANTIGENS ON ACTIVATED DONOR T CELLS, Terry L. Delovitch and John F. Harris, University of Toronto, Toronto, Canada M5G 1L6

During a GVH reaction induced by the transfer of A.SW ($H\text{-}2^s$) donor thymocytes into irradiated (800 R) A/WySn ($H\text{-}2^a$) hosts, 30-50% of donor alloactivated T cells derived from host spleens acquire host Ia antigens on their surface. Radioimmune binding assays performed with monoclonal anti-Ia antibodies indicate that about 10^4 $I\text{-}A^k$ and $I\text{-}E^k$ coded Ia molecules of host origin appear on activated donor T cells. 2-D gel electrophoretograms demonstrate that both Ia α- and β- radioiodinated polypeptide chains are transferred from host cells to donor cells. Biosynthetic studies show that activated donor T cells do not synthesize Ia antigens of the donor haplotype. Functional analyses were carried out with activated donor T cells that were sorted with a FACS into subpopulations that were either surface positive or negative for host-derived $I\text{-}A^k$ coded Ia molecules. During an in vitro primary anti-SRBC response, donor T cells that bear host Ia antigens preferentially cooperate with unprimed host B cells whereas donor T cells that do not bear host Ia antigens preferentially help unprimed donor B cells. This apparent H-2 restriction of donor helper T cell mediated interaction suggests that self-Ia and allo-Ia antigen receptors are expressed by different functional T cell subpopulations and that these self- and allo-receptors may be structurally different molecules. If either donor or host-derived antigen-primed B cells are used, donor T cells irregardless of their surface host Ia phenotype display no H-2 restricted interaction. This suggests that occupancy of helper T cell Ia antigen receptors is required during interaction with unprimed non-dividing B cells but not with antigen-primed dividing B cells.

179 MURINE LEUKEMIA VIRUS BINDS TO SURFACE IMMUNOGLOBULIN ON THE B CELL LEUKEMIA BCL_1. M.S. McGrath & I.L. Weissman, Department of Pathology, Stanford Univ., Stanford, CA

Murine leukemia virus induced T-lymphomas bear surface receptors specific for the leukemogenic virus which induced them. In specific cases, interruption of this MuLV-receptor interaction with monoclonal anti-Thy 1 causes non-complement mediated lymphoma cell death in cell cycle phase G_1. The observation that anti-μ and anti-λ inhibited in vitro growth and LPS stimulation of the B cell lymphoma BCL_1 prompted experiments to determine the role of surface stimulation in BCL_1 proliferation. We have found that the in vivo derived BCL_1 cell line produces a retrovirus which binds to a high degree to the BCL_1 cell surface. These cells also bind several thymotropic MuLV's to an equal degree; this demonstrates a generalized polyspecific binding similar to the radiation induced T-lymphoma, L691. Isolated BCL_1 immunoglobulin (μλ) has been shown to contain the MuLV binding activity. Anti-μ, anti-λ, and anti-BCL_1-Ig idiotypic sera all block MuLV binding to BCL_1-immunoglobulin whereas only the anti-idiotype binds to and blocks MuLV binding to L691 cells. This idiotypic expression is being used to investigate surface expression of B- and T-lymphoma type specific receptors.

180 IDIOTYPES ON MHC RESTRICTED VIRUS-IMMUNE CYTOTOXIC T CELLS, Ursula R. Kees, ICRF Tumour Immunology Unit, Dept. of Zoology, University College London, Gower St., London WC1E 6BT, England.

For the first time idiotypes have been demonstrated on naturally occuring cytotoxic T lymphocytes (CTLs), which indicates that the response against virus-infected syngeneic cells is sufficiently restricted to allow the detection of such determinants. Antisera were raised in CBA or B10 mice against Newcastle Disease virus (NDV)-immune CTLs, which show an H.2 restricted response. Secondary CTLs were generated in vitro, purified and used to immunize syngeneic mice. Sera from animals given four to six injections were screened in a radioimmunoassay using CTLs as target cells. The reactivity pattern of three antisera indicated that idiotypic determinants were recognized on NDV-immune CTLs used for immunization. In an attempt to assess the functional activity of these three antisera, blocking studies were carried out by measuring the cytotoxic activity of relevant CTLs in the presence of anti-idiotypic antisera (anti-id). No reduction of cytotoxicity was observed with any serum. In a second test CTLs were treated with anti-id antisera and complement before measuring the residual cytotoxicity in comparison with NMS controls. All three anti-id antisera consistently reduced the cytotoxicity of CTLs used for immunization. The effect of a CBA antiserum raised against NDV-immune CBA CTLs varied considerably between individual experiments (45-91% reduction). However, the antisera did not react with syngeneic influenza-immune CTLs, syngeneic alloreactive CTLs or allogeneic NDV-immune CTLs (B10 anti B10 NDV). These results are more consistent with a one receptor model for H.2 restriction of CTLs.

181 THE INDUCTION OF NUCLEASE-SPECIFIC HELPER T CELLS WITH ANTI-IDIOTYPIC ANTIBODIES. Paul I. Nadler, Geraldine Miller, David H. Sachs, and Richard J. Hodes, National Institutes of Health, Bethesda, MD 20205.

A comparison of the abilities of antigen or anti-idiotypic antibodies (anti-ID) to induce antigen-specific T cell responses was studied employing a system of T cell dependent in vitro antibody responses to TNP-Staphylococcal nuclease (Nase). The in vivo antibody response to Nase has previously been demonstrated to result in the generation of anti-Nase antibodies bearing cross-reactive idiotypes. Nase priming also generates antigen-specific T helper (T_H) cells as demonstrated functionally in in vitro antibody responses to TNP-Nase. Priming was idiotype-specific in that T_H cells expressed the same anti-Nase-specific ID normally present on the anti-Nase antibodies of each strain tested. Purified, heterologous anti-ID administered in vivo in the absence of antigen was also found to induce a a population of Nase-specific T_H cells. However, anti-ID was able to induce Nase-specific T_H cells regardless of the idiotype normally present on the anti-Nase antibodies of the strain tested. These T_H cells induced by anti-ID expressed the Nase ID corresponding to the specificity of the anti-ID used in priming, and not necessarily the ID normally expressed on either anti-Nase antibodies or Nase primed T_H cells in that strain. These findings demonstrate that anti-ID may mimic antigen in its ability to induce a population of antigen-specific, ID-bearing T_H cells. Furthermore, such induction of ID expression on T_H cells which would not normally express that ID suggests that regulatory genes may be involved in determing the observed genetic restriction of T cell idiotype expression.

182 SELF- AND ALLO-IA RECOGNITION BY T CELL CLONES FROM LONG TERM MLC, Bruce E. Elliott, and Zoltan Nagy, Cancer Research Division, Department Pathology, Queen's University, Kingston, Ontario, Canada K7L 3N6

Highly selected alloreactive T cell lines were developed by repeated restimulation of A($H\text{-}2^a$) or B10.D2 ($H\text{-}2^d$) responder cells with B10($H\text{-}2^b$) stimulator cells. The lines proliferate strongly against $I\text{-}A^b$ determinants, but not $H\text{-}2^{d,f,q,r,or\ s}$ stimulators. Clones derived from the line were shown to bind $H\text{-}2^b$ membrane vesicles with high affinity (i.e., at low concentration) via $I\text{-}A^b$ but not K^b or D^b determinants. T blasts from long term MLC were Ia-negative; however, low affinity binding of self-Ia in soluble or vesicular form (at high concentrations) was observed. Ia specificity of binding was confirmed by inhibition with monoclonal anti-self-Ia antibodies; whereas antibodies against H-2K/D, or non-H-2 determinants were non-inhibitory. No binding of third party Ia material was observed. Acquisition of self-Ia by T blasts did not interfere with binding of stimulator vesicles; however, self-Ia bearing T blasts were susceptible to stimulator vesicle binding inhibition with monoclonal anti-self-Ia antibodies. Antibodies against Thy-1, Lyt-1, Lyt-2 and Ly-6 antigens were not inhibitory. These results strongly suggest the existence of specific self-Ia acceptor sites closely linked to the receptors for stimulator alloantigens on allo-Ia specific T cell clones.

Immunoregulatory Circuits and T Cell Subpopulations

183 INACTIVATION OF HELPER CELLS BY SUPPRESSOR CELLS, Diane D. Eardley, Department of Microbiology, Harvard School of Public Health, Boston, MA, 02115

The activation of suppressor T cell activity can occur in the absence of B cells. However, the role of B cells or immunoglobulin during the effector phase of suppressor T cell activity has been difficult to assess in systems where antibody production is the assayed parameter. In order to focus on the cell biology of helper cell inactivation by suppressor T cells, an intermediate culture system has been devised where activated helper and suppressor cells are mixed together in the absence of B cells. After an appropriate length of time, the helper cells are retrieved and tested for residual helper activity for the anti-erythrocyte response by adding to in vitro cultures of B cells. The kinetics, specificity, genetic restrictions, and requirements for idiotypic recognition are described for this intermediate culture system.

184 ANTIGEN SPECIFIC, H-2 RESTRICTED T CELL HYBRIDS, John W. Kappler, Barry Skidmore, Janice White and Philippa Marrack. Nat. Jewish Hosp. & Res. Ctr., Denver, CO 80206

We have produced hybrids between the AKR thymic leukemia, BW5147 and normal B6D2F$_1$ T cells. One cloned hybrid, FS6-14.13 was shown to produce the growth factor, interleukin-2 (IL-2), in response to Con A. We fused an azaguanine resistant subline of FS6-14.13 to normal T cells enriched in antigen specific cells by in vivo immunization followed by in vitro challenge with antigen and expansion in IL-2. A number of cloned hybrid cell lines were established that responded to a challenge with antigen and the appropriate I-region bearing antigen presenting cells with the production of IL-2. One hybrid line, AO-40.10, responded to chicken ovalbumin (OVA) plus I-A^k. An azaguanine variant of AO-40.10 was fused to normal T cells specific for various other antigens and I-region types. A number of doubly specific hybrids were found. In every case the reactivity of these hybrids was for OVA plus I-A^k and for the second antigen/I-region combination, but no hybrids reacted to OVA plus the second I-region type or to the second antigen plus I-A^k. These results will be discussed in relation to the various theories concerning antigen plus H-2 recognition. In the course of these fusion studies, a number of hybrids were detected with unexpected antigen-independent reactivities to I-region antigens. These hybrids will be discussed in relation to the various theories on the relation between antigen plus self-H-2 recognition versus allo-H-2 recognition.

185 PHENOTYPIC AND FUNCTIONAL INTERRELATIONSHIPS OF DISTINCT LYl T CELL SUBSETS, J. S. McDougal, F. W. Shen, J. A. K. Nicholson, S. P. Cort, and J. Bard, Centers for Disease Control, Atlanta, GA 30333 and Sloan Kettering Cancer Institute, New York, NY 10021

Several of the inducer-cell functions which have been ascribed to the Lyl T cell set are examined with respect to 1) whether they are performed by distinct subpopulations in terms of IJ and Qa-1 phenotypes, 2) the requirement for immunoglobulin in the induction or effector phase, and 3) idiotype-specific or restricted function. To do this we have examined streptococcal A vaccine (SAV) primed T cells which are derived in vitro from cortisone resistant thymocytes cultured with SAV pulsed macrophages. In this system, the Lyl precursor:effector relationships have been well characterized and 3 functionally distinct Lyl T cell sets have been described: the Lyl helper effector (Lyl:HE), the suppressor inducer (Lyl:SI) and the helper inducer (Lyl:HI) which induces the differentiation of antecedent Lyl23 T cells to become functional Lyl cells.

The Lyl suppressor inducer is Qa-1$^+$:IJ$^+$. It acts on non-immune Lyl23 T cells to induce suppression of an expected B cell PFC response. The suppression is potentiated if Qa-1$^-$:IJ$^+$ Ly23 T cells are present. The interaction of the Lyl:SI with Lyl23 T cells is restricted by the IgH heavy chain locus, specifically the variable gene region. However, this restriction can be overcome if greater numbers of Lyl:SI are assayed, and the suppressive circuit can be activated by aggregated immunoglobulin having an antigen specificity (or idiotype) which is irrelevant to the B cell response being measured.

Lyl helper effectors can be induced by anti-idiotypic antibodies, however, the helper activity of anti-id induced Lyl:HE is not confined to B cells bearing the idiotype. Lyl:HE are Qa-1$^-$:IJ$^-$ and we were unable to demonstrate a significant synergy between this population and the Lyl:Qa-1$^+$:IJ$^+$ cell set in mediating the helper effect. Moreover, analysis of log-log dose response curves do not indicate that more than one helper cell population is active in this system whereas similar analysis of T cells educated in vivo or in vitro in the presence of B cells indicate that 2 helper cell populations exist. Therefore, in this system, only one helper cell population appears to be generated; an antigen-specific, anti-id inducible but not idiotype restricted, MHC restricted, Qa-1$^-$:IJ$^-$ Lyl helper cell.

186 THE ROLE OF HELPER CELLS IN THE GENERATION OF ANTI-QA-1 CYTOTOXIC T CELLS

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There are currently available two Qa-1/T1a congenic pairs of mice 1) A/J, A.T1a^b, 2) B6.T1a^a, B6. We have shown previously that a secondary anti-Qa-1^b CML response can be generated using the first combination, A/J↷A.T1a^b, but cannot be generated using the second combination, B6.T1a^a↷B6.

However, anti-Qa-1^b cytotoxic T cells (CTL) can be generated in the nonresponding congenic pair, B6.T1a^a↷B6, if B6.T1a^a female mice are immunized with B6 male spleen cells or ARS haptenated B6 female spleen cells. It is mandatory for the second antigenic difference, H-Y or ARS, to be present on the same cell as the Qa-1 alloantigen. No extra antigenic difference, in addition to the Qa-1 alloantigen, is required for in vitro restimulation. Thus, the extra antigenic difference, H-Y or ARS, may be viewed as a "carrier" determinant, functioning to sensitize obligatory helper cells.

Since the H-Y determinant need not be present for in vitro restimulation and no helper cell activity could be demonstrated that augments the in vitro phase of the response, we suggest that the helper cell activity, for anti-Qa-1 CTLs, functions at the level of the CTL precursor in vivo.

187 T-HELPER CELLS EXHIBIT VARIANT EXPRESSION OF THE Ly1, 2 ALLOANTIGENS, D.B. Thomas and R.A. Calderon, National Institute for Medical Research, Mill Hill, London, NW7 1AA, England.

The Ly alloantigens provide convincing evidence that T-helper (Ly1$^+$2$^-$) and T-suppressor (Ly1$^-$2$^+$) cells are distinct populations with an independent differentiation lineage from thymus to peripheral effector cells. There is an implicit assumption that an Ly phenotype exhibits invariant expression during differentiation. Evidence is presented that T-helper cells exhibit a reciprocal change in phenotype, from Ly1$^+$2$^-$ to Ly1$^-$2$^+$, during an immune response. The Ly1$^-$2$^+$ helper cells cooperate with "activated" B-cells but suppress the induction of competing B-cell clones.

188 RELATIONSHIP BETWEEN HAPTEN-SPECIFIC AND CARRIER-SPECIFIC REGULATION, Takeshi Tokuhisa*, Leonard A. Herzenberg and Leonore A. Herzenberg, Genetics Dept., Stanford Univ. Sch. of Med., Stanford, CA, 94305 (*currently at Lab. for Immunol., Chiba Univ. Sch. of Med., Chiba, Japan)

We have shown that carrier-primed mice exposed subsequently to a hapten on the priming carrier develop a specific and persistent suppression of IgG anti-hapten responses that, once induced, suppresse in situ responses to hapten on the priming or an unrelated carrier. This "hapten-specific" suppression mechanism does not interfere either with anti-carrier antibody responses or with the development of anti-hapten memory B cells. It shows isotype and allotype specificity in that it can selectively suppress individual IgG isotype or allotype anti-hapten responses. The induction of this suppression (in contrast with its operation) is carrier-specific since it requires exposure of carrier-primed animals to the hapten on the priming carrier.

This carrier-specificity suggests a functional relationship between the cells that induce hapten-specific suppression and the traditional carrier-specific suppressor T cells (CTs). CTs studies have shown that Lyt-2$^+$, I-J$^+$ splenic T cells from carrier-primed donors produce a carrier-specific suppressor factor which stimulates Lyt-1,2$^+$ acceptor T cells to induce a second (resting) Lyt-2$^+$ population in carrier-primed spleen to actively suppress responses. These final suppressor cells are also carrier-specific in that they suppress responses only in the presence of the carrier. Studies presented will focus on the question of whether CTs participate in hapten-specific suppression induction.

189 FUNCTIONAL AND BIOCHEMICAL CHARACTERIZATION OF IMMUNOREGULATORY FACTORS: Mark C. Horowitz, Douglas R. Green, Donal Murphy and Richard Gershon. Yale University School of Medicine and The Howard Hughes Medical Institute, New Haven, Conn. 06510.

Neonatal spleen cells cultured for 6 days develop a unique immunological activity, distinguished by their ability to block suppressor T cell function. These cells have been termed contrasuppressors. Cell free supernatant of spleen cell cultures from neonatal mice contain a factor which mimics the cells activity. We have designated this material contrasuppressor factor (CSF). CSF delivers no direct helper activity to purified B cells nor any amplifying activity to Lyl^+ spleen cells. Augmentation of antibody production, in the presence of suppressor T cells is only seen with the addition of CSF.

Supernatant material contains at least two functional activities: 1) To reduce the effector phase of suppressor T cell activity and 2) Modulate the generation of suppressor T cells. T cells educated for suppression in the presence of culture supernatant are less suppressive and are more sensitive to contrasuppression. Whether these two activities are mediated by the same molecules is under investigation.

CSF activity is heat stable, resistant to RNase, does not bind to SRBC and appears to bear determinants encoded in the I-J subregion of the MHC.

Coexisting with CSF is a separable factor characterized by its capacity to suppress a primary in vitro anti-SRBC response. In contrast to CSF, suppressor factor can be absorbed on SRBC.

190 THYMOPOIETIN MODIFIES IMMUNOREGULATION IN MAN. Gideon Goldstein, Patrick C. Kung, Catherine Y. Lau, John Schindler and Tapan Audhya, Ortho Pharm. Corp., Raritan,NJ 08869.

Distinct immunoregulatory abnormalities have been defined in human diseases utilizing monoclonal antibodies reactive with differentiation antigens restricted to functional subsets of human T cells (OKT series). OKT4 reacts with inducer/helper T cells and OKT8 reacts with a distinct subset containing cytotoxic/suppressor cells. We term $OKT4^+/OKT8^+$ an immunoregulatory index. This index is elevated (relative reduction of suppressor cells) in the active phase of autoimmune diseases such as rheumatoid arthritis, multiple sclerosis, SLE, and primary biliary cirrhosis and depressed (relative increase in suppressor cells) in certain immunodeficiencies and infections. TP-5 (Arg-Lys-Asp-Val-Tyr), a biologically active synthetic fragment of the thymic hormone thymopoietin is immunoregulatory in a number of animal model systems and is now being shown to have similar effects in human disease with demonstrable immunoregulatory abnormalities. An interesting point is that TP-5 appears homeostatic, restoring the immunoregulatory state towards normal whether its initial perturbation is in the direction of excessive or deficient suppression.

191 INFLUENCE OF ULTRAVIOLET IRRADIATION AND PHORBOL ESTERS ON THE SHEDDING OF CELL SURFACE ALLOANTIGENS, J. Pretell, S. G. Emerson, R. K. Gershon and R. E. Cone

Previous studies in our laboratory have suggested that the requisite shedding and turnover of histocompatibility antigens may serve as an important means of communication between cells of the immune system. Several laboratories have shown that treatment of immunocompetent cells with glutaraldehyde, ultraviolet irradiation and phorbol esters inhibits the development of mixed lymphocyte reactions. Our initial studies suggested that glutaraldehyde fixation and UV irradiation of cells led to an apparent block in the shedding of lactoperoxidase-catalysed 125-I labelled Ia antigens. Recent studies, however, have shown that UV irradiation actually causes a rapid loss of both H-2K and Ia within minutes, which is then followed by a total block in shedding of the remaining surface antigens. Polyacrylamide gel analysis of the Ia antigens before and after UV irradiation reveals a rapid loss of both I-A and I-E with a predominance for the I-E remaining when compared to the mock UV-treated cells. Treatment of lymphocytes with the tumor promotor, 12-O-tetradecanoylphorbol-13-acetate (TPA), causes a continuous acceleration of the shedding of H-2 and Ia antigens with no effect on IgM. An analog of TPA, 4-O-methyl-TPA, which is inactive as a tumor promotor, has no effect on shedding. Pretreatment of the cells with TPA followed by its removal results in a block of the shedding of the remaining H-2 antigens. The use of agents which alter the turnover of surface antigens and receptors, whether reversibly or irreversibly, may be an important tool in dissecting out the necessary communication signals, either membrane-bound or in soluble form, which are required for immunoregulation.

192 MONOCLONAL ANTIBODIES TO HUMAN T LYMPHOCYTE SUBPOPULATIONS, Robert W. Knowles, Bai Yan, and Walter F. Bodmer, Imperial Cancer Research Fund, London WC2A 3PX, U.K.

Monoclonal antibodies have been produced which bind to subpopulations of human thymocytes and peripheral T lymphocytes. Three T cell lines, MOLT-4, CEM, and HSB-2, derived from patients with acute lymphocytic leukemia, were used for immunization. Spleen cells from immunized mice were fused with a mouse myeloma cell line. Hybrids were screened for the production of antibodies which bound to T cell lines but did not bind to lymphoid cell lines of B cell origin. Immunofluorescence was used to demonstrate that these antibodies bind to subpopulations of thymocytes and peripheral T lymphocytes.

193 ACCESSORY CELLS AND SUPPRESSOR T CELLS BEAR DIFFERENT I-J SUBREGION CONTROLLED DETERMINANTS, Donal B. Muprhy, Katsumi Yamauchi, Sonoko Habu, Diane D. Eardley, and Richard K. Gershon, Yale University, New Haven, Connecticut 06510.

Studies of the I-J subregion of the murine H-2 gene complex have shown that this chromosomal segment controls determinants expressed in immunocompetent cells and associated with immunoregulatory molecules. The number of different antigens controlled by the I-J subregion has not been fully resolved. Data presented in this report show that T cells involved in the generation of suppressor activity bear an I-J determinant not found on non T:non B accessory cells, and vice versa. T cells examined include the Ly-1 inducer and Ly-1,2 acceptor T cells in the in vitro SRBC feedback suppression system. Non T:non B accessory cells examined include accessory cells involved in concanavalin A induced T cell proliferative responses and in in vitro antibody responses to SRBC. These results provide evidence for genetic and serologic complexity of the I-J subregion.

194 IMMUNOGLOBULIN (Ig) GENE RESTRICTED AUGMENTATION OF IN VITRO SECONDARY ANTIBODY RESPONSE BY NOVEL LYMPHOCYTE (Thy-1$^-$, Lyt-1$^+$), Ko Okumura, Kyoko Hayakawa and Tomio Tada Department of Immunology, Faculty of Medicine, University of Tokyo, Tokyo, Japan

Newly discovered lymphocyte subpopulation ("T"h3: Thy-1$^-$, Lyt-1$^+$) which tends to adhere to nylon wool column and anti-mouse immunoglobulin (Ig) coated Petri dish was revealed to augment in vitro secondary anti-DNP antibody response in the presence of limited number of carrier(KLH) primed helper T cells of either nylon wool column passed (Th1: Thy-1$^+$, Lyt-1$^+$, I-J$^-$) or nylon wool adherent (Th2: Thy-1$^+$, Lyt-1$^+$, I-J$^+$) cell fraction. The augmentation was only observed when the Ig-allotype of "T"h3 source was matched with that of DNP-primed B cell source (prepared by the treatment with the mixture of anti-Thy-1 and anti-Lyt-1 plus C). Namely, the addition of "T"h3 from unprimed CSW(Igj/j) could augment anti-DNP response of CSW but not that of Ig-allotype congenic CWB(Ig$^{b/b}$) when cocultured with limited number of KLH-primed helper T cells (Th1 or Th2) from either CSW or CWB. Experiments utilizing other Ig-allotype congenic mice, such as BALB/c (IgCH$^{a/a}$, IgVH$^{a/a}$), C.B20 (IgCH$^{b/b}$, IgVH$^{b/b}$) and BAB/14 (IgCH$^{b/b}$, IgVH$^{e/a}$), suggested that the identity of both IgCH- and IgVH-gene between "T"h3 and B cell source was required for the efficient augmentation, however, the marginal augmentation was also observed when only IgVH-gene of "T"h3 and B cell was identical. This augmenting activity of "T"h3 was abolished by the cytotoxic treatment with anti-Lyt-1 but not with anti-Lyt-2, anti-Ia and anti-ThB. Furthermore, such "T"h3 subpopulation was also found in the spleen cells from athymic nude (nu/nu) mouse.

195 REGULATORY T CELL INTERACTIONS INVOLVED IN SUPPRESSION OF DNFB ACTIVATED DELAYED-HYPERSENSITIVITY T CELLS (T_{DH}), Stephen D. Miller and Henry N. Claman, University of Colorado Medical Center, Denver, CO 80262

Our past work has shown that the i.v. injection of syngeneic DNP-modified lymphoid cells (DNP-LC) leads to specific unresponsiveness of the T cell-mediated DNFB contact sensitivity response in mice. This unresponsiveness is due, in part, to the activation of antigen-specific suppressor T cells in the spleen and lymph nodes of tolerant mice 7 days post tolerization. These Ly 2,3$^+$, I-J$^+$ Ts have been shown to block the efferent limb of sensitivity as measured by their ability to suppress the passive transfer of sensitivity mediated by syngeneic DNFB immune T cells (T_{DH}) in cotransfer experiments. Based on the failure of Ts to suppress DNFB-immune T_{DH} derived from cyclophosphamide (Cy)-treated donors, we have shown that this suppressive circuit requires the presence of an antigen-activated, Cy-sensitive, I-J$^+$ T cell which we have termed the auxiliary Ts (Ts-aux) cell. Recent experiments have shown that the interaction between the Ts and Ts-aux cell population is both hapten-specific and MHC restricted. In addition, this interaction is restricted by genes linked to the Igh-1 allotype locus, and the Ts-aux population is sensitive to lysis with mouse anti-DNP serum (idiotype) and C'. However, once the suppressor circuit is activated, it appears that the final suppressive signal is neither hapten, MHC, or allotype restricted. The data indicates that the Ts focus to passively bound DNP-H-2K/D complexes associated with the T_{DH} population, Ts-aux then appears to interact with Ts via an idiotype-anti-idiotype linked interaction to deliver the final nonspecific suppressive signal. (Supported in part by NIH-USPHS Grants AI-12685 and AI-14913.)

196 A FUNCTIONAL REGULATORY CIRCUIT CAN BE INDUCED TO CONTROL GROWTH OF THE MURINE T-CELL LYMPHOMA, RLO$^{\circ}$1, J. Buxbaum, R. Basch, S. Zolla-Pazner, Departments of Medicine and Pathology, Manhattan V.A. Medical Center and N.Y.U. Medical School, New York, N.Y. 10010. 10^6 RLO$^{\circ}$1 tumor cells, or their cloned, cultured counterparts injected subcutaneously, kill 90% of female BALB/c recipients. Cells selected in vitro for the absence of surface Thy 1.2 kill only 20% (Somatic Cell Genetics 3:1). Animals surviving inoculation with the Thy negative cells are protected against a subsequent lethal challenge with the positive cells (J. Immunol. 125:673). The sera of the protected animals contain IgM antibody which is cytotoxic for the Thy(-) cell, the Thy(+) cell and normal helper T-cells. Spleen cells obtained from the protected animals show little cell mediated cytotoxicity against the tumor cells. They respond normally to all T and B cell mitogens and are fully active in in vitro suppression systems. However, they show a 30% reduction in MLR response and fail to provide anti-SRBC help as measured by IgG plaque formation. We suggest that the Thy 1 negative cells stimulate the production of an IgM autoantibody directed against a helper T-cell antigen (TH_1). The antibody prevents the host from undergoing the switch from cytotoxic IgM to possibly enhancing IgG antibody formation, thereby negatively regulating its own growth. The Thy 1.2 positive cell does not induce a similar respone either because the Thy 1 antigen itself is immunosuppressive, as suggested by Miller and Esselman, or because its presence in the tumor cell membrane blocks recognition of the relevant antigen. The availability of cells variant in the expression of specific cell surface antigens has allowed the delineation of a new lymphoid differentiation antigen and an additional class of lymphoid regulatory circuit.

197 DECREASED ABILITY TO DEMONSTRATE SUPPRESSOR T CELL ACTIVITY DURING TOLERANCE, D. Elliot Parks, Scripps Clinic and Research Foundation, La Jolla, CA 92037

Antigen-specific suppressor T lymphocytes can be induced during the establishment of immunologic unresponsiveness in helper T and B lymphocytes by the soluble protein antigen, human gamma globulin (HGG). Suppressor cells appear soon after tolerization in A/J mice given a single injection of 2.5 mg of deaggregated HGG as detected by in vivo adoptive cell transfer with normal spleen cells or by in vitro co-culture with HGG-primed spleen cells. However, these suppressor cells appear to be only transiently associated with this tolerant state. In contrast with antigen-specific B cells which remain completely unresponsive for at least 7 weeks and helper T cells which remain unresponsive for more than 6 months, suppressor cells cannot be detected in vivo or in vitro 5 weeks after the induction of tolerance. When attempts are made to reinduce suppressor cell activity by a second injection of 2.5 mg of deaggregated HGG into tolerant animals, the ability to reinduce suppressor cells decreases as the initial, transient suppressor cell activity diminishes. By 9 weeks following tolerization, mice appear to regain the ability to generate suppressor cells upon the reinjection of deaggregated HGG. These data suggest 1) that the maintenance of the unresponsive state to HGG is not dependent upon the presence of detectable levels of antigen-specific suppressor cells and 2) that the ability to induce suppressor cells diminishes as the unresponsive state deepens. This diminished capacity to generate active suppression resulting from a disruption of the suppressor T cell circuit could reflect the inactivation of mature suppressor cells, their precursors, or their inducers by mechanisms comparable to those which induce tolerance in helper T cells.

198 CONTRASUPPRESSION CAN CONVERT A TOLEROGEN INTO AN IMMUNOGEN. D.R. Green, W. Ptak, R.K.Gershon. Yale U. Sch of Med., and Howard Hughes Medical Institute at Yale, New Haven, Ct 06510 and Copernicus Sch of Med., Cracow, Poland.

A cell which bears the surface phenotype $Ly1^+2^-;I\text{-}J^+$ and adheres to the lectin V. villosa, has the specialized capacity to block the expression of activated suppressor cells. Six day cultures of spleen cells from neonatal animals yield high numbers of these contrasuppressor cells. Previous work had shown the potent activity of these cells in in vitro systems. In order to examine their in vivo relevance, we asked if injection of these contrasuppressor cells could change a tolerogenic signal into an immunogenic one. TNP coupled to peritoneal exudate macrophages acts as a potent tolerogen for delayed type hypersensitivity when injected intravenously. We injected TNP-macrophages into adult CBA mice, i.v. with or without cultured neonatal CBA spleen cells. Animals injected with TNP-macrophages alone were completely unresponsive to subsequent challenge by skin painting with picryl chloride. Animals which had also received cultured neonatal cells were fully immunized, showing responses as high as those of normally sensitized animals. The immunity was specific for the antigen presented on the macrophage, and the cultured cell responsible for the conversion to immunity was an $I\text{-}J^+$, V.villosa adherent T cell. Thus, an effector of contrasuppression can convert the tolerogenic signal produced by i.v. injection of antigen coupled macrophages into a potent immunogenic one in vivo. In addition, these findings indirectly supports the notion that suppressor cells are involved in the induction of tolerance in this system.

199 DISSECTION OF THE MOLECULAR COMPONENTS OF AN Ly-1 CELL SUPPRESSOR INDUCER "FACTOR". K. Yamauchi, D.B. Murphy, and R.K. Gershon. Yale University Sch of Med., Dept. of Pathology, Howard Hughes Medical Institute at Yale University, New Haven, Ct. 06510.
The T cells and their cell free products that are involved in suppressing antibody responses are organized into a series of interactions that have been referred to as "the suppressor circuit". In this circuit, activated Ly-1;I-J^+; Qa-1^+ cells (suppressor inducer cells) activate a resting population of Ly-1,2; I-J^+;Qa-1^+ cells (suppressor acceptor cells) to generate antigen specific suppression. Cultured suppressor inducer cells release cell free material (Ly-1 TsiF) into the culture supernatants that can replace the cells function. Communication between Ly-1 TsiF and its acceptor cells is controlled by genes linked to the variable region of the heavy chain immunoglobulin locus. Ly-1 TsiF can be separated into two different moieties; one that binds antigen and is I-J^+ and another that does not bind antigen and is I-J^-. Neither the I-J^- moiety nor the I-J^+ moiety can suppress antibody responses. A mixture of these two, however, yield suppressive activity. The question whether both moieties are released by the same cell has not yet been resolved. However, since horse red blood cell specific I-J^- material interacts equally well with I-J^+ material from either sheep red blood cell or horse red blood cell immunized Ly-1 cells it is clear that two different cells are at least capable of combining products to make a single multi-chain antigen specific suppressor inducer factor.

200 T-T CELL INTERACTION IN H-2 LINKED IR GENE CONTROLLED SUPPRESSION TO HEN EGG-WHITE LYSOZYME. Barbara A. Araneo, Robert L. Yowell and Eli E. Sercarz. UCLA Los Angeles, CA. 90024.
Footpad priming of nonresponder, B10, mice with hen egg-white lysozyme (HEL) initiates a T helper cell population in the draining LN whose effector function can be blocked by antigen-specific suppression. Suppressor cell activity in the lymph node is dependent upon collaboration between two physically distinct T cell subsets. The first cell type has been operationally defined as the T-suppressor inducer (TsI) and is distinguished from the helper T cell by I-J alloantigen expression. The second cell type, provisionally named the suppressor effector, gains functional competence later after immunization, e.g. 21 days; this antigen-specific suppressor T cell bears the same predominant idiotype as that found on secreted antibody. The I-J^+ inducer not displaying this idiotype is responsible for the functional activation of the Ts. Thus, the antigen-specific helper and the suppressor effector are subsequently regulated by the I-J^+ inducer. Furthermore, the Ts effector may be derived from a splenic population since a potent HEL specific Id^+ suppressor can be obtained from primed splenic T cells.

201 SUPPRESSOR AND EFFECTOR CIRCUITS IN THE AZOBENZENARSONATE (ABA) SYSTEM, Mark I. Greene, Ira J. Fox, Joathan S. Bromberg and Man-Sun Sy, Harvard Medical School, Boston, MA 02115
The ability to divert a response into a suppressor or an effector mode has been studied. Ligand coupled cells can be used to activate antigen-reactive cells of either suppressor or effector type. The cell surface receptor on Ts is idiotypic, since the cells can be lysed by anti-idiotypic antibodies and complement, whereas one type of in vivo effector T cell, namely the T_{DH} cell, cannot be inactivated by similar treatment. The Ts ligand induced cell (Ts_1) requires two distinct types of signals. The first signal is ligand in association with certain H-2 encoded structures. The second is a differentiative activation-type signal that can be delivered by an anti-I-J allogeneic effect. This anti-I-J allogeneic effect and ligand activated idiotypic Ts cell is Lyt $1^+2^-3^-$, and appears to induce or help the generation of anti-idiotypic Ts_2 type cells. The generation of certain effector T cells specific for ligand requires presentation of ligand on viable I-A+ antigen presenting cells (APC). However, the presentation of ligand on I-A+ APCs treated briefly in vitro with ultraviolet (UV) irradiation renders them incapable of activating T cells participating as T proliferating (T_P) or T_{DH} reactions but can still activate Ts. Thus ligand I-A antigen signaling for effector cell activation also requires an additional signal abrogated by UV treatment. It appears, therefore, that predeliction for suppressor circuits or effector circuits may be determined by discrete types of second signals independent of ligand antigen receptor interactions.

202 SUPPRESSOR T CELLS INDUCED BY A SUBOPTIMAL DOSE OF ANTIGEN PLUS AN I-J SPECIFIC ALLOGENEIC EFFECT, Jonathan S. Bromberg, Man-Sun Sy and Mark I. Greene, Harvard Medical School, Boston, MA 02115

Spleen cells derivatized with trinitrobenzene sulfonic acid (TNBS) at concentrations of 1 mM or 10 mM and injected syngeneically i.v. can both tolerize a recipient mouse and induce a set of suppressor cells (T_S) which can suppress the contact sensitivity response to TNP in an MHC unrestricted manner. Spleen cells derivatized with 0.01 mM TNBS cannot tolerize a recipient mouse; however, they can induce a set of cells which, under the influence of an allogeneic effect, can suppress in an MHC unrestricted manner. Thus, 0.01 mM TNBS-BALB/c → BALB/c induces cells which can transfer suppression to CBA, B10, or BALB/c (provided additional allogeneic cells are given to these latter recipients to induce an allogeneic effect). The allogeneic effect has been mapped to I-J. Allogeneic effects at K, D, I-A, or Mls are unable to provide a suitable activational or differentiational signal to the suppressor cell. The cell is antigen specific and is Thy-1^+. $P \rightarrow F_1$ and $F_1 \rightarrow P$ studies have shown that it is necessary and sufficient for the allogeneic effect to be directed solely against the T_S; bystander allogeneic effects are ineffective in causing activation. These results have been extended to the azobenzenearsonate (ABA) system, where response to this antigen is under the control of a major idiotype (CRI) in the A/J mouse. The T_S induced by lightly haptenated (1.26 mM ABA) spleen cells are exactly analogous to those in the TNP system. In addition, these cells have been shown to be Ly 1^+2^-, to suppress the afferent but not efferent limb of the immune response, and to be allotype restricted. We suggest that I-J mediated signals may be an effective way of modulating immune responsiveness.

203 THE CELLULAR BASIS OF NEONATALLY INDUCED TOLERANCE TO SOLUBLE ANTIGENS, Bruce Acres and Alastair Cunningham, Ontario Cancer Institute, 500 Sherbourne Street, Toronto, Canada M4X 1K9.

Tolerance to either of two soluble antigens was induced in mice by neonatal injection. At specific intervals during the time that mice were tolerant, lymphoid tissues were assayed for specific regulatory cells, T helper cell function and B cell function.

With both antigens, splenic B cell function was diminished several fold for the duration of tolerance. Tolerant mice were shown to have a specific T helper cell dysfunction, also for the duration of tolerance. Spleen cells and thymus cells of tolerant mice were shown to contain specific suppressor cells only if the tolerant mice were more than six weeks old.

Experiments to determine the role of thymus cells in the induction and maintenance of neonatally induced tolerance will be discussed.

204 FUNCTIONAL NEUTRALIZATION OF ANTIGEN-SPECIFIC SUPPRESSOR T CELLS BY IDIOTYPE-SPECIFIC SUPPRESSOR T CELLS. Byung S. Kim, Northwestern Univ. Medical Sch. Chicago, IL 60611

Specific tolerance to phosphorylcholine (PC) can be induced in BALB/c mice by neonatal injections with either PC-containing antigen (100 ug of pneumococcal C-polysaccharide) or anti-T15 idiotype antibody recognizing the major idiotype of anti-PC antibody. Spleen cells from these tolerant mice exhibited T-cell mediated active suppression of anti-PC response when they were co-cultured with normal spleen cells. The possible interaction of the two different types of suppressor cells was examined by co-culturing normal spleen cells (1×10^7 cells/culture) with a mixture (1×10^7 nylon wool-purified T cells) of the different suppressor T cell types in varying cell ratios in the presence of T-independent PC-antigen, R36a. Although the cell number of one suppressor cell type required to neutralize the other differed depending on the individual mice, exposure of antigen-induced suppressor T cells to anti-idiotype-induced suppressor cells resulted in a complete cancellation of their suppressor function. Further analyses of the specific receptors of these suppressor T cells using either PC- or T15-coated plates revealed that receptors of antigen-induced suppressor T cells recognize PC, while receptors of anti-idiotype-induced suppressor T cells react with the idiotype. These results suggest that network regulation may also occur among suppressor T cell populations.

205 GENERATION OF SUPPRESSOR T CELLS AND CELL LINES BY HAPTEN-MODIFIED SELF, Timothy Darrow, Mark Ling, and David W. Scott, Duke Medical Center,Durham, N.C. 27710
We have previously demonstrated that suppressor T cells can be elicited by exposure to hapten-modified self (HMS) H-2 molecules. Such HMS-induced T suppressor cells act at an early stage of the humoral immune response and are hapten-specific. Whether idiotype-specific suppressor cells are generated in this system is under investigation. HMS-induced suppressor cells do not appear to be H-2 restricted, although the recognition of H-2K or D-end moieties is required for their generation. In analogy to studies in the delayed hypersensitivity system, hapten-modified allogeneic cells induce suppressors restricted to the strain of the stimulators. More definitive analysis of the restriction and mechanism of action of such suppressor cells is being sought by the preparation of long-term T cell lines or somatic cell hybrids of these specific suppressor cells. Once cloned, these suppressor lines will allow detailed analysis of the factors and cell interactions involved in the regulation of the humoral response. (Supported by U. S. P. H. S. grant no. CA-22845).

206 EVIDENCE THAT ANTIGEN "PROCESSING" BY SPLENIC ADHERENT CELLS IS REQUIRED TO TRIGGER THE HELPER T CELL PATHWAY INVOLVED IN CYTOLYTIC T LYMPHOCYTE INDUCTION. Steven J. Burakoff, Ofra Weinberger, Steven Herrmann, Matthew F. Mescher. Sidney Farber Cancer Institute and Harvard Medical School, Boston, MA 02115
Purified H-2K^k antigen incorporated into liposomes has been used to evaluate the helper T cell pathway involved in allogeneic cytolytic T lymphocyte (CTL) induction. It has been demonstrated that H-2K^k antigen pulsed to Ia positive splenic adherent cells (SACs) (syngeneic to the responder) stimulate CTL 10-20-fold more efficiently than if H-2K^k antigen is added directly to culture, suggesting that Ia positive SACs are involved in antigen presentation. SACs appear to present antigen to an Ly 1$^+$2$^-$ helper T cell that produces a helper factor, interleukin II. This factor provides one of the signals required for CTL activation. In order for SACs to trigger helper T cells, SACs must interact for at least 12 hrs with the H-2K^k antigen. If the SACs are inactivated by UV irradiation after 12 hrs of interaction, they are still able to stimulate helper T cells if phorbol myristic acetate (PMA) is added to culture. Preliminary experiments suggest that membranes from syngeneic Ia positive SACs that have interacted with the H-2K^k alloantigen for 12-24 hrs can also trigger helper T cells in the presence of PMA. If the helper T cell pathway is bypassed, CTL can be triggered by Ia positive or Ia negative SACs that have been inactivated by UV irradiation prior to their interaction with antigen. These results suggest that helper T cells see "processed" antigen in the content of self Ia antigens, while there is no requirement for antigen processing in order to trigger CTLs.

207 IDIOTYPE ANTI-IDIOTYPE INTERACTIONS IN THE REGULATION OF DTH RESPONSE TO AZOBENZENE-ARSONATE (ABA) CONJUGATED SPLEEN CELLS (SC). Man-Sun Sy, Baruj Benacerraf, Muneo Takaoki, and Mark I. Greene. Harvard Medical School, Boston, MA 02115.
Subcutaneous injection of ABA-SC induces a T cell mediated delayed type hypersensitivity (DTH) response. The administration of ABA-coupled cells intravenously (i.v.) leads to the generation of cross-reactive idiotype (CRI) bearing first order suppressor cells (Ts_1). CRI bearing Ts cells can be lysed with treatment with anti-idiotype and complement. It is possible to induce second order Ts (Ts_2) by either administration of CRI+ I-J+ factor (TsF) derived from ligand binding CRI+ Ts_1, to naive mice, or alternatively to couple CRI antibody to cell surfaces and administer such coupled cells intravenously. Such induced Ts are termed anti-idiotpic ($\propto$ ID) based on their binding to idiotype coated plates, but not ligand coated plates. In vitro treatment of the DTH effector T cells (T_{DH}) with anti-idiotypic antibodies and complement does not diminish their ability to transfer ABA-specific T cell dependent immunity to naive syngeneic recipients. However, after such treatment the same population of T_{DH} cells becomes refractory to suppression by anti-idiotypic second order suppressor T cells (Ts_2) as demonstrated in a co-transfer experiment. Therefore, an idiotype bearing T cell (Ts_3), which interacts with id Ts_2, resides in the immune lymph node population and is absolutely required for the expression of Ts_2 function. Furthermore, in mixing experiments, it was found that the interactions between Ts_2 and Ts_3 is restricted by genes linked to the Igh-1 locus. Nevertheless after such interactions have occurred, the final suppression appears to be idiotype nonspecific.

208 REGULATORY ROLE OF ABA-SPECIFIC HELPER AND SUPPRESSOR T CELLS IN THE IN VITRO ANTIBODY RESPONSE TO ABA-PROTEINS. Gino Doria and Luciano Adorini,Laboratory of Radiopathology, C.S.N. Casaccia (Rome) Italy.

An in vitro system has been developed to analyze the immunoregulatory circuits operating in T cell-dependent antibody responses to ABA-proteins. Popliteal lymph node cells from ABA-KLH footpad-primed mice when cultured in vitro with TNP-ABA-KLH give a sizeable anti-TNP PFC response. Spleen cells from ABA-MouseIgG primed mice not only fail to give anti-TNP PFC response when stimulated in vitro with TNP-ABA-KLH but also suppress the anti-TNP response of lymph node cells from ABA-KLH primed mice. The induction of suppressor cells is dependent on the dose of ABA-MouseIgG injected and is ABA-specific. The T cell nature of suppressor cells was demonstrated by their enrichment after passage over anti-MouseIg coated plates. Spleen cells from ABA-MouseIgG primed mice suppress the anti-ABA PFC response of lymph node cells from ABA-KLH primed mice stimulated in vitro with TNP-ABA-KLH or ABA-KLH. Experiments with separated and recombined helper, suppressor, and B cells induced by ABA-proteins indicate that ABA-primed B cells need to be present in culture to obtain suppression of the anti-TNP response. These findings establish the conditions to raise in vitro antibody response to ABA-proteins and to analyze immunoregulation by ABA-specific cell populations as mediated by antigen and idiotype recognition.

209 IDIOTYPE SPECIFIC SUPPRESSION OF PLAQUE FORMING CELL RESPONSES, David H. Sherr, Shyr-Te Ju, Judah Z. Weinberger, Baruj Benacerraf and Martin E. Dorf, Harvard Medical School, Boston, MA 02115

The ability of suppressor cells induced by the i.v. administration of 4-hydroxy-3-nitrophenyl acetyl (NP) modified syngeneic cells to reduce an idiotypic B cell response was studied in both an in vivo and an in vitro system. Idiotype positive B cells were assayed by the ability of guinea pig anti-idiotypic antiserum to specifically inhibit idiotype positive plaque formation. It was found that up to 57% of the PFC response in vivo and 100% of the PFC response in vitro was inhibitable with anti-idiotypic antiserum. The expression of these idiotype positive B cells could be suppressed by the transfer of spleen cells from mice treated 7 days previously with NP coupled syngeneic cells. T cells are both required and sufficient for the transfer of idiotype specific suppression. The induction of these idiotype specific T suppressor cells directly with antigen suggests that recognition of unique determinants on cell surfaces is important for regulation of lymphoid cell interactions.

Network Regulation by Extrinsic Idiotype Manipulations

210 SPONTANEOUS APPEARANCE OF AUTO ANTI(ANTI A48Id) ANTIBODIES(Ab_3) LEADS TO THE ACTIVATION OF A SILENT CLONE, Constantin A. Bona, Department of Microbiology, Mount Sinai School of Medicine, New York, N.Y. 10029.

Syngeneic anti A48Id(Ab_2), anti(anti A48Id)(Ab_3), and anti[anti(anti A48Id)] (Ab_4) antibodies, have been prepared in BALB/C mice. The analysis of this series of complementary anti Id antibodies showed a lack of symmetry in the network system. While Ab_2 and Ab_4 react with Id determinants of $\beta(2 \rightarrow 6)$ fructosan binding ABPC48 myeloma protein, Ab_3 does not bind bacterial levan. There is a striking difference in the affinity of Ab_2 and Ab_4 for A48Id determinants. While the binding of ^3H-Ab_3 to Ab_2 was inhibited with 10ng of Ab_1, the binding of ^3H-Ab_4 was inhibited with 3µg Ab_1. Four out of five BALB/C mice in response to immunization with A48 showed the appearance of Ab_2 followed by Ab_3. An inverse fluctuation was observed between the production of Ab_2 and Ab_3 indicating that Ab_1 induced the synthesis of Ab_2 and then that Ab_2 elicited the production of Ab_3. The appearance of Ab_3 suppressed the synthesis of Ab_2. Interestingly, the spontaneous appearance of auto Ab_3 in response to immunization with Ab_1, or intentional induction of Ab_3 in response to immunization with Ab_2 was followed by activation of $A48Id^+$ anti $\beta(2 \rightarrow 6)$ fructosan clone(s). This clone(s) is not activated in normal BALB/C mice as well as in Ig-1^a congenic or recombinant mice strains in response to immunization with bacterial levan or bacterial levan conjugates.

The only other situations in which we have observed the appearance of measurable amounts of the A48Id in anti BL antibodies were in the response to BL of nude mice that have been previously treated with anti E109IdX antibodies and in 1 day old mice injected with 0.01µg anti A48Id antibodies.

It seems likely that two mechanisms contribute to the regulation of the $A48Id^+$ anti BL response: activation of precursor by Ab_2 and elimination of Ab_2-suppressor T cells by Ab_3.

211 THE IMMUNE RESPONSE TO THE VARIABLE (V) DOMAINS OF MYELOMA PROTEIN 315 IS CONTROLLED BY H-2 LINKED GENES, K.Hannestad and T.Jørgensen, Institute of Medical Biology, University of Tromsø School of Medicine, Tromsø, Norway.

The present study was aimed at learning more about recognition of V regions by T helper cells (Th). For this purpose we have used the V-domains of the IgAλ2 myeloma protein produced by the BALB/c plasmacytoma MOPC 315 as carriers for anti-hapten (NIP) responses. Various strains of mice were injected with 100 μg of V_H-315 or V_L-315 in CFA (Th priming) and 200 μg of NIP-BSA (B-cell priming). The primed cells were transferred to irradiated recipients which were boosted with 200 μg NIP_2-Fab-315 in saline. The strains that developed high anti-NIP responses were defined as responders; those strains in which the anti-NIP response (Farr assay) was very low were considered as non-responders to the carrier. Mice of the d (BALB/c, DBA/2), b (C57BL) and s haplotypes were responders to V_L-315, while mice of the k (CBA,C3H,AKR) haplotype were non-responders. A different genetic pattern was observed for Th responses to V_H-315: Mice of the k and s haplotypes were responders, whereas mice of the b and d haplotypes were non-responders. (C3HxBALB/c)F1 mice (kxd) were responders to both domains. In contrast to the distinct response patterns to the isolated domains, all strains were responders to Fv which embodies both the V_H and the V_L domains. Mice of the congenic strain BALB.K ($H\text{-}2^k$) responded to V_H-315 and did not respond to V_L-315, thus behaving like mice of the k haplotype. These results provide strong evidence that distinct genes within MHC control the Th responses to these V-domains. The nature of the V-domain epitopes recognized by the Th is probably related to idiotypes. Evidently, the lesser antigenic complexity of individual domains offers certain advantages in the dissection of the immunogenicity of complete Igs. We propose that the Th primed with individual V-315 domains encounter and recognize the same domains in an assembled form when the animals are boosted with NIP-Fab-315, and that these Th focus on immunogenic sites shared by free and assembled V-regions. According to this hypothesis each member of the M315 pair of V-domains contains sufficient structural information by itself to be recognized by Th. The V-region specific Th of the present study may be envisioned to be able to operate directly on the Ig receptors of B lymphocytes like the idiotype specific set of Th. Hence, V_H and V_L may also function as independent targets in the idiotype network, and the same Th may be able to perform both carrier- and idiotype-specific help.

212 ANTI-IDIOTYPE ANTIBODIES REGULATE CONTACT HYPERSENSITIVITY BY TWO DISTINCT MECHANISMS, John W. Moorhead, University of Colorado Medical School, Denver, Colorado 80262.

Contact hypersensitivity to DNFB in mice is maximal 6 days after sensitization but declines rapidly. We have shown that this rapid decline in immunity is due to anti-idiotypic antibodies produced by the host (J.I. 123:1979). We have investigated the mechanism(s) of this regulation using an auto-anti-idiotypic antiserum (anti-Id) raised in BALB/c mice by repeated immunization with DNFB-immune lymph node (LN) T cells from BALB/c mice. The activity of this antiserum is specifically adsorbed by and can be recovered from columns conjugated with affinity-purified mouse anti-DNP antibody, thus establishing its anti-idiotypic specificity. Treating DNFB-immune LN cells with this anti-Id, with or without complement (C), inhibits the transfer of sensitivity to naive recipients. Inhibition of transfer by anti-Id without C requires Ia^+ T cells in the immune population. Depleting the immune LN cells of Ia^+ cells rendered them insensitive to inhibition by anti-Id alone although the same population was inhibitable by anti-Id plus C. This cell population could be rendered sensitive to inhibition by anti-Id alone by addition of untreated DNFB-immune LN cells. These results indicate that anti-Id antibodies regulate the effector limb of the contact hypersensitivity response by two distinct mechanisms. One involves complement-mediated lysis of the effector T cells. The second mechanism, which is complement independent, appears to be mediated by anti-Id activation of Ia^+ T cells in the immune population. This latter subset is most likely analogous to the Ts-auxiliary cells we have previously identified in the DNFB-immune population (J.E.M. 149:1979). Supported by USPHS Grant AI-12993.

213 EXPRESSION OF A MAJOR CROSS-REACTIVE IDIOTYPE (CRI) IN ANTIBODIES OF BALB/c MICE TO P-AZOPHENYLARSONATE: RELATIONSHIP OF THE BALB/c CRI WITH A MINOR CRI OF A/J MICE, Alan R. Brown, Edmundo Lamoyi and Alfred Nisonoff, Rosenstiel Research Center, Brandeis University, Waltham, MA 02254

A major cross-reactive idiotype (CRI_C) defined by heterologous rabbit anti-idiotype (anti-id) is present in antibodies to p-azophenylarsonate (Ar) of BALB/c mice. All BALB/c mice tested express CRI_C; in individual mice 20%-65% of BALB/c anti-Ar antibodies bear CRI_C determinants. Inheritance of CRI_C is linked to the Igh-1^a allotype group (based on congenic strain analysis); however, not all strains with this allotype express CRI_C. In addition, CRI_C is expressed in some strains of other allotype groups. Serological studies showed that CRI_C is completely unrelated to the major CRI expressed in A/J strain mice (CRI_A), but is related idiotypically to a minor CRI population (5-10% of the anti-Ar response) in A/J mice. Expression of CRI_C can be suppressed in adult BALB/c mice by pretreatment with anti-CRI_C, but not by treatment with an anti-id prepared against an A/J CRI_A(+) hybridoma. CRI_C expression in BALB/c could be suppressed, however, by treatment with antiserum raised against A/J serum anti-Ar which contained anti-id specific for the minor CRI of A/J as well as the major CRI. CRI_A and CRI_C expression in A/J and in F_1(A/J X BALB/c) mice should be a useful model for studying immunoregulation. Further analysis of these idiotypes specific for the same hapten may enhance understanding of the interrelationships and scope of immune networks. (Supported by grants AI-12907 and AI-12895 from the NIH.)

214 IDIOTYPE EXPRESSION IN MICE TREATED WITH ANTI-H-2 ANTI-IDIOTYPIC ANTIBODIES, Jeffrey A. Bluestone, Suzanne L. Epstein, Susan O. Sharrow, Keiko Ozato, and David H. Sachs, Immunology Branch, National Cancer Institute, NIH, Bethesda, MD 20205

The effects of in vivo treatment of mice with purified xenogeneic anti-idiotypic antibodies directed against two IgG2a monoclonal anti-H-$2K^k$ antibodies have been investigated. BALB/c mice immunized against H-$2K^k$ antigens by conventional means did not produce detectable levels of either idiotype as tested in a hemagglutination inhibition assay. However, similar animals treated with anti-idiotypic antibodies produced high titers of idiotype positive immunoglobulin. The idiotypes expressed by these treated mice were identical or closely related to those of the original monoclonal anti-H-$2K^k$ antibodies, although they were found to be present on immunoglobulins of both IgG1 and IgG2 subclasses. Assessment of H-$2K^k$ antigen binding activity by flow cytofluorometry indicated that antigen-binding and nonantigen binding idiotype-positive molecules could be induced by anti-idiotype treatment. The genetics of idiotype production in anti-idiotype treated mice has also been studied. Linkage of this response to the heavy chain allotype linkage group was determined by a formal backcross analysis. Furthermore, BABL/c, B.C8 and BAB.14 (Igh-V^a) mice all produced the idiotype, while C57BL/6, C.B20 (Igh-V^b), and A.TL (Igh-V^e) mice failed to do so, suggesting that idiotype expression in anti-idiotype treated mice was controlled by V_H-linked structural genes.

It thus appears that the effects of in vivo administration of heterologous anti-idiotype in animals of appropriate Igh genotype can lead to the production of antibodies directed against an H-2 antigen to which the animals have never been exposed. These findings may have important applications in attempts to manipulate the immune response to H-2 antigens.

215 INDUCTION AND REGULATION OF SILENT IDIOTYPE CLONES. D.Juy,D.Primi, P.-A.Cazenave Institut Pasteur,Unité d'Immunochimie Analytique,28 rue du Dr.Roux,75015 PARIS.

Allotype linkage of idiotype expression in the course of the immune response has been described in several systems.The demonstration that idiotypic manipulation can result in the expression of normally silent idiotypes indicates that the allotype linkage phenomenon has to be explained by regulatory mechanisms.In these studies we have followed the expression of anti-TNP M460 idiotype in B cells of various strains of mice cultured for a prolonged period of time under low density conditions in the presence of optimal mitogenic doses of LPS.This approach takes advantage of the polyclonal B cell activator LPS's ability to reveal the total V gene repertoire of B cells. The M460 idiotype is normally expressed on 20% of the anti-TNP antibodies induced by TNP antigens in mice carrying the $Igh1^a$ locus. Our results show the following:1)B cells of all strains of mice cultured in the absence of T cells synthetized anti-TNP plaque forming cells (anti-TNP-PFC)a part of which was inhibitable by syngeneic anti-M460 antibodies.2) anti-idiotypic antibodies play an important role in B cell clonal regulation. This was shown by the fact that B cells of BALB/c ($Igh1^a$) or DBA/2 ($Igh1^c$) cultured in the presence of anti-M460 hybridoma cells showed a decrease of the number of anti-TNP PFC expressing M460 while the total anti-TNP response remained unaffected. On the other hand cultures of B cells from anti-M460 treated mice showed an enhancement of the number of the PFC 460^+.An increase of the M460 clonotype was also obtained by adding anti-anti-M460 antibodies to B cell cultures from normal mice.Taken together these results show that allotype linkage of the M460 idiotype expression is related to regulatory mechanisms and that even in the absence of T cells B cells are competent to exhert regulatory functions mutually controlling clonal expression.

216 IDIOTYPE-SPECIFIC T-CELL INHIBITION OF MOPC-315 SECRETION IN VITRO. Gary L. Milburn and Richard G. Lynch, Washington Universtiy School of Medicine, St. Louis, MO 63110

BALB/c mice immunized with the IgA anti-TNP protein (M315) produced by plasmacytoma MOPC-315 develop idiotype (Id^{315})-specific suppressor T cells that inhibit M315 secretion from MOPC-315 cells *in vivo* (J.Immunol. 122:2011, 1979). In the present studies we have used an *in vitro* system to: i) further characterize the suppressor T cells and ii) begin to analyze how secretory inhibition is achieved. We have determined that the suppressors are $Ly_t1^-2^+$ cells with surface membrane receptors that recognize idiotypes on M315 but not on M460, another IgA anti-TNP myeloma protein. Secretory inhibition: i) is T cell dose-dependent; ii) does not require macrophages at the effector stage, and iii) is first detected 6 hours after co-culture of specific T cells and myeloma targets. Secretory inhibition occurs without any detected effect on MOPC-315 growth, viability or surface membrane expression of M315. Secretory inhibition is not *simply* due to engagement of surface membrane M315 molecules by T cell anti-idiotype because engagement of surface membrane M315 by anti-Id^{315} antibodies does not influence M315 secretion (Fed. Proc. 39:572, 1980). Preliminary results indicate that M315 synthesis is markedly reduced after MOPC-315 cells interact with Id^{315}-specific suppressor T cells.

These studies clearly demonstrate that idiotype-specific, $Ly_t1^-2^+$ cells suppress immunoglobulin expression by directly operating on the actual antibody-secreting cell. The MOPC-315 system provides a powerful tool that should prove useful in the effort to understand the molecular events that occur in cells when they are regulated by idiotype-specific T cells.

217 NEONATAL TREATMENT WITH LOW DOSE OF ANTIIDIOTYPIC ANTIBODY LEADS TO THE EXPRESSION OF A SILENT CLONE, Jacques Hiernaux, Constantin Bona, and Phillip J. Baker, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, Maryland 20205.

A48 idiotype of the β (2→6) polyfructosan binding ABPC48 myeloma protein is not expressed in the immune response of various strains of mice to bacterial levan (BL). However, neonatal treatment of BALB/c mice with .01μgr of affinity purified BALB/c anti-A48Id antibody, followed by immunization with 20μgr BL one month later, leads to the expression of the $A48Id^+$ component of the anti-BL response. Indeed, 50% of anti-BL splenic PFC were inhibited by BALB/c anti-A48 antibody. This effect was specific since the A48Id was not detected in neonate mice treated with affinity purified BALB/c anti M384Id antibody. Furthermore, the $M460Id^+$ component of the anti-TNP response was not altered in BALB/c mice neonatally pre-treated with affinity purified BALB/c anti-A48 antibody and immunized one month later with TNP-Ficoll. By contrast, neonatal treatment with higher doses of affinity purified BALB/c anti-A48Id antibody (1-10μgr) leads to the suppression of the total anti-BL response (highest inhibition was obtained with 10μgr). These results show that neonatal treatment with a single low dose of anti-A48 antibody leads to the activation of $A48Id^+$ anti-β(2→6) polyfructosans clone(s) which belong to the silent anti-BL repertoire. Studies, now in progress, are designed to understand the cellular basis of this unexpected phenomenon. Similar results were obtained using the M460 system.

218 TWO T CELLS INTERACT TO EFFECT IDIOTYPE-SPECIFIC SUPPRESSION OF M315 SECRETION BY MOPC-315 CELLS *IN VIVO*, James W. Rohrer, John Kemp, Richard Gershon, and Harvey Cantor Univ. of S. Ala. Med. Coll., Mobile, AL 36688, Yale Univ. Med. Sch.,New Haven,CT 06510, and Harvard Univ. Med. Sch., Boston, MA 02115.

MOPC-315, a TNP-specific IgA(M315)-secreting BALB/c myeloma, is composed of small, nonsecretory lymphocytic stem cells(LC) which differentiate into large M315-secreting plasmacytes(PC) during *in vivo* growth in peritoneal diffusion chambers(DC) implanted in normal BALB/c mice. MOPC-315 cells within peritoneal DC implanted in M315-immune BALB/c mice grow and synthesize M315 normally and undergo normal LC to PC differentiation, but are specifically inhibited from secreting M315 by T cells induced by M315 immunization. Those M315-immune T cells can adoptively transfer this M315-specific secretory blockade to normal BALB/c mice. We now report that those M315-immune T cells are resistant to 1100 R *in vitro* X-irradiation and 50 mg/kg *in vivo* cytoxan(CTX) treatment and are Ly $1^+,2^-$ T cells. While transfer of M315-immune Ly 1 T cells to normal mice results in M315-specific suppression of M315 secretion by peritoneal DC-enclosed MOPC-315 PC, passage of the same T cells to lethally irradiated, BM-reconstituted mice or to CTX-treated mice results in an enhanced frequency of M315-secreting MOPC-315 cells. Restoration of M315-specific secretory blockade in irradiated or CTX mice occurs if normal splenic T cells are injected with the M315-immune Lyl T cells. It thus appears that the T cells that transfer M315-specific secretory blockade do so by activating a radiosensitive,CTX-sensitive suppressor T cell precursor which then effects the inhibition of M315 secretion. In fact, the Ly 1 T cell induced by M315 immunization enhances M315 secretion when it directly interacts with the MOPC-315 cells. (Supported by research grant CA28708).

219 STRUCTURAL BASIS FOR THE MOPC 173 IDIOTYPE AND MULTIGENIC CONTROL OF THE ANTI-IDIOTYPIC REPERTOIRE. Michel Fougereau, Claudine Schiff, Claude Boyer, and Michèle Milili. Centre d'Immunologie INSERM-CNRS de Marseille-Luminy, Case 906, 13288 Marseille Cedex 9, France.

Production of anti-Id antibodies against the MOPC Id is multigenic: i) Balb B and C57 L (H2b) are non-responders, although they possess the anti-Id-repertoire, Balb K and C 58 (H2k) are high responders, and Balb/c (H2d) give a scattered response; ii) (Balb B x Balb K) F1 behave as responders, whereas (Balb B x C 57 Bl 10) F1 do not, thus suggesting that the IgC_Ha-H2b association strictly controls the non-response, and may be overcome in heterozygotes at the H2-locus; iii) Non-IgC_Ha strains (A/J, CBA, C 57 B/6, C 57 B1/6 H2k) are good responders, whatever their H2 genes, although some scattering in Ab titers might suggest an additional influence of Background genes. Anti-Id sera prepared in IgC_Ha strains recognize Id determinants that require the presence of both 173 H and L chains, whereas those prepared in some non-IgC_Ha strains recognize a determinant primarily associated with the H chains, which is expressed upon combination with normal Balb/c L chains, suggesting the existence of 2 discrete sets of Id determinants. Hybrid molecules built from X24 H chains (a myeloma with anti-galactane activity) which differed from 173 by a very limited number of amino-acids, and from 173 L chains do not express either determinant, which is compatible with the implication of H residues of the D-J area to 173 conformational Id-determinants.

220 ATTEMPTS TO ELICIT EXPRESSION OF LATENT ALLOTYPES, Hamida B. Abdi and Rose G. Mage, Laboratory of Immunology, NIAID, NIH, Bethesda, MD 20205

We have attempted to confirm and extend the report of Mandy and McCartney-Francis (Abstr. #2.2.03 4th Int. Congr. Immunol. 1980) that expression of latent allotypes can be elicited in cultures of rabbit splenocytes. There are four b locus alleles, b4, b5, b6 and b9 which are markers for kappa light chain constant regions. For this study we selected homozygous b^4b^4 animals. Fluoresceinated (Fl.) $F(ab')_2$ fractions of affinity purified anti-b4 and -b5 reagents were used to stain for surface immunoglobulin allotypes and detected by flow microfluorometry (FACSII). Controls were Fl normal $F(ab')_2$ fragments. Fl. sheep anti-rabbit immunoglobulin was used to measure total content of surface immunoglobulin positive (slg^+) cells. Normal Fl. sheep immunoglobulin was the control for non specific staining. The normal fresh spleens had a total of 50-60 percent slg^+ cells and 30-40 percent were $b4^+$. No b5+ cells were detected. When the cells were cultured for 5-6 days in a modified Iscove's medium in the presence of LPS and b5-anti-b4 serum, the percent $b4^+$ was lowered but the cells showed significant staining for slg of b5 allotype. The total percent slg^+ was within the low to normal range. Studies to detect secretion of labelled Ig during culture are in progress.

221 IDIOTYPE-ANTI-IDIOTYPE INTERACTIONS IN EXPERIMENTAL MYASTHENIA GRAVIS, Donard S. Dwyer and John F. Kearney, University of Alabama in Birmingham, Birmingham, AL 35294

We have developed a murine model of the autoimmune disease, myasthenia gravis, using monoclonal antibodies against the nicotinic acetylcholine receptor. About 50% of the mice which receive purified monoclonal antibody or the antibody-secreting hybridoma display characteristic symptoms of weakness and fatigue. However, since the disease is not initiated in all mice and may be transient in those who do develop myasthenia, we feel that regulatory circuits exist which modulate or prevent immunopathologic responses. Recently, we have produced monoclonal antibodies which react with idiotypic determinants found on the myasthenia- inducing antibody. We are using these anti-idiotypic antibodies to learn about the cellular interactions involved in this disease.

As a result of these studies, I am very interested in immunoregulatory circuits which operate through idiotypic interactions. It would appear that such circuits are instrumental in controlling the autoimmune response in myasthenia gravis. In addition to this project, I am interested in B cell ontogeny in mice, especially those events which occur at the level of the pre-B cell.

222 FREQUENCY OF B LYMPHOCYTES RESPONSIVE TO ANTI-IMMUNOGLOBULIN REAGENTS, Anthony L. DeFranco, Elizabeth Raveche, and William E. Paul, Natl. Institutes of Health, Bethesda, MD 20205.

It is now well established that B cells can be stimulated to divide by anti-Ig antibodies providing a model for the activation of specific B cells by antigen. Sieckmann, et al. , (J. Exp. Med. 148, 1628-1643, 1978), have shown that B cells from mice with the xid defect are unresponsive to anti-Ig antibody suggesting that responsiveness of normal B cells is a feature of the $Lyb5^+$ subset. We show here that small, resting B cells can be stimulated in serum-free medium by affinity-purified goat anti-μ antibody and that all B lymphocytes, from normal or xid mice,undergo early phases of the activation process. This is indicated by the fact that virtually all small resting B cells increase their cell volume within one hour after the beginning of exposure to anti-μ and continue to increase their volume over the next 24 hours. However, only 50% of normal B lymphocytes and virtually none of the xid B lymphocytes enter S phase, as measured by flow microfluorometric analysis of DNA content of individual cells. These results suggest independent regulation of activation of the G_1 and S phases of the response to anti-μ. All B cells appear capable of responding to anti-μ as measured by size enlargement, but only a subset, presumably the $Lyb5^+$ cells, actually proliferate in response to exposure to anti-μ.

223 AN ALLOTYPESPECIFIC ELISA TO FOLLOW INDUCTION OF RABBIT LATENT ALLOTYPES, Susan Jackson, John A. Sogn and Thomas J. Kindt, Laboratory of Immunogenetics, NIAID, NIH, Bethesda, MD 20205

The possibility that an allotype-mediated network operates in rabbits has been suggested by experiments in which 10-20 fold increases in latent allotype levels were induced by injection with purified anti-allotype antibodies (Abs). Levels of both V_H (a2) and C_L (b6) latent allotypes of antistreptoccocal Abs were found to markedly increase in rabbits treated with the respective anti-allotype Ab and subsequently challenged with antigen (Yarmush et al., J. exp. Med., 153, 1981). Accurate measurement of latent allotype levels was not easily accomplished throughout the entire induction period because anti-allotype Ab interferes with the solid phase radioimmune assay used. This interference, which was eliminated by cumbersome procedures such as absorption of test samples on Ig columns, can now be totally circumvented by the use of a recently developed allotype-specific ELISA which is not affected by anti-allotype Ab and which can quantitate rabbit allotypes in a linear range of 50-500 nanograms/ml. The assay is being used to obtain more complete data concerning latent allotype induction in anti-allotype treated rabbits and to routinely screen sera for presence of latent allotype. It will have further use in screening for rabbit-mouse hybridomas, including those potential latent allotype producers, prepared from the spleens of induced animals.

224 THE ROLE OF IDIOTYPE RESTRICTED T LYMPHOCYTES IN THE IN VITRO ANTIBODY RESPONSE TO (α-1,3) DEXTRAN, Linda B. Buck, Constantin A. Bona, and Benvenuto G. Pernis, Columbia University, College of Physicians and Surgeons, New York, NY 10032 and Mount Sinai School of Medicine, New York, NY 10029

Numerous studies have shown that the majority of antibodies produced in vivo in Balb/c mice immunized with B1355 (α-1,3) dextran bear idiotypic determinants (IdX) also found on two (α-1,3)dextran-binding myeloma proteins, J558 and MOPC104E. A large proportion of such antibodies also bear an idiotypic determinant (IdI) present on J558, but not MOPC104E, molecules. We are currently studying the role of idiotype bearing (id^+) and idiotype specific (anti-id^+) T lymphocytes in the antibody response to dextran. For this purpose, we have established an in vitro system for assaying B cell responses to the antigen. Although the in vivo response to dextran is T independent, the majority of the in vitro response appears to require T help. Using antibodies specific for IdX, IdI, or anti-IdI to positively or negatively select T cells bearing idiotypic or anti-idiotypic determinants, we are examining the role of id^+ and anti-id^+ T helper and suppressor populations in the in vitro response to (α-1,3) dextran.

225 INTERNAL IMAGES IN THE IMMUNE NETWORK, Oberdan Leo, Moncef Slaoui, Bernard Mariamé and Jacques Urbain, Laboratory of Animal Physiology, University of Brussels,Belgium.

Network concepts led to the view that an immune system already contains within it imperfect images or homobodies of antigens. During a study of the specificity of idiotypic reactions in the tobacco mosaïc virus system we found that one antiidiotypic serum contained antibodies with strange properties. 1. these "antiidiotypic antibodies" were recognizing not only the idiotypic anti-TMV antibodies used for immunization (as it is expected) but were reacting also with part of anti-TMV antibodies from all rabbits immunized against TMV. No linkage with allotypes of the a group was apparent since anti-TMV antibodies from a1/a1,a2/a2,a3/a3 rabbits are recognized. These special Ab2 never react with antibodies from rabbits immunized against other antigens. They are therefore exquisitely specific for antibodies specific for TMV. 2. the epitopes which are recognized by these "peculiar" antiidiotypic antibodies are not species specific since a similar reactivity was discovered with anti-TMV antibodies from all mice tested, horses, goats, chickens... In other words, these antiantibodies react with any anti-TMV serum. 3. these same antiidiotypic antibodies when injected into mice, in the total absence of TMV, induce the appearance of anti-TMV antibodies. In summary, these special Ab2 behave like the antigen :they react with anti-TMV antibodies and they are also able to promote the synthesis of anti-TMV antibodies. They are therefore good candidates for being considered as the internal image of Tobacco Mosaïc Virus. These results also strongly support the assertion that the immune system is a functional idiotypic network, since the immune system can be triggered by elements of the system itself,without intervention of external antigens.

226 MONOCLONAL ANTI-IDIOTYPE REAGENTS FOR THE STUDY OF A B CELL TUMOR. David G. Maloney, Ronald Levy and Joseph Haimovich, Stanford University, Stanford, CA, 94305.

Surface IgM (κ) immunoglobulin from the chemically induced murine B cell tumor 38C-13 was obtained by somatic cell hybridization and used as an immunogen to produce xenogeneic rat monoclonal anti-idiotype antibodies. Five idiotype specific antibodies and several μ chain specific antibodies were produced. No antibodies directed against the κ light chain were produced. The anti-idiotypes were of the rat IgG1, IgG2a and IgG2c classes. Idiotype specificity of the antibodies was demonstrated by radioimmunoassay on cells and immunoglobulin coated plates, indirect immunoprecipitation of ^{125}I surface labeled cells and immunofluorescence by microscopic and FACS analysis. Cross blocking studies demonstrated that there are at least two separate idiotype determinants recognized by this population of monoclonal anti-idiotypes. These anti-idiotype antibodies were not capable of effecting complement mediated lysis of tumor cells in vitro. Also, culturing of the B cell tumor in the presence of anti-idiotype or anti-μ antibodies produced no change in cell proliferation as determined by ^{3}H-thymidine incorporation. However, the amount of antigen (surface immunoglobulin) was found to decrease during incubation with monoclonal reagents. This modulation was found to be dependent on anti-idiotype concentration and, to a limited extent, the length of incubation. Modulation was not complete and expression of immunoglobulin idiotype returned following removal of the anti-idiotypes. The anti-idiotype antibodies were also used to detect and localize idiotype bearing cells in tumor-bearing mice and to monitor the concentrations of free serum idiotype with disease progression.

227 NATURE OR NURTURE? ON THE DIFFERENCE BETWEEN HELPER AND SUPPRESSOR T CELLS IN THE FRAMEWORK OF THE SYMMETRICAL NETWORK THEORY, Geoffrey W. Hoffmann, University of British Columbia, B.C., Canada, V6T 1W5.

Work with the Ly markers has led to the present orthodoxy that helper and suppressor T cells have different Ly markers, and are thus distinctly different cell types. Within the framework of the symmetrical or "plus-minus" network theory (Contemp. Top. in Immunobiol. (N. Warner, Ed.), vol. 11, 1980, pp. 185-226) the system can function perfectly well with just one basic type of T cell, which mediates both roles. The difference between T cells that tend to preferentially help or suppress can be most simply ascribed to a difference in their network environment, rather than to a difference in the properties of the cells themselves. Whether they are helpers or suppressors is determined by a quantitative parameter called connectivity, that measures the extent of their idiotypic interactions with the rest of the network. Cells with high connectivity tend to suppress, while cells with low connectivity help. The connectivity of a T cell can be changed drastically in limiting dilution experiments. The recent limiting dilution experiments of Eichmann et al. (J. Exp. Med. 152, 477, 1980) will be discussed in these terms.

Regulation of Immunoglobulin Quality

228 HELPER T CELLS WITH V-REGION RELATED SPECIFICITY REQUIRED FOR EXPRESSION OF BOTH DOMINANT AND MINOR IDIOTYPES IN PHOSPHORYLCHOLINE SPECIFIC RESPONSES, Maurice Zauderer, Denise A. Faherty, David R. Johnson, Joseph F. Sproviero and Michael J. Imperiale, Columbia University, New York, NY 10027

We have previously described antigen dependent selection in cultures of KLH primed lymph node cells of a highly enriched population of helper T cells with immunoglobulin variable region related specificity.[1] Different T cells were shown by limiting dilution analysis to be required for expression of dominant (T15+) and minor (T15-) BALB/c idiotypes in phosphorylcholine specific antibody responses in vitro. Titration of these T cells revealed that even in normally T15 dominant BALB/c the KLH induced population includes more T cells restricted to cooperate with PC specific B precursors that express minor than dominant idiotypes. This argues strongly that although T cells with V-region related specificity are required for induction and/or maturation of B cells in response to a T-dependent antigen, they do not themselves mediate idiotype dominance.

It is particularly striking in these experiments that T cells restricted to cooperate in a response of defined idiotype are induced at a very high frequency by randomly chosen antigens. As many as 1 in 100 viable cells recovered from 18 day cultures of KLH or FYG primed lymph node cells is restricted to cooperate in a T15+ response. These T cells are induced in the absence of specific selection with either PC or PC specific paratopes, and, as noted above, their specificity does not reflect T15 idiotype dominance of PC specific responses. We suggest, therefore, that such T cells are efficiently induced by randomly chosen antigens because they are specific for V-region related determinants that are widely represented in BALB/c clones. On the assumption of random representation in a KLH induced population, we estimate that there are of the order of 100 such determinants with regulatory significance in BALB/c. This estimate is an upper limit since other T cells, in particular a carrier specific subpopulation, are also recovered in these cultures. Experiments are in progress to define more precisely the number and representation of independent V-region related determinants by cloning T cells with V-region restricted function.

[1] Zauderer, M., Sproviero, J.F., Cosenza, H. and Imperiale, M.J. Cooperation subsets of antigen-specific helper T cells. In "Regulatory T Lymphocytes", Pernis, B. and Vogel, H.J. editors, pp. 186-200, Academic Press, Inc. (1980).

229 REGULATION OF THE PRODUCTION OF HIGH AFFINITY ANTIBODY. Gregory W. Siskind, Rosemary H. DeKruyff, Tova Francus, Edmond A. Goidl, Kathleen A. Haines, David H. Sherr, and Myron R. Szewczuk, Department of Medicine, Cornell University Medical College, 1300 York Avenue, New York, New York 10021

I will describe a series of studies on factors regulating the production of high affinity antibodies. The efficient shift, with time after immunization, from the production of mainly low affinity antibody to the production of high affinity antibody generally requires T-cell help. T-cell tolerance or depletion of helper T-cell activity inhibits efficient selection for high affinity antibody production. In the mouse the T-cell population matures, to be capable of facilitating the selection of high affinity antibody secreting cells, within 3 or 4 days after birth. On the other hand, the ability of the B-cell population to produce high affinity antibody matures between 7 and 10 days after birth in the LAF_1 mouse. This antigen independent event, in the ontogeny of the B-cell population, is dependent upon a cell or factor produced by the thymus. The thymus cell population acquires the capacity to mediate the differentiation of the B-cell population to be able to produce high affinity antibody secreting cells between 7 and 10 days of age. This thymic differentiation event is distinct from the acquisition of the capacity to provide helper activity for the antigen-dependent selection of high affinity antibody producing cells since this latter T-cell function matures earlier. Thus, evidence for a role of the thymus in the ontogeny of the function of the B-cell population will be presented. (Supported by NIH grants AI-11694 and CA-20075.)

230 ANALYSIS OF T CLONES PROVIDE EVIDENCE FOR TWO DISTINCT POPULATIONS OF HELPER T CELLS (TH1 AND TH2) ONE OF THEM PARTICIPATING TO THE ISOTYPIC REGULATION OF THE ANTIBODY RESPONSE. M. Seman and J. Morisset. Groupe d'Immunodifférenciation - I.R.B.M. - Tour 43 - Université Paris VII - C.N.R.S. - 2, Place Jussieu - 75221 PARIS Cédex 05 (France).

The isotypic pattern of the antibody response to various antigens is depending upon the antigen dose, the protocole of immunization, the route of administration or upon genetic parameters. This leads to recognize antigen and class specific regulations in the antibody response. IgG2a and SRBC specific suppressor T cells can be identified, for example, in low IgG2a responders to SRBC. However, cloning of T lymphocytes activated by various antigens allow to obtain antigen specific clones of T helper cells. When added to B cells primed under different protocoles each of the helper clones induces an antibody response the isotypic pattern of which is identical to the one obtain in a polyclonal T helper cell situation. This shows that most of these T helper cell clones allow the expression of the memory B cells whatever the isotype they have been committed to. This helper activity is also MHC restricted and requires a cross-linkage between the carrier and the hapten. Thus, they can be classed as TH1 cells. However class specific Ts2 cells can be demonstrated in IgG2a low responder to SRBC or in animals primed with different carriers by oral immunization. These Ts2 requires only the presence of the carrier to suppress IgG response. IgA or IgG specific TH2 can also be detected under appropriate circumstances. However, the cloning conditions which allow the proliferation of TH1 clones cannot be used for TH2 cloning. This cloning might require the presence of TH1 cells or TH1 factors since the TH2 activity is not detected in the absence of TH1 cells.

231 T-T CELL INTERACTIONS IN CLASS SPECIFIC IgE ANTI-HAPTEN ANTIBODY SUPPRESSION, Zoltan Ovary, Department of Pathology, NYU Medical Sch., 550 First Avenue, New York, N.Y. 10016

In certain strains of mice IgE antibody production is rapidly abrogated. However, when the mice are irradiated, IgE antibody production is prolonged, but when unprimed spleen cells are injected in these irradiated mice the IgE antibody production is selectively abrogated (1).The cells responsible for the suppression of IgE antibody class are T cells from the Lyt-1 subclass (2). Advantage was taken of the carrier effect (3) to study this suppression using adoptive transfer. $3x10^7$ cells from mice immunized with 1γ DNP-KLH + 0.2γ Egg albumin + 1 mg Al(OH)3 and $5x10^7$ unprimed spleen cells were injected iv in irradiated recipients and the mice were challenged with 0.2γ Ea. High titer anti-DNP IgE and IgG antibodies were produced. If the donors were primed with 1γ DNP-KLH and 10γ Ea and 1 mg Al(OH)3, IgE antibody production was selectively abrogated. Omission of injection of unprimed spleen cells did not abrogate the production of IgE anti-DNP antibody. In other experiments, recipients were injected with $1.5x10^7$cells from donors primed with 0.1γ DNP-Ea (donor 1), with $5x10^7$spleen cells from donors hyperprimed with 10γ Ea (donor 2) and $5x10^7$ unprimed spleen cells (donor 3) and then challenged with 0.1γ DNP-Ea, IgE antibody production was selectively and significantly suppressed. Omission of cells from donor 2 or donor 3 does not cause suppression of IgE antibody production. If donor 2 is primed with an unrelated carrier (KLH), suppression of anti-DNP IgE production occurs if the free carrier (KLH) is also injected at challenge. Injection of the free carrier is necessary for suppression. Treatment of the spleen cells with anti-Thy-1 and complement of either donor 2 or 3 abrogates the suppressive effect. In conclusion: T-T cell collaboration is demonstrated in this system. The induction and expression of the suppression is antigen-specific, but the action is not. (Grants: NIH AI-03075 and National Cancer Institute CA-16247)

(1) Watanabe et al. J. Exp. Med. 143: 833, 1976 (2) Watanabe et al. J. Immun. 118:251, 1977 (3) Ovary and Benacerraf Proc. Soc. Exp. Biol. & Med. 114: 72, 1963.

232 T CELLS RECOGNIZING Igh LINKED GENE PRODUCTS ARE REQUIRED IN THE NORMAL RESPONSE TO SHEEP RED BLOOD CELLS, Henry H. Wortis, Nan Nutt, Jerome Haber, Tufts University School of Medicine, Boston, MA 02111.

Mice congenic for the Igh haplotypes Igh^j and Igh^b make equivalent responses to SRBC. CBA.nude mice (Igh^j) supplemented with peripheral T cells of either Igh^j or Igh^b genotype produce equivalent high γ_3 γ_1 γ_{2b} and γ_{2a} responses. Therefore T cell-B cell mismatching for the Igh haplotype is not in itself a bar to the generation or expression of help. In contrast, T cells primed in an environment that lacks Igh^j linked products are inefficient helpers for Igh^j B cells. These results suggest that antigen primed B cells or their products initiate a B_1-T-B_2 helper pathway. B_1-B_2 Igh matching is required for the expression of this help.

233 SELECTIVE SUPPRESSIVE EFFECTS OF ANTI-IG ON IG SECRETION BUT NOT ON EXPRESSION OF MEMBRANE-BOUND IG, Louise T. Adler, Tetsufumi Inoue and Frank L. Adler, St. Jude Children's Research Hospital, Memphis, TN 38101.

Spleen cells of adult rabbits with known kappa chain allotypes (*b* locus) were treated with affinity-purified rabbit anti-allotypic antibodies *in vitro*. Following antibody pulse treatment of 2 or 24 hours at 37°, allotypic Ig was no longer detectable on the cell surface. However, complete or nearly complete recovery of surface Ig expression occurred on subsequent culture of these cells, while at the same time their ability to secrete allotypic Ig into the culture fluid was impaired. Inhibition of Ig secretion on subsequent culture of the cells was dependent on the antibody concentration used and was more pronounced following a pulse for 24 hours than for 2 hours. Such selectively suppressed cells provide a possible model for B cells with a secretory block found in chronically allotype-suppressed rabbits. Furthermore, selective suppression of Ig secretion but not of membrane Ig expression appears to be relevant to recent reports that membrane and secreted IgM molecules have different structures and are encoded by separate mRNA's. (This work was supported by U.S. Public Health Service Grants AI-13159, CA-21765, and CA-23709, and by ALSAC.)

234 GENETIC CONTROL OF THE IMMUNE RESPONSE TO FERREDOXIN: LINKAGE AND MAPPING OF DETERMINANT SPECIFICITY TO THE MHC OF MICE, Lydia K.J. Sikora and Julia G. Levy, University of British Columbia, Vancouver, British Columbia, Canada, V6T 1W5.

The genetics of the immune response to Ferredoxin (Fd) in mice were studied at the one determinant level. Previous investigations demonstrated that Fd bears two major antigenic determinants the N terminal heptapeptide and the C terminal pentapeptide. The response has been shown to be under MHC linked Ir gene control mapping at least to the I-A subregion. The present work involved the use of the Fd molecule functionally devoid of either one of the two determinants by the use of selective enzymes. The assessment of determinant specificity of anti-Fd serum antibody was carried out with the use of the two non-immunogenic unideterminant molecules in a 'hapten' inhibition assay on the native Fd ELISA. Results demonstrate that the determinant specificity of the anti-Fd response is H-2-linked: high responder mice ($H\text{-}2^k$) make a predominantly C-determinant directed response (60-70%) as compared to intermediate responder strains ($H\text{-}2^b$ and $H\text{-}2^s$) which make 30-45% C-determinant directed antibody. This determinant selection process clearly maps to the I-A subregion. These results form the basis for studies concerning the role of Ir genes in idiotype selection, in the response to each determinant of Fd.

235 PREFERENTIAL ISOTYPE ASSOCIATION OF IDIOTYPES OF ANTI-DNP ANTIBODIES, Mitchell G. Scott and Julian B. Fleischman, Washington University, St. Louis, MO.

DNP and TNP conjugates of T-dependent, T-independent-1, and T-independent-2 antigens elicit different IgG isotypes in the murine responses to these categories of antigens. We are currently studying whether there is also a difference in the idiotypes elicited by these categories of antigens and whether there are preferential idiotype-isotype associations. Rabbit antisera were prepared against idiotypes of four IgG3 anti-DNP-Ficoll (DNP-F) hybridomas and against MOPC 460. By RIA, the MOPC 460 IdX was found on one IgG1 anti-DNP-OVA hybridoma and at significant levels in Balb/c immune sera to DNP-proteins but was much less evident, or undetectable in immune sera to DNP-F. By inhibition of isotype facilitated, Ag-specific PFC's, we have shown that 30-40% of IgG1 PFC's (and 20-35% of IgM PFC's) from mice immune to DNP-BSA bear this 460 IdX. This antiserum had little effect on the small IgG3 component of PFC's from these mice and did not inhibit the large IgG3 and IgM components of PFC's from mice immune to DNP-F or TNP-LPS. Conversely, two idiotypes (8-11 and 7-17) expressed on IgG3 anti-DNP-F hybridomas were found in all immune sera to DNP-F but were absent or barely detectable in immune sera to DNP-proteins. Antisera to either of these two IdX's inhibited 40-45% of the IgG3 PFC's (and 25-40% of IgM PFC's) of mice immune to DNP-F and TNP-LPS. Simultaneous inhibition with anti-8-11 and anti-7-17 gave an additive effect inhibiting 75% of IgG3 PFC's (and 55% of IgM PFC's) from these mice. These antisera did not inhibit the IgG1 or IgM PFC's of mice immune to DNP-BSA. These results suggest isotype restriction of idiotype expression in that 460 IdX is preferentially associated with IgG1 and with the response to DNP-proteins (TD) and the 8-11 and 7-17 IdX's are associated with IgG3 and with responses to DNP-F (TI-2)and TNP-LPS(TI-1

236 HELPER T CELLS RESTRICTED TO INDEPENDENT I REGION ENCODED RESTRICTION ELEMENTS. CONTRIBUTION OF I-A AND I-E/C SUBREGIONS TO INDEPENDENT AND TRANSCOMPLEMENTING RESTRICTION ELEMENTS, Joseph F. Sproviero, M.J. Imperiale, Maurice Zauderer, Columbia University, New York, N.Y. 10027

Clones of KLH specific, MHC restricted F_1 hybrid helper T cells were isolated and expanded in limiting dilution cultures. In ($H\text{-}2^k$ x $H\text{-}2^b$)F_1 hybrids, different clones with helper activity restricted to either parental or unique F_1 hybrid MHC determinants were found to occur at approximately equal frequency.

This system has been extended to evaluate the independent contributions of the I-A and I-E subregions to formation of F_1 hybrid restriction elements. Clones of KLH specific helper T cells were selected in F_1 hybrids between strains which differ only in the I-A or the I-E subregions. These experiments demonstrate the potential for multiple I region restriction elements especially in heterozygotes.

237 Differential Idiotype Expression in Dextran Specific Precursors Responding to TI and TD Antigens. R. Ward, J. Kearney and H. Kohler. La Rabida-Univ. of Chicago Inst., Chicago, IL 60649 and Univ. of Alabama, Birmingham, AL 35294.

The frequencies and idiotype compositions of B cell precursors responding to TI and TD Dextran antigens were analyzed using the splenic fragment culture technique. Primary splenic B cells taken from normal adult BALB/c mice gave rise to 1.75 precursors per 10^5 B cells responding to Dextran B1355S in unprimed fragment cultures. The frequency of primary B cells responding to Dex-hemocyanin in fragment cultures from hemocyanin-primed BALB/c was three times higher ($5.04/10^5$ B cells). Monofocal Dextran antibodies were analyzed with hybridoma anti-idiotypic antibodies specific for J558 and M104E IdI and the J558-M104E crossreacting IdX idiotypes. The J558 IDI idiotype was found on 5.10% of the TD precursors and on 5.56% of the TI precursors, while the M104E IDI was found on 1.91% of the TD precursors and 7.41% of the TI precursors. In contrast to the low IDI expression, the IDX idiotype was found on 91% of the TI precursors and on 21.6% of the TD precursors. Co-expression of either IdI idiotype with IdX idiotype occurred in only a small number of clones. These findings demonstrate that the expression of the Dextran-specific idiotype repertoire is different for TD and TI precursors, and that the differences are more pronounced in the Dextran specific repertoire than in the differential T15 expression of precursors responding to TD and TI phosphorylcholine antigens (J. Immunol. 125:640). (Supported by USPHS grants GM-07183, AI-11080, AI-14782 and AI-00338).

238 THE Tα CELLS INDUCED BY IgA-SECRETING PLASMACYTOMAS ARE θ^+, $Ly_t1^-2^+$, ADULT-THYMECTOMY SENSITIVE LYMPHOCYTES. R.G. Hoover, B.K. Dieckgraefe, J.P. Lake, J.D. Kemp, R.K. Gershon and R.G. Lynch. Washington University, St. Louis, MO and Yale University, New Haven, CT

We have previously shown that BALB/c mice with the IgA secreting plasmacytomas MOPC-315 (α,λ_2), TEPC-15 (α,κ), McPc-603 (α,κ) and MOPC-167 (α,κ) develop large numbers of circulating θ-bearing lymphocytes with Fc receptors for IgA (Tα cells) (J.Immunol. 125:1280, 1980). The Fc receptor present on these Tα cells is class specific and does not react with IgG or IgM. The increase in Tα cells is directly related to the high level of IgA paraprotein present in the tumor-bearing animals. In preliminary studies Tα cells have been induced in mice given daily injections of large quantities of IgA myeloma protein. Using monoclonal antisera specific for the Lyt 1.2 and Lyt 2.2 alloantigens we have determined that the Tα cells are $Ly_t1^-2^+$ cells. Tα cells were not detected in nude BALB/c or adult-thymectomized BALB/c mice with MOPC-315 tumors. When MOPC-315 was grown in nude BLAB/c mice that had been previously grafted with a neonatal BALB/c thymus Tα cell expansion occured as in normal BALB/c mice with MOPC-315.

Collectively, the present studies have further characterized the Tα cells in myeloma as a distinct subpopulation of T lymphocytes. Phenotypically these Tα cells are immunoregulatory T cells and their increase in myeloma may reflect an exaggerated, but otherwise appropriate, immunoregulatory response.

239 INDUCTION OF IgG PRODUCTION IN HUMAN B-LEUKEMIC CELLS BY NORMAL ALLOGENEIC T CELLS, OSAMU SAIKI, TADAMITSU KISHIMOTO, YUICHI YAMAMURA, Osaka University Medical School, Fukushima-ku, Osaka, Japan

The molecular basis for the differential expression of immunoglobulin C genes by antibody-secreting cell is now being intensively investigated at the DNA level. Now everyone expects to see genetic solutions to the problems of switching in isotype expression and double producers. Nevertheless at the biological level of these processes, our understanding lies far behind. In vitro stimulation of B-leukemic cells with normal allogeneic T cells plus PWM induces IgM and IgG production. Induction of IgM and IgG productions in B-CLL cells with T cells was demonstrated by the presence of the same idiotype in induced immunoglobulin as that present in the monoclonal IgM protein in the patient's serum. Variations existed among T cell donors in the capabilities to induce differentiation of the same leukemic cells, suggesting the requirement of matching of acceptors on B-CLL cells and T effector molecules for the induction of immunoglobulin production in B-CLL cells. PWM induced, antigen-nonspecific helper factors were also effective in the induction of differentiation of the leukemic cells. The capability of factor to induce differentiation is absorbed by culture cell lines derived from the patient which has the same idiotype as the leukemic cells.

240 THE INFLUENCE OF THE lpr GENE ON AUTOANTIBODY PRODUCTION, D.S. Pisetsky, P. Stewart, J.B. Roths and E.D. Murphy, Durham VA Hospital, Durham, NC and The Jackson Laboratory, Bar Harbor, Maine.

In MRL congenic autoimmune mice, the mutant lpr gene is associated with an acceleration of disease and an augmentation of the anti-DNA antibody response. To determine whether this gene leads to autoantibody production in mice of another background, anti-DNA antibody levels have been measured in serial bleedings from B6-lpr/lpr mice. This strain was developed by successive crosses to transfer the lpr gene from the MRL-lpr/lpr strain to the B6 background. Male B6-lpr/lpr mice failed to produce significant levels of anti-DNA up to the age of 9 months at times when male MRL-lpr/lpr mice had highly significant titers. In contrast, female B6-lpr/lpr animals produced high levels of anti-DNA over this same interval. These results indicate that the action of the lpr gene can lead to anti-DNA production in a background other than the MRL, although its action may be influenced by the sex of the animal in other strains. In addition, antibodies to a component of rabbit thymus extract, tentatively identified as RNP, were found in sera from some of the B6-lpr/lpr mice, suggesting that the lpr gene may be responsible for stimulating a variety of autoantibody responses in addition to anti-DNA.

241 SUPPRESSION OF ANTI-HAPTEN IgE ANTIBODY RESPONSE IN BALB/C MICE BY ISOLOGOUS ANTIIDIOTYPIC ANTIBODIES AGAINST ANTI-HAPTEN AND ANTI-CARRIER ANTIBODIES, K. Blaser, A.L. de Weck, University of Berne, Berne, Switzerland

Recently, we have investigated a syngeneic model to study the regulation of the IgE antibody response with antiidiotypic antibodies (aId). Isologous aId have been raised against purified anti-hapten (benzylpenicilloyl (BPO), phosphorylcholine (PC)) antibodies and anti-carrier (ovalbumin) antibodies from BALB/c mice. Anti-hapten aId showed suppressive effects on the corresponding anti-hapten IgE antibody level independent of the carrier conjugate used to mount an IgE antibody response, while the formation of anti-carrier IgE antibody was not affected. Anti-carrier aId on the other hand showed a longlasting depression of both the corresponding anti-carrier and the anti-hapten IgE response provided the haptens have been bound to this carrier. Similar suppressive effects have been observed also on the IgG antibody production. Studies indicating that IgE antibody regulation by aId acts on a cellular basis and not merely by complexing antibodies of the corresponding idiotypes are in progress and results will be discussed.

242 BALB/c *a1* RESTRICTED λ_1 Id^+ ANTI-α(1→3) DEXTRAN RESPONDER B CELLS SHOW A κ Id^- CLONE IN A NON-*a1* BACKGROUND. Clara Bell. College of Med., Univ. of Illinois, Chicago, Ill.

The immune response (Ir) reflects the balance of the structural gene complement available for response and the mechanism regulating its expression. Multiple genes code for the V and C regions of Ig H and L chains, giving B cells potential to respond to antigens (Ag) by Ab with a variety of V and C regions. But altho exceptions of κ gene expression (mRNA and κ related peptides) are reported in 104E λ_1 myeloma proteins (MP), generally a differentiated B clone secretes 1 type only V_H and L chain, each from 1 of its 2 alleles. If this relates to the functional λ_1 L chain, rather than to its κ or λ_1 isotype, a preponderance of κ or λ_1 products would relate to events during differentiation of B cells with nominal specificity. The mouse Ir to α(1→3) dextran (dex) was studied. This Ir is coded by genes mapping to the $V_{\lambda 1}$ and a V_H-DEX gene, apparently linked to the *a1* allotype of the C_H chain. *a1* mice have $1/5.5 \times 10^4$ dex-specific B precursors, of which >96% are λ_1, and non-*a1* mice have $1/11 \times 10^4$ dex-specific precursors almost all κ. BALB/c (*a1*) mice show a high (0.5-5.0 mg/ml) λ_1 restricted Ir to dex, sharing idiotypes (Id^+) with 3 dex-specific λ_1 MP 104E, J-558 and UPC-102. Non-*a1*, which apparently lack the V_H-DEX, show a low Id^- κ Ir (20-60 μg/ml). In an allogeneic *b* background, *a1* B cell prototypes differentiate to generate more κ than λ_1 dex-specific clonotypes, the last mostly Id^-, in contrast to >96% mostly Id^+ λ_1 for *a1* B cells in *a1* syngeneic background. The proportion of κ to λ_1 was higher in athymic *a1* B cells under allogeneic conditions. It is concluded that the λ_1 is more consonant with clonal differentiation in *a1*, and is inactive in *b*, where the κ chain is more functional. Hence, the choice of λ_1 or κ response is dictated by the functionality for the complementary Ag, rather than by the genetic encodement. (NIH CDA 70235)

243 SUPPRESSION OF THE ANTI-HAPTEN IgE RESPONSE BY TOLEROGENIC CONJUGATES OF THE HAPTEN WITH POLYVINYL ALCOHOL (PVA), Robert J. Schwenk, Weng Y. Lee and Alec H. Sehon, Department of Immunology, University of Manitoba, Winnipeg, Canada R3E 0W3.

The capacity of $B_6D_2F_1$ mice to produce anti-hapten (DNP, NP, NIP) IgE antibodies, as a result of immunization with hapten-OA conjugates in the presence of $Al(OH)_3$, was specifically suppressed by treatment of mice with tolerogenic conjugates of the hapten coupled to PVA prior to or after immunization. This mode of suppression was selectively hapten-specific, since it did not affect the immune response of the host to the carrier. Moreover, with regard to the NP/NIP response, the suppression was also shown to be partially heteroclitic. The transfer of spleen T cells from mice, which had been treated with hapten-PVA conjugates 7 to 21 days prior to sacrifice, into normal nonirradiated syngeneic mice resulted in the impairment of the recipients' capacity to mount the appropriate anti-hapten IgE response upon immunization with hapten-OA conjugates. However, the cell fraction containing B cells of the mice treated with these conjugates was devoid of suppressive activity and of the capacity to cooperate with T helper cells in producing an IgE response in X-irradiated recipients. Hence, these results are interpreted as indicating that suppression of IgE responses following the administration of hapten-PVA conjugates was due, at least in part, to the activation of hapten-specific and/or idiotype-specific suppressor T (T_S) cells. Sonication of these T_S cells yielded a soluble suppressor factor (T_SF) which adequately mimicked the suppressive activity of the parent T_S cells. Experiments are being conducted to establish if these T_S cells and their T_SF are hapten-specific and/or idiotype-specific and to study the interactions between these two cell types. (Studies supported by MRC of Canada and NIH, Bethesda, MD (Grant No. AI14526.))

244 LONG-TERM CULTURE OF NORMAL MOUSE B LYMPHOCYTES, Maureen Howard and William E. Paul, Laboratory of Immunology, NIAID, NIH, Bethesda, MD 20205.

The development of long-term in vitro T cell clones has prompted speculation that the same technology may be possible for B lymphocytes. However, while it has previously been possible to propagate individual B cell clones in vivo, and to isolate B cell clones in short-term in vitro assays, attempts of long-term cell culture of B lymphocytes have failed to exceed time intervals greater than 4 weeks. We have developed a procedure for the continuous in vitro propagation of normal mouse B lymphocytes using previously defined lymphokines or monokines as sources of B cell growth factors. FACS-sorted immunoglobulin-positive splenic B lymphocytes were cultured for several weeks with LPS, and then transferred into serum-containing medium supplemented with a T-hybridoma derived source of interleukin 2 (IL2). Continuous feeding with IL2 led to the establishment of cell lines which could also be propagated in IL2-free medium containing interleukin 1, but not in culture medium alone. Cell lines propagated in this manner have the appearance of small, dense lymphocytes, and express surface immunoglobulin detectable by rosetting procedures. The immunocompetence of these cells after 6 months culture resembles that of anti-immunoglobulin stimulated B cells in that they are unresponsive to LPS, proliferate in response to IL2, and are activated to more rapidly-dividing immunoglobulin-secreting blasts when exposed to supernatants containing the T cell-derived AFC helper factor termed TRF.

245 REGULATION OF THE SPECIFICITY AND CLONALITY OF THE ANTIBODY RESPONSE TO A PROTEIN DETERMINANT, Phillip R. Morrow, Donna M. Rennick and Eli Benjamini, U.C. Davis School of Medicine, Davis, CA 95616

Mice of several strains were immunized with tobacco mosaic virus protein. Serum antibodies were tested for binding with the whole protein, with a decapeptide representing residues 103 to 112 of the protein, and with a panel of synthetic peptides representing the native sequence and substituted analogues in the region of amino acids 93 to 117 of the protein. The binding with the whole protein was similar for all strains. In contrast, the pattern of binding with the peptide panel differed among the strains, indicating that distinct antibody specificities are produced by different strains. Moreover, a remarkable constancy of binding with certain peptides was noted within certain strains. These findings, coupled with the observation that allotype congenic strains showed different binding patterns, suggest that the peptide panel affords a method for characterizing V region gene expression. This method can be compared with idiotypic analysis. Detection of anti-decapeptide antibodies from individual mice by isoelectric focusing showed restricted heterogeneity.

Unexpectedly, F_1 mice of parental strains which exhibit different anti-decapeptide specificities express the specificity of one or the other parent but never of both. These findings indicate that regulatory events are maintaining the restricted response.

246 MACROPHAGE PRODUCTS ARE REQUIRED FOR MITOGEN-INDUCED CLONAL B LYMPHOCYTE PROLIFERATION
Gayle D. Wetzel and John R. Kettman. Univ.Tx. Health Science Center, Dallas, Tx. 75235.

The role of macrophages and their products in B cell proliferation was examined using a microculture system allowing observation of culture wells receiving a single input B lymphocyte. Some fetal bovine sera (FBS) supported lipopolysaccharide (LPS) plus dextran sulfate (DXS)-induced growth of about 80% of the input splenic B cells. Other FBS, however, provided less support and B cell cloning efficiencies of 10% or less were observed. The presence of a few clones of irradiated, macrophage-like, adherent splenocytes restored LPS+DXS induced clonal B cell growth to levels obtained in fully supportive FBS. The presence of macrophages also extended the duration of LPS+DXS induced B cell growth resulting in larger average clone size. Adherent, macrophage-like cells, free of lymphocytes, obtained from two or more week old cultures of T depleted spleens were activated with LPS for two days. Supernatants were precipitated with ammonium sulfate and tested for their ability to replace macrophages in our B cell cloning system. In deficient FBS, activated macrophage supernatants increased the frequency of LPS+DXS induced B cell growth several fold. Similarly treated supernatants from the macrophage-like P388D1 cell line also improved clonal B cell growth. T cells did not appear to be required since T depleted splenocytes were induced to grow as were isolated B cells. These observations suggest that a macrophage factor(s) is required for mitogen-induced clonal B cell growth. The factor(s) may render B cells responsive to mitogens or act as a growth factor.

247 LONG TERM CHIMERA ARE OBTAINED IN A CELL TRANSFER SYSTEM. Tova Francus and Gregory W. Siskind, Cornell Medical College, New York, N.Y. 10021

The allotype congenic pair C57BL/6 (Igh^b) and B.C-9 (Igh^a) was used in a cell transfer system (a) to verify the state of chimerism in this system, and (b) to learn whether the ontogeny of the immune response to dinitrophenylated-bovine gamma globulin (DNP-BGG) is linked to the heavy chain allotype. Lymphoid cells from donor mice of ages varying from neonate to adult were used to reconstitute lethally irradiated syngeneic or allotype congenic recipients. Recipients were immunized with DNP-BGG either one day or four months after cell transfer, and their anti-DNP antibody response and the allotype of their anti-DNP antibodies were assayed by a solid state radioimmunoassay. All of the anti-DNP antibodies in each of the recipients studied, and regardless of the age of the donor or the length of time between the cell transfer and the priming, were of donor cell allotype. Similarly, the secondary responses were also exclusively of donor allotype. A state of long term chimerism was clearly established. The ontogeny of the response to DNP-BGG by B.C-9 (Igh^a) was studied in this system, using lymphoid cells from donor mice of different ages. It was found that the adult-like response, as measured by the level of the anti-DNP antibodies in the sera of the recipients, and by the affinity of their splenic PFC, appears at about 4 weeks of age, which is also the age at which the response matures in the allotype congenic partner C57BL/6 (Igh^b), thus suggesting absense of linkage to the heavy chain allotype. Sup. NIH grant no. AG01855.

Endogenous Idiotypic Regulation

248 PRODUCTION OF AUTO-ANTI-IDIOTYPIC ANTIBODY DURING THE NORMAL IMMUNE RESPONSE, Edmond A. Goidl, A. Faye Schrater, G. Jeanette Thorbecke and Gregory W. Siskind from the Department of Medicine, Cornell University Medical College, New York, N.Y. and the Department of Pathology, New York University School of Medicine, New York, New York

We have previously presented evidence that hapten-augmentable plaque-forming cells (PFC) are cells whose secretion of antibody is specifically inhibited by auto-anti-idiotype antibody, which can be displaced by hapten. We have shown that the primary immune response to a thymic-independent (TI) antigen, trinitrophenylated-lysyl-Ficoll (TNP-F), is down-regulated by the production of auto-anti-idiotypic antibody. We now present evidence that the primary and secondary immune responses to a thymic-dependent (TD) antigen, TNP-bovine gamma globulin (TNP-BGG), are down-regulated by the production of auto-anti-idiotypic antibody. Our data indicate that the secondary immune response to the TI antigen TNP-F is down-regulated by auto-anti-idiotypic antibody. In the secondary response to TNP-F, the rate of appearance of auto-anti-idiotypic antibody is faster and the number of hapten-augmentable PFC is greater than in the primary response, suggesting an anamnestic auto-anti-idiotypic antibody response. With the TD antigen TNP-BGG, the kinetics and magnitude of the auto-anti-idiotypic antibody response are relatively similar in the primary and secondary responses. Both direct and indirect hapten-augmentable PFC are seen suggesting that the secretion of both IgM and IgG antibodies is regulated by auto-anti-idiotypic antibody. It should be emphasized that in the studies reported here the response to the TNP determinant, especially when presented on BGG, is highly heterogeneous. Thus, auto-anti-idiotypic antibody production can be involved in the regulation of even highly heterogeneous responses. (Supported by NIH grants AI-11694, AG-01885, AG-02347 and AI-3076.)

249 RESCUE OF A CLONE SUPPRESSED BY AUTOGENOUS IDIOTYPE, Donald A. Rowley, La Rabida-University of Chicago Institute and Departments of Pathology and Pediatrics, University of Chicago, Chicago, IL 60637

Specific suppression is produced by immunization of A/He mice with the phosphorylcholine (Pc) binding IgA myeloma protein secreted by the plasmocytoma HOPC-8 (H8). Such actively immunized mice are unresponsive to immunization with Pc containing antigens. Conversely, A/He mice actively immunized with Pc are unresponsive to immunization with H8 (1,2). The process of suppression makes tolerant or eliminates mature B cells of the suppressed clone. Presumably specific unresponsiveness mediated by a prior complementary response must occur in nature and be disadvantageous to the individual under some circumstances. If this is so, then the objective is to devise ways to suppress the prior response and rescue the suppressed response.

Termination of suppession presumably occurs because the suppressor system is exhausted or eliminated. Sublethal irradiation, or other procedures which destroy lymphocytes, may temporarily eliminate lymphocytes involved in suppression. Following irradiation, treatment with antibody of the specificity produced by the suppressed clone might neutralize remaining complementary antibody or cells, and also tolerize or eliminate regenerative lymphocytes bearing complementary receptors. In this way the passively given antibody might permit regeneration of mature cells from stem cells or from immature progenitor cells of the suppressed clone.

Therefore, A/He mice suppressed by prior immunization with H8 were treated with X-irradiation (300R) alone, with αPc antibody alone, or with both. (The αPc antibody was IgM, T15 idiotype, secreted by a hybridoma designated M2, produced and kindly supplied by Patricia Gearhart.) The αPc was injected I.P. beginning the day after irradiation. A total amount equivalent to that produced by primary immunization of normal mice with Pc in adjuvant was given over a 7 to 10 day period. Combined treatment with x-ray and αPc caused recovery of the capacity to respond to active immunization with Pc. Recovery was slow, requiring 1 to 2 months. The recovered response was of T15 idiotype. Treatment with irradiation alone or αPc alone was totally ineffective.

The capability for specifically suppressing one response and then rescuing an autogenous complementary response may be a clinical objective for treating some autoimmune diseases and cancer. The present model may provide a conceptual basis for achieving this objective.

1. Rowley, D.A., et al. J. Exp. Med. 144, 946, 1976; 2. Rowley, D.A., et al. J. Exp. Med. 148, 148, 1978.

250 CORRELATION OF ENDOGENEOUS AUTO-ANTI-IDIOTYPIC ANTIBODY SYNTHESIS WITH IDIOTYPIC REGULATION IN OUTBRED RABBITS. L. Scott Rodkey. Division of Biology, Kansas State University, Manhattan, KS. 66506

Studies were initiated to determine if outbred rabbits were capable of mounting auto-anti-idiotypic (AAI) antibody responses specific for autologous antibody idiotypes that had been synthesized earlier by the same individual. Rabbits were immunized with p-aminophenyl-N-trimethylammonium chloride (TMA) coupled to keyhole limpet hemocyanin and the anti-TMA antibodies were specifically purified. Anti-TMA antibodies were reinjected into the same individual that synthesized the antibodies. Radioimmunoassays (RIA) were used to show the presence of AAI antibodies after reinjection of autologous anti-TMA antibodies. The reactions were hapten-inhibitable. The specificities of AAI antibodies were compared with those of isologous anti-idiotypic antibodies and RIA showed that the two kinds of antibodies recognized the same idiotopes. Studies designed to detect natural AAI antibody production following immune responses were initiated. One rabbit was identified which appeared to have modified an immune response by the natural production of AAI antibodies following multiple immunizations with M. lysodeikticus. A dilution-induced precipitation reaction was observed which was caused by the interaction of idiotypic antibodies specific for M. lyso., AAI antibodies, and an Fc-specific rheumatoid factor. The AAI antibody was present in alternate rounds of immunization. The quantity of AAI was found to be 3-fold higher in 4th-round sera than in 2nd round. AAI peaked in quantity at 7 weeks in both 2nd and 4th rounds and AAI appeared to be synthesized cyclically. Radioautographic isoelectric focusing results showed that AAI synthesis caused substantial shifts in antibody clonotype patterns. Antibody clonotype patterns of 1st-round sera, with antibodies focusing in the range of pH 6.5 to 7.5, were modified after AAI synthesis in 2nd-round by elimination of 1st-round clonotypes and replacement of these by a new clonotype set focusing at pH 5.5 to 6.5. Third-round sera contained antibodies characteristic for both 1st- and 2nd-round clonotypes. Fourth-round sera contained AAI and showed a deletion of antibodies similar to the results from 2nd-round. Further results showed that clonotype shifts occurred frequently in rabbits given multiple injections of M. lyso. Clonotype shifts have been observed ranging from complete loss of all clonotype followed by replacement with all new clonotypes to loss of only one clonotype when 2nd- and 1st-round sera were compared. NSF-PCM-7921110

251 THE DEVELOPMENT AND FUNCTIONS OF IMMUNOGLOBULIN DEPENDENT T CELLS. Charles A. Janeway, Jr., Kim Bottomly, Elaine A. Dzierzak, Diane Eardley, Scott Durum, and Dick Gershon. Department of Pathology, Yale University School of Medicine, New Haven, CT 06510.

These studies involve T cell functions in mice deprived of B cells by means of repeated injections of antibody to u chains. In such mice, T cells responding to major histocompatibility complex antigens, or providing helper function to B cells via a hapten-carrier bridge are present at normal levels. By contrast, a second helper T cell whose activity is particularly important in the early phases of an antibody response is missing or grossly deficient in such mice. This cell has been characterized in responses to the haptens DNP and phosphorylcholine and to sheep red blood cells. It has the surface Lyt antigen phenotype Lyt-$1^+,2^-$ and is specific for antigen, but does not require a hapten-carrier bridge. Its activity on B cells requires the presence of a second helper T cell that is present in anti-u treated mice and operates via a hapten carrier bridge. These mice are also missing or deficient in Lyt-$1^+,2^-$ cells necessary for the induction of feedback suppression. Taken together, these facts suggest an intimate relationship between this helper T cell and the inducer cell in the feedback circuit. Since the helper cell is responsible for activation of idiotype-bearing B cells, the inducer may be important in down-regulation of idiotype expression. We have tested this further by examining a factor that activates both activities when incubated with Lyl cells, by determining the time course of expression of an idiotype associated with the antibody response to DNP, and by anti-u treating MRL-lpr-lpr mice, which accumulate Lyl cells in their lymph nodes. These experiments support the general concept that a major subpopulation of T cells is immunoglobulin-dependent and perhaps also immunoglobulin-specific.

252 T LYMPHOCYTE REGULATION OF MYELOMA CELLS IN VITRO. Abul K. Abbas, Steven J. Burakoff and M.I. Greene. Harvard Medical School, Boston, MA 02115

The use of myeloma cells as targets for specific immunologic regulation permits analysis of the surface determinants recognized by regulatory cells and the mechanisms of action of such cells. Suppressor T cells are induced by immunizing mice i.v. with syngeneic splenocytes to which the IgA,λ 2 protein of MOPC 315 cells is covalently coupled. Such suppressor cells inhibit IgA secretion by the myeloma following 3-5 days of co-culture. The suppressor cells can be specifically bound to dishes coated with the relevant myeloma protein. Moreover, suppressor specific for M315 (IgA,λ2) or MPC 11 (IgG2b,k) inhibit only the relevant parent line. When co-cultured with a somatic cell hybrid produced by fusing MOPC 315 and MPC 11 cells, each suppressor inhibits secretion of only that immunoglobulin whose idiotype is recognized on the hybrid cell surface.

In a different system BALB/c trinitrophenol (TNP)-reactive cytolytic T lymphocytes (CTL) when cultured with TNP-binding MOPC 315 cells and TNP-proteins, inhibit antibody secretion by the myeloma. This interaction of CTL and myeloma targets is hapten-specific, H-2 restricted and requires specific receptor on the target. Under these conditions, CTL inhibit secretion of both IgA and IgG by the hybrid myeloma line.

Thus, myeloma targets can be regulated by idiotype- and antigen-specific T lymphocytes, and can be used not only to distinguish the functional effects of different types of regulatory T cells but also to analyze their mechanisms of action.

253 IDIOTYPIC DOMINANCE AND SELECTIVE ISOTYPE SWITCHING IN A PRIMARY ANTIBODY RESPONSE, John D. Conger, George K. Lewis and Joel W. Goodman, Univ Calif, San Francisco, 94143

The hyperimmune response of A/J mice to the antigen ABA-KLH (p-azobenzenearsonate-keyhole limpet hemocyanin) includes Abs bearing a major cross-reactive idiotype (CRI; Nisonoff and coworkers). Little is known about the primary (1^0) response to this Ag. Difficulties in obtaining a detectable 1^0 response have now been overcome. Two points of particular interest have emerged from our studies of this early response. 1) The idiotypic dominance in the 1^0 is much greater than in secondary (2^0) or hyperimmune (H) responses. In the 1^0, IgM and IgG PFC regularly surpass 80% CRI^+ (by anti-CRI inhibition of PFC). In 2^0 or H responses, IgG PFC are $\geq$ 10x higher in numbers but only 30-40% CRI^+. The lower numbers of IgM PFC remain surprisingly high in CRI content (about 70%). 2) The kinetics suggest that selective IgM to IgG isotype switching based on idiotype occurs in the 1^0. In one study, IgG PFC first appeared on day 6 and were 100% CRI^+ while the IgM PFC on that day were 60% CRI^+ (significant difference, $p<.01$). In another study, the first IgG PFC (day 8) were 76% CRI^+ and increased to 90% CRI^+ (day 13). During the same period, IgM PFC declined from 62% to 30% CRI^+. This data is consistent with the idea that CRI^+ IgM PFC are selectively switched to CRI^+ IgG PFC. A possible explanation is that CRI^+ IgM PFC receive more T help than CRI^- IgM PFC and thus differentiate faster. The additional help might derive from Id-specific T_h cells, for which we have independent evidence. A limiting dilution analysis of anti-ABA precursors should indicate exactly how much the CRI is being "up regulated" in the 1^0 response.

254 HOMEOSTATIC CONTROL OF THE B-LYMPHOID SYSTEM, Juraj Ivanyi and Michael Ratcliffe, Dept. Exp. Immunobiology, The Wellcome Research Laboratories, Beckenham, Kent, UK.

The main attribute of the immune system rests in the specificity of its cellular and humoral elements: thus, specific regulatory mechanisms are a corollary expected from such a polymorphic biological system. However, alternative "natural" surveillance functions have been clearly implicated in reactions towards alloreactive, hemopoietic, and neoplastic cells. The existence of surveillance which selectively operates within the B lymphoid system is suggested on the basis of extensive studies of allotype-marked B cell interactions in the chicken. Isotype-specific markers (IgM-1 and IgG-1) with allelic exclusion in all B cells and allotype-disparate sublines of inbred strains were used. An antagonistic B-B cell relationship was demonstrated in two experimental models: 1. The recovery of B cells in cyclophosphamide treated juvenile birds was suppressed following the transfer of allotype disparate B cells. 2. Injection of anti-IgM-1 allotype antibodies to embryonal or neonatal hosts produced allotype suppression only in genetic (i.e. heterozygous) or somatic chimeras. Since T cells were not effective in any of the experimental protocols it is concluded that suppression of compromised (by cyclophosphamide or antibody) B cells is exercised by alternative normal B cells. Homeostatic mechanisms may mitigate apparently "suppressor cell"-mediated effects or allotype-linked specific manifestations, and the scope of this type of regulation is apparent from the stability (more than 1 year) of suppression of the relevant B cells. Homeostatic regulation plays an important role in controlling B cell recruitment during ontogenic development and may determine the hierarchy (i.e. clonal dominance) within functionally mature cell populations.

255 THE IDIOTYPE(S) OF MONOCLONAL ANTIBODIES, SPECIFIC FOR THE HEMAGGLUTININ OF MEASLES VIRUS, Jan Gheuens and Dale E. McFarlin, NIB, NINCDS, NIH; Bethesda, MD 20205

The hemagglutinin (HA) of measles virus is a glycoprotein (MW 76,000), that is localized on the surface of the virion and the membrane of infected cells. It is a major target for the immune response against the virus. Syngeneic anti-idiotypic sera were prepared against 3 monoclonal anti HA antibodies of BALB/c origin. Each antiserum defines a distinct idiotype, or set of idiotypic determinants (Id(s)), on the respective monoclonal antibody against which it was raised. The Id(s) of these murine anti HA antibodies have been termed the HAMM-1, HAMM-2 and HAMM-3 Id(s). Some (but not all) of the HAMM-1 Id(s) were expressed in the sera of 2 out of 12 BALB/c mice, hyperimmunized with measles virus. No HAMM-2 or HAMM-3 Id(s) were detected in the same sera. Anti-idiotypic sera specifically inhibited some of the biological activities of HAMM-1 and HAMM-3 Id(s) bearing monoclonal antibody. These include: (a) neutralization of measles virus in vitro; (b) binding to persistently infected cells; (c) inhibition of HA mediated Rhesus monkey erythrocyte agglutination. These functions of the HAMM-2 Id(s) bearing antibody could not be inhibited with anti-idiotypic sera. Autoanti-idiotype is believed to have an important role in immunoregulation. If autoanti-idiotypic antibodies are raised in an immune response against an epitope, present on a pathogen, our results indicate that such antibodies can interfere significantly with the effector function of idiotype bearing structures at the level of the pathogen. This should be considered along with the role of autoanti-idiotype in immunoregulation.

256 IDIOTYPE-SPECIFIC HELP ALONE CANNOT ACCOUNT FOR T15 DOMINANCE IN PRIMARY ANTI-PC RESPONSES, J. Quintáns, M. Loken, and Z.S. Quan, La Rabida-University of Chicago Institute, Chicago, IL 60649

(CBA/N x BALB/c) F_1 hybrid male mice are unable to mount anti-PC IgM PFC responses because they carry the CBA/N X-linked immune defect of B lymphocyte differentiation. Transplantation of splenic B cells from BALB/c mice restores responsiveness to thymus-dependent and thymus-independent PC antigens up to 8 months after cell transfer. Cytotoxicity studies demonstrate the donor origin of PFC generated in reconstituted NBF_1 mice. Although responsiveness to PC is restored permanently, we detect a shift in idiotype expression that leads to the loss of T15 idiotypic dominance 3 months after cell transfer. This shift originates from Ig- cells because Ig+ splenic cells purified in a Fluorescence Activated Cell Sorter maintain T15 dominance. Therefore, the Ig+ cells have a remarkable capacity to maintain responsiveness to antigens and can perpetuate idiotypic dominance if the stem cell pool is removed.

257 ONTOGENY OF T15 AND NON-T15 CLONES IN THE NBF_1 TRANSPLANTATION MODEL, Z.S. Quan and J. Quintáns, La Rabida-University of Chicago Institute, Chicago, IL 60649

We have examined the emergence of responsiveness to phosphorylcholine (PC) using the (CBA/N x BALB/c) (NBF_1) transplantation model. Earlier studies had established that the immunodeficient NBF_1 male is a permissive host for both T15 and non-T15 anti-PC clones. By determining the PFC responses of BALB/c fetal/neonatal liver cells transplanted into sublethally irradiated NBF_1 males, we have shown that fetal/neonatal liver does in fact contain progenitors for PC-specific B cells, which appear quantally in a well-defined sequence. In this system, PFC responses to thymus-dependent (TD) forms of PC emerge before those to putative thymus-independent (TI) class II PC antigens; both are preceded by responsiveness to TNP. In both TD and TI anti-PC responses, T15 dominance is initially seen, but it is eventually superseded by non-T15 dominance. However, reconstitution with neonatal liver cells of almost exclusively non-T15 (CBA/CaJ x CBA/N)F_1 mice do not exhibit the same pattern: non-T15 dominance can be reproduced in the NBF_1 host. The loss of T15 dominance cannot be reversed by pretreating the recipients with either parental serum; instead, the serum serves as a general immunosuppressant, with no effect on the idiotypic profile. Transplanted adult BALB/c B cells will also shift idiotype over a long period of time, as will neonatally tolerized or sublethally irradiated BALB/c mice. Based on this, we conclude that, in ontogeny, the T15 clone emerges first while non-T15 clones appear subsequently, and that T15 dominance in the BALB/c mouse cannot be solely due to a T helper-B cell loop.

258 THE EXPRESSION OF POLYCLONALLY DISTRIBUTED RECEPTORS WHICH RECOGNIZE THE M460 IDIOTYPE ON B CELLS. D.Primi, D.Juy, C.Le Guern and P.-A.Cazenave,Institut Pasteur, Unité d'Immunochimie Analytique, 28 rue du Docteur Roux, 75015 PARIS.

Some monoclonal anti-idiotypic reagents have been shown to induce polyclonal B cell activation.[1] One of these F6(51) (1) is specific for determinants expressed by the TNP binding MOPC-460 myeloma protein. Because of the importance of these findings we studied whether complementary polyclonal receptors capable of recognizing M-460 idiotype are also expressed on B cells.Thus 10µg/ml of M460 protein induced *in vitro*, strong B cell polyclonal activation, while anti-anti-M460 (Ab3), XRPC-25 and XRPC-24 myeloma proteins had no effect. Moreover MOPC-460 protein was found to trigger B cells from various strains of mice, thus excluding any linkage to Igh loci. Fab fragments of M-460 were more active than the intact protein. Activation was specifically inhibited by addition to cultures of DNP-glycine, thus excluding the possibility that contaminants are responsible for the triggering effect. The specificity of the receptor was studied by constructing the following hybrid molecules:(L_{460}-H_{X25}), (L_{X24}-H_{460}) (H_{460}-L_{X24}). As demonstrated by RIA only the first hybrid retained the M-460 idiotype. Interestingly when these hybrids were tested for polyclonal activation we found that only (L_{460}-H_{X25}) had B cell triggering activity. Finally indirect, fluorescent studies indicated that 20% of spleen cells and 90% of LPS blasts could be specifically stained by M-460 Fab fragments. Taken together the results indicate the possible existence of a network of triggering receptors.Biochemical isolation and characterization of the polyclonal anti-M460 receptor is currently in progress.

(1)Coutinho A., Forni,L. and Barnabé,R.R. (1980) Springer Seminars in Immunopath.3, 171.

259 MALE F_1 MICE CARRYING CBA/N IMMUNE DEFECT PERMIT EXPRESSION OF T15 AND NON-T15 IDIOTYPES. A. M. Stall, J. Quintans, Z. S. Quan, and M. R. Loken. La Rabida - University of Chicago Institute. Chicago, Ill. 60649

The ratio of T15:non-T15 clones (idiotype profile) of the anti-PC response in female (CBA/N x Balb/c) (NBF_1) hybrids differs from that observed in F_1 crosses between CBA/N and animals allotype congenic with Balb/c: C.AL-20, BAB/14, and C.B-20. (CBA/N x C.B-20)$_{F1}$ female mice immunized with PC-KLH give 80% $T15^+$ anti-PC IgM PFC whereas (CBA/N x C.AL-20)$_{F1}$ female mice give 20% $T15^+$ PFC. This is in contrast to the 1:1 ratio of T15:non-T15 clones observed in NBF_1 females.

Splenic B cells from these F_1 females upon transfer to unirradiated male F_1 mice carrying the CBA/N immune defect are able to respond to the thymus dependent antigen PC-KLH. The idiotype profile of the anti-PC IgM PFC response is the same whether observed in the intact donor or after transfer into the unirradiated host. The high T15 expression of (CBA/N x C.B-20)$_{F1}$ hybrid B cells is retained after transfer into the unirradiated NBF_1 male mice. Similarly the 1:1 ratio of T15:non-T15 clones found in the NBF_1 female or BNF_1 B cells is maintained after transfer to (CBA/N x C.B-20)$_{F1}$ or (CBA/N x C.AL-20)$_{F1}$ male mice.

Under these conditions allotype restricted help is not observed. These data indicate that the immunodefficient males do, however, provide adquate help for the PC response and do not change the idiotype representation.

260 CHARACTERIZATION OF REGULATORY T CELL POPULATIONS INVOLVED IN ANTI-PHENYLTRIMETHYLAMMONIUM (TMA) ANTIBODY RESPONSE, Clifford J. Bellone and S. Jayaraman, St. Louis University School of Medicine, St. Louis, MO 63104

There are two distinct, functionally specialized sets of Lyt.1$^+$ helper T cells which act synergistically in the activation of memory B cells to produce an idiotype positive PFC response to TMA in vitro. One of these helper T cell (T_{h1}) is carrier-specific and acts via an antigen bridge in activating B cells. The other helper T cell (T_{h2}) has specificity both for idiotype and for carrier, but does not require a hapten-carrier conjugate to activate B cells. In addition, treatment of the helper T cell population with anti-idiotypic antisera and C' abolished helper function specific for TMA, suggesting either the presence of idiotypes on the T_{h1} carrier-specific population or a third T_h population which is specific for TMA and bears the associated idiotype. Further evidence for the presence of an idiotype-bearing T_h population comes from experiments in which anti-idiotypic antiserum induces a T_h population which dramatically enhances the PFC response to TMA.

261 REGULATION OF HAPTEN-SPECIFIC DELAYED-TYPE HYPERSENSITIVITY BY L-TYROSINE-P-AZOPHENYLTRIMETHYLAMMONIUM (TYR(TMA) INDUCED SUPPRESSOR T CELLS, S. Jayaraman and Clifford J. Bellone, St. Louis University School of Medicine, St. Louis, MO 63104

Syngeneic spleen cells coated with the diazonium salt of phenyltrimethylammonium induced a hapten-specific T-cell-mediated immunity in A/J mice. Since we have already observed that A/J mice primed for six weeks with tyr(TMA) develop suppressor T cells which selectively shut down idiotype$^+$ anti-TMA plaque forming cells (1), we wanted to know if the same suppressor population would also shut down TMA specific DTH. These suppressor T cells inhibited both the afferent and efferent phases of TMA specific DTH. The T_s cells were Lyt.2$^+$ and H-2 restricted. In addition, adsorption experiments indicate that these suppressor T cells are specific for idiotypes and not for TMA antigen. The regulation of DTH to TMA possibly occurs via idiotype-anti-idiotype interactions. Genetic analysis revealed that the ability of anti-idiotypic antibodies to induce TMA-specific DTH was linked to the IgH-1 heavy-chain allotype.

(1) Alevy, Y.G., and C.J. Bellone. J. Exp. Med. 151: 528, 1980

262 THE RECOGNITION OF MECHANISMS REGULATING IDIOTYPE PRODUCTION VIA TRANSFER OF IDIOTYPE SPECIFIC IMMUNE REACTIVITY TO PROGENY, Joan C. Olson and Gerrie A. Leslie, University of Oregon Health Sciences Center, Portland, Or 97201. Id-1, a public idiotype associated with rat anti-group A streptococcal carbohydrate (anti-SACHO) antibodies appears to be coded by germline genes, but its pattern of inheritance is complex. Some of this complexity could be attributed to the immune status of mothers which had been immunized with antigen prior to birth of progeny. Foster mother studies confirmed that the observed influence could be induced by idiotype-specific immune reactivity of females, independently of gene transfer. The relationship between maternal and progeny Id-1 production, however, was not a direct one. Females which produced extremely high Id-1 concentrations (>10 mg/ml) had progeny with lower Id-1 levels, while females with low Id-1 produced progeny having either suppressed or enhanced Id-1 levels. We later observed that the type of influence induced by low Id-1 females could be related to the mechanism responsible for their low Id-1 levels. That is, females suppressed for Id-1 by neonatal injections of anti-Id-1 had progeny with enhanced Id-1 production. In contrast, >50% of the progeny of females either naturally producing low Id-1 levels or suppressed for Id-1 by injection as adults with anti-Id-1 were also Id-1 suppressed. In regard to Id-1 expression, all females in these studies were identical (10 µg/ml Id-1 vs. 3-6 mg/ml anti-SACHO) yet their transfer of opposite influences to progeny indicates that at least 2 functionally distinct mechanisms for Id-1 suppression were involved. The different influences may be a reflection of a "stage" of Id-1 suppression in females or of the tenuous balance of Id-1 regulatory factors required to maintain Id-1 suppression. Thus, alteration of this balance upon transfer of factors to the neonate can lead to enhanced, rather than suppressed, Id-1 production.

263 Altered Idiotype Expression in CBA/N and (CBA/N X BALB/c)F_1 Male Immune Defective Mice following Immunization with PC-KLH. JAMES J. KENNY, GRETCHEN GUELDE and IRWIN SCHER, Uniformed Serv. Univ. of the Health Sci. and Naval Med. Res. Inst., Bethesda, MD 20014.

Our studies demonstrate that immune defective mice produce anti-phosphocholine antibodies following immunization with the T dependent antigen PC-KLH. However, this response is distinct from that of immunologically normal mice in that the majority of immune defective mice (group 1,

~65%) fail to produce the normally predominant T15 idiotype. The remaining defective mice are capable of producing anti-PC antibodies of the T15 idiotype and can be divided into two groups, those that make very low levels (group 2, ~25%) and those that produce levels of T15 idiotype which are indistinguishable from the normal mice (group 3, ~10%). The kinetics of the immune response and the class of anti-PC antibodies in this high T15 responder group are also indistinguishable from normal mice in that IgM, anti-PC antibodies are produced seven days after a primary injection of antigen. Like their normal F_1 female littermates, these high responder males have detectable levels of T15 antibody in their preimmune sera. On the other hand, the immune defective mice which fail to produce the T15 idiotype or produce only low levels of this idiotype: 1) exhibit a poor primary anti-PC response; 2) produce a secondary response which is 1/3 that of the normal controls and/or the high T15 responder males; 3) lack T15 idiotype positive antibodies in their preimmune sera even though substantial amounts of non-T15-anti-PC antibodies are present; and 4) fail to make IgM anti-PC antibodies. Further studies suggest that the high T15 responder group of immune defective mice may have Lyb5 positive B cells, and that this population of B cells is necessary for preferential expression of the T15 idiotype.

264 REGULATION OF SERUM ANTI-DNA ANTIBODY AND DNA-BINDING CELLS IN SYSTEMIC LUPUS ERYTHEMATOSUS (SLE) BY ANTI-IDIOTYPIC ANTIBODY. Nabih I. Abdou, University of Kansas Medical Center, Kansas City, Ks. 66103.

Regulation of the immune response by a network of antibodies has been postulated and shown. We tested the ability of normal (n=21) or SLE sera from active (n=14) or inactive (n=17) SLE to block ^{3}H-DNA binding to active SLE sera or to DNA-binding lymphocytes. The blocking sera were depleted of anti-DNA by DNA-cellulose immunoadsorbents and of DNA by sepharose-DNase treatment. The depleted blocking sera were then incubated with active SLE sera for 1 hr at 37°C and then 18 hr at 4°C. Controls blocking sera were normal sera or human serum albumin (HSA) processed similarly and at same protein concentration. Autologous sera from inactive SLE when compared to the control HSA blocking effects were more efficient in suppressing the binding of ^{3}H-DNA to active SLE sera; suppression = 71 $\pm$ 17% (mean $\pm$ SD) ($p=<0.01$). Active SLE sera were ineffective ($p>0.05$). Though 19% of normal sera were effective in blocking, the majority were not; mean of the group = 27 $\pm$ 14% ($p>0.1$). IgG but not IgM isotype and $F(ab)_2$, but not Fc fragments of the adsorbed inactive SLE sera were effective in blocking. The same IgG was capable of blocking blood mononuclear cell binding to ^{3}H-DNA ($p<0.05$) and in presence of complement decreased the number of DNA-binding cells as tested by autoradiography ($p<0.02$). Autologous IgG of inactive SLE sera can regulate binding of ^{3}H-DNA to serum anti-DNA antibody and to DNA-binding cell clones. The mechanisms responsible for anti-anti DNA production and its relationship to regulation by suppressor cells are unknown.

265 STIMULATION OF SPECIFIC ANTIBODY-FORMING CELLS IN ANTIGEN-PRIMED NUDE MICE BY THE ADOPTIVE TRANSFER OF SYNGENEIC ANTI-IDIOTYPIC T CELLS. Jan Cerny & Michael Caulfied, Harvard School of Public Health, Boston, MA 02115.

Previous experiments from this laboratory had demonstrated that the primary antibody plaque forming cells (PFC) response to Pn vaccine (S. pneumoniae R36a) in the spleens of euthymice mice is cyclical and that it is associated with a recipricol expansion of spleen cells bearing receptors for the T15 idiotype(i.e. anti-idiotypic cells). In particular, the second cycle of the PFC response on d 12 after immunization was preceded by a sharp peak of anti-idiotypic cells on d 9. In contrast, nude mice responded to Pn vaccine with only one cycle of the PFC response; both the second peak and the expansion of anti-idiotypic cells were absent.

In the present study, groups of euthymic BALB/c and nude mice (on the BALB/c background) were immunized with Pn vaccine. When the first cycle ofthe Pn-specific PFC response subsided (d 9 after immunization), splenic T cells from the euthymic mice were adoptively transferred to Pn-primed nude mice without an additional antigen challenge. The transferred T cells induced a second PFC peak three days later that was 3-10 fold higher than the response of control (Pn-primed)nude mice that received no T cells. This enhancement could be induced with T cells from either Pn-primed or normal donors; however, the induction of the second cycle of PFC required priming of the recipients. The T cells involved in the PFC stimulation were specific for the T15 idiotype since they could be removed on dishes coated with TEPC-15 myeloma proteins but not with other myeloma proteins (MOPC-315 or McPC-603). The results indicate that anti-idiotypic T cells may interact with antigen-primed B cells *in vivo* in a manner that leads to triggering of specific antibody production.

266 ANTIGEN ACTIVATED B-CELLS PRIME T-HELPER CELLS THAT ARE RESTRICTED TO COOPERATE WITH B-CELLS EXPRESSING THE PRIMING IgvH LOCUS ENCODED STRUCTURES, Johanna L'age-Stehr, Dept. of Virology, Robert Koch-Institut, Nordufer 20, 1000 Berlin 65, Germany

Transfer of antigen (SRBC) activated B-cells of BALB/c mice in CB 20 and BAB 14 mice (congenic to BALB/c at the Ig locus) indicate that T-helper cells are primed by B-cell products encoded for

by genes linked to Igv_H loci. The thus primed T-cells give rise to clones of antigen specific helper cells that are restricted to cooperate with B-cells displaying Igv_H locus encoded structures similar to those of the priming B-cells. These findings demonstrate that in the course of an immune response recognition of Igv_H gene linked products by e.g. complementary T-cell receptors leads to specific T-helper cell induction.

267 APPEARANCE OF A PREDOMINANT CROSS-REACTIVE IDIOTYPE IN THE PRIMARY RESPONSE IS PRECEEDED BY A LARGE POPULATION OF IDIOTYPE NEGATIVE PFC: Christopher D. Benjamin, Linda S. Wicker and Eli E. Sercarz, Dept. of Microbiology, University of California, Los Angeles, CA 90024. The murine secondary humoral anti-hen eggwhite lysozyme (HEL) response is characterized by a predominant idiotype (IdX-HEL) appearing on approximately 90% of HEL specific antibody. In this work, we show that IdX-HEL is essentially absent from IgG plaque forming cells (PFC) obtained from the draining lymph nodes 8 days after HEL immunization. This was established by incorporation of rabbit anti-IdX-HEL into a Cunningham PFC assay. By day 12 however, 80-100% of the PFCs are inhibitable with rabbit anti-IdX-HEL. Previous results from our laboratory have demonstrated that an idiotype specific T helper cell is critical to the development of an in vitro secondary anti-HEL PFC response. It is conceivable that this cell also plays a critical role in the primary selection of IdX-HEL bearing B cells.

Affinity maturation also occurs during the course of the primary anti-HEL response. Affinity is assessed by HEL inhibition and by selecting for high affinity PFC using red cells coated with suboptimal concentrations of HEL. Preliminary studies on the primary PFC response indicate that while IdX-HEL negative PFC dominate the response, most of the highest affinity PFC (15% of total response) are inhibitable by anti-IdX-HEL. Thus the selection of IdX-HEL positive B cells occurs after an initial burst of IdX-HEL-negative B cells. Concomitantly, there is a maturation of affinity for HEL in the population. We are currently examining the relationship between these two selection processes. Supported by AI 07126-03(CB), AI 07116-03 (LW) and ACS #IM-263.

268 ANTI-IDIOTYPIC AUTO-ANTIBODIES IN PATIENTS WITH FUNCTIONING RENAL GRAFT, Tetsuya Miyajima, Reiko Higuchi and Shigeyoshi Fujimoto, National Cardiovascular Center, Suita Osaka, and Department of immunology, Kochi Medical School, Kochi, Japan. Anti-idiotypic auto-antibodies directed to T cells which react with the donar MLR antigen(s) were detected in the sera of two kidney grafted recipients who have good renal function in spite of the withdrawal of an immunosuppressive drug, azathiopurine. These antibodies were identified by the following experiments. 1) The sera of the renal recipients specifically inhibited the mixed lymphocyte culture reaction (MLR) of a certain combination of third individual lymphocytes having the same mismatched HLA-DR antigens as those found between donar and recipient combination of renal grafting. 2) The inhibition of the MLR was found to be mediated by IgG antibodies of the patients' sera by the study of DEAE-cellulose column chromatography. 3) The inhibition of the MLR was only observed by the treatment of the responding but not of the stimulating cells with the antibodies. 4) The antibody activity was absorbed with the responding but not the stimulating cells. 5) Fifty to seventy percent of T cell blasts of the responder which stimulated with the donar cells by MLR were stained with the antibodies by indirect immunofluorescence, whereas any T cell blasts of irrelevant responders which stimulated with the same donar cells were not stained at all with the antibodies.
The antibodies in the patients' sera may have an important role for the acceptance of HLA-mismatched renal grafts.

269 EVIDENCE FOR IDIOTYPIC RECOGNITION BY T CELLS IN THE RESPONSE OF BALB/c MICE TO APOCYTOCHROME c, H.M. Etlinger, A. Accolla, C. Bron and G. Corradin, Basel Institute for Immunology, 4005 Basel, Switzerland, Ludvig Institute for Cancer Research, Epalinges, Switzerland, and Institut de Biochemie, University of Lausanne, Epalinges, Switzerland.

A Balb/c monoclonal antibody, A-1-59, specific for beef apocytochrome c, was prepared by cell fusion techniques utilizing the myeloma P3x63Ag8.653. A-1-59 was employed to generate a Balb/c hybridoma, Id-46, which secretes antibody specific for A-1-59 idiotope.

Serological analysis of the antibody response of Balb/c mice to beef apocytochrome c demonstrated that A-1-59 carries a major or common idiotopic determinant. Utilizing a T cell-dependent proliferation assay, lymph node cells from Balb/c mice primed with beef apocytochrome c were stimulated in vitro with beef apocytochrome c, A-1-59 or Id-46. Also, lymph node cells obtained from Balb/c mice primed with A-1-59 were stimulated in vitro with A-1-59.

Collectively, these data indicate that the B cell response to beef apocytochrome c in Balb/c mice is characterized by a major idiotope which is also expressed on T cells. Furthermore, the finding of both idiotypic and antigen-specific T cells in antigen-primed mice implies idiotypic regulation of the beef apocytochrome c response.