B LYMPHOCYTE DEVELOPMENT

Organizers: Roger M. Perlmutter, Max D. Cooper and Irving L. Weissman

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Hematopoiesis and Early B Cell Development

M 001 TRANSGENIC MICE AS TOOLS FOR DISSECTING MULTI-STEP HEMATOPOIETIC MALIGNANCIES, Suzanne Cory, Alan W Harris, David Vaux, Andreas Strasser, Helen Rosenbaum, S Peter Klinken, Warren Alexander, M L Bath, Judy McNeall and Jerry M Adams, The Walter and Eliza Hall Institute of Medical Research, Post Office, Royal Melbourne Hospital, Victoria 3050, Australia

To facilitate dissection of haematopoietic malignancy, transgenic mice predisposed to specific tumours have been developed using oncogenes coupled to the immunoglobulin heavy chain enhancer [Eμ], which promotes expression in lymphocytes and certain myeloid cells. Pre-B and B lymphomas were elicited by either a *myc* or N-*myc* transgene; plasmacytomas by v-*abl*; pre-B and T lymphomas by *bcr*-v-*abl*; and T cell and macrophage tumours by a mutant N-*ras* gene. In the preneoplastic state, both *myc* and N-*myc* promoted overproduction of cycling pre-B cells, whereas *bcl*-2, which favours cell survival, yielded an excess of resting B cells. Surprisingly, N-*ras* and v-*abl* had little effect on B cell development.

The transgenic strains are providing insights into oncogene collaboration. Most notably, most of the plasmacytomas of v-*abl* mice bear a *myc* gene that is rearranged, apparently by translocation to the IgH locus. The collaboration between v-*abl* and *myc* appears to be stage specific, since mice bearing both transgenes succumb rapidly to plasmacytomas but not pre-B or B lymphomas. A minority of Eμ-*myc* lymphomas bear *ras* mutations and a v-*ras* gene could hasten the lymphoma onset as well as transform the preneoplastic bone marrow cells. To provide access to other genes that can collaborate with a trans-oncogene, a retrovirus without an oncogene is being exploited as an insertional mutagen.

M 002 COMPLEXITY OF CELLULAR INTERACTIONS WITHIN THE BONE MARROW MICROENVIRONMENT, Paul W. Kincade, Kensuke Miyake, Shin-Ichi Hayashi, Kay Medina, Carolynn E. Pietrangeli, Anthony Namen, Charles Underhill and Jeffrey M. Gimble. Oklahoma Medical Research Foundation, 825 N.E. 13th Street, Oklahoma City, OK 73104.

Multiple adhesion, induction and recognition mechanisms are involved in the differentiation of stem cells to functional lymphocytes. Long term culture techniques have made it possible to effectively investigate these with murine bone marrow and to isolate cloned stromal cells which interact with B lineage precursors. However, culture models do not faithfully reproduce all aspects of the *in vivo* microenvironment and patterns of expression of the known cytokines do not explain why cells of a particular lineage grow. Analysis of cloned stromal cell lines has revealed that they have differentiation potential, most notably with respect to their ability to become fat cells. They are also responsive to a number of cytokines, including four of at least nine that they elaborate themselves. Of these, interleukin 7 is probably a necessary, but not sufficient, stimulus for long term lymphocyte replication. This conclusion results from studies with freshly isolated bone marrow cells and stromal dependent lymphocyte clones, which seem to fall into three categories. One clone is stromal cell dependent and dies after a brief response to IL-7, a second can grow continuously in IL-7 alone, and another can grow autonomously at high cell density. As is frequently the case with long term cultured lymphocytes, the phenotypes of the clones are unusual. However, they do provide valuable indicators of stromal cell derived activities. A number of known adhesion molecules are expressed on stromal cells and/or lymphocytes in long term bone marrow cultures. These include MEL-14, LFA-1, N-CAM, N-cadherin and CD44/Pgp-1. Antibodies to the latter totally block lymphopoiesis and myelopoiesis in culture and we have identified one probable ligand for this cell surface glycoprotein. Interactions of some lymphoid cells with hyaluronate coated dishes or stromal cells are prevented by one antibody to CD44, or by hyaluronate added to the medium. Although cell interactions mediated by this receptor - ligand combination may be critical to B lymphocyte formation, there is reason to believe that additional adhesion molecules must be involved. Experiments underway are designed to investigate that possibility.

M 003 RETROVIRUSES AS TOOLS TO MARK LYMPHOID STEM CELLS AND TO IDENTIFY DEVELOPMENTALLY REGULATED GENES IN STEM CELLS. R.A. Phillips, B. Holowachuk and F. Sablitzky, Research Institute, Hospital for Sick Children, and Department of Medical Genetics, University of Toronto, Toronto, Canada, M5G 1X8.

Studies on stem cells are limited by the necessity of having to assay their function by transplantation into recipient animals. Often after transplantation, the stem cell uses up its proliferative potential in reconstituting the recipient. Many investigators have recently used retroviruses to mark stem cells to follow their development within recipient mice. We have used this approach to follow the differentiation in immune deficient *scid* mice of stem cells obtained from long-term bone marrow cultures. We have found that such stem cells are deficient for myeloid reconstitution, but have lymphoid-restricted stem cells. More importantly, we have found that retroviruses prefer to integrate to developmentally regulated regions of the mouse genome. Thus, retroviruses can be used to mark transcriptionally active genes of the cell which they infect. Furthermore, retroviruses often come under the control of the transcriptional unit into which they integrate. By using retroviruses with weak promoters requiring enhancers, we have designed an enhancer trap retrovirus to identify transcriptionally regulated genes in hematopoietic stem cells.

M 004 CLONAL LYMPHOID PROGENITOR CELL LINES EXPRESSING THE BCR/ABL ONCOGENE RETAIN FULL DIFFERENTIATIVE FUNCTION. Peggy A. Scherle,* Kenneth Dorshkind,+ and Owen N. Witte* Department of Microbiology, Molecular Biology Institute, and Howard Hughes Medical Institute, University of California Los Angeles, 405 Hilgard Avenue, Los Angeles, California, 90024; and + Division of Biomedical Sciences, University of California, Riverside, California, 92521. The early stages of hematopoiesis have been difficult to study due to problems in obtaining homogeneous populations of progenitor cells which retain both self-renewal and differentiative capacities. We have developed an in vitro system in which transformation of murine bone marrow cells with the BCR/ABL oncogene, a gene product associated with stem cell leukemias, leads to the outgrowth of clonal lines which have an early lymphoid progenitor cell phenotype. The progenitor cells retain Ig heavy and light chain genes in a germline configuration. They give rise in vitro to pre-B cells which have diverse D-J rearrangements. On transfer to SCID mice, the progenitor cells differentiate to surface IgM^+, Ig-secreting B cells which respond to T cell help, function in an antigen-specific fashion and are of a diversity grossly comparable to B cells in normal BALB/c mice. Although growth-stimulated by BCR/ABL, the progenitor cells remain dependent for continued growth on a stromal cell derived soluble factor distinct from the pre-B cell growth factor, interleukin 7. These findings demonstrate that BCR/ABL can promote proliferation of an early hematopoietic progenitor cell without preventing its differentiation. This system provides a unique means of studying the complete B cell developmental process from a clonal progenitor cell to the end-stage plasma cell.

Control of Gene Expression During B Cell Development

M 005 MOLECULAR ANALYSIS OF TRANSCRIPTION FACTORS WHICH BIND BOTH THE IMMUNOGLOBULIN HEAVY CHAIN ENHANCER AND PROMOTER, Kathryn Calame, Chris Roman, Suso Platero and Nicole Avitahl, Department of Microbiology, Columbia University College of Physicians and Surgeons, New York, N.Y. 10032

At least three transcriptional activator proteins bind to sites in both the immunoglobulin heavy chain (IgH) enhancer and the V_{H1} promoter: Oct-2, uEBP-E and uEBP-C2(uE3). We have purified uEBP-E and uEBP-C2 to homogeneity and cloned cDNAs which encode them. Purified uEBP-E is a 44 kD protein which binds to the IgH enhancer and promoter sites with high affinity. Analysis of the predicted protein sequence derived from cDNA clones shows that uEBP-E contains a basic region immediately upstream of a heptad repeat of leucines, an arrangement shared by a family of DNA binding proteins including c-fos, c-jun, C/EBP, CREB and GCN4. By analogy, the leucine repeats of uEBP-E are probably dimerization domains and the basic region interacts with DNA. We are currently studying which members of this family may form heterodimers with uEBP-E. The uEBP-E gene is single copy in the genome and expressed at moderate levels in all tissues examined. Purified uEBP-C2 exists as either a dimer or a tetramer of 42-45 kD subunits. Amino acid sequence derived from cloned cDNAs shows that uEBP-C2 contains a basic region upstream of a "myc homology" region followed by a heptad repeat of leucines and finally by a proline rich region. "Myc homology regions" are shared by the myc proteins, Myo D, several drosophila proteins involved in neural development and USF transcription factor. Both the myc homology and leucine repeats are potential protein-protein interaction domains. We are testing the possibility that tetramers of uEBP-C2 could promote the formation of intrastrand loops between IgH promoters and the enhancer. The uEBP-C2 gene is single copy in the genome and is expressed at low levels in all tissues examined except brain, where its expression is strikingly elevated. We are exploring the possibility that this protein may play an important role in neural development or function. Negative regulation is also important for IgH transcription. A negative regulator, NF-uNR has recently been shown to bind four sites in the IgH enhancer. We show that this protein binds to the V_{H1} promoter as well.

M 006 REGULATION OF IMMUNOGLOBULIN GENE EXPRESSION DURING LYMPHOID DIFFERENTIATION

Rudolf Grosschedl, Thomas Jenuwein, Andre Darveau, and Adam Travis, Dept. of Microbiology and Immunology, Howard Hughes Medical Institute, University of California, San Francisco, CA.

Immunoglobulin (Ig) heavy and light chain genes are expressed only in B lymphocytes. Moreover, the genes are expressed in a temporally-ordered manner during cell differentiation. Transcription of the μ heavy chain gene precedes expression of the κ light chain gene. To examine the role of the enhancer and promoter for the temporal regulation of Ig gene expression, we generated transgenic mice carrying chimeric κ/μ genes in which the enhancer or promoter of the μ gene were replaced with the corresponding regulatory sequences from the κ gene. Replacement of the μ enhancer with the intragenic κ enhancer resulted in an altered temporal expression pattern. The level of expression of the chimeric transgene was low in pre-B cells that were derived from transgenic mice and could be induced to a high level by stimulation of the pre-B cells with bacterial lipopolysaccharide. The κ enhancer, however, was not sufficient to confer upon the μ gene the temporal expression pattern of the endogenous κ gene.

We also examined the functional importance of individual factor binding sites in the promoter and enhancer of the μ gene. In particular, μ genes carrying point mutations in the octanucleotide binding site of the lymphocyte-specific transcription factor Oct-2 were introduced into the mouse germ line and analyzed for expression. Mutation of the octanucleotide in the promoter reduced the level of μ gene expression in splenocytes by two orders of magnitude. By contrast, in fetal liver pre-B cells, the transcriptional effect of the octanucleotide mutation was only ten-fold. This suggests that the dependence of μ gene expression on a functional binding site for Oct-2 increases during normal cell differentiation. We examined the effects of the mutation of the Oct-2 binding site in the enhancer on the occupancy of other factor binding sites by genomic footprinting. The mutation of the octanucleotide abrogated factor binding at an adjacent site indicating a putative cooperative interaction between factors normally bound at both sites. The occupancy of other factor binding sites in the enhancer, however, was not abrogated by the mutation of the Oct-2 binding site.

M 007 OCT-2, A POU-HOMEOBOX TRANSCRIPTION FACTOR EXPRESSED IN B CELLS, Louis M. Staudt, Timothy Behrens, Sharon Doll, Patricia Fast, Jane Jensen, Hon-Sum Ko, Mitchell Rosner and Wayne Tsang, Alessandra Vigano, Metabolism Branch, National Cancer Institute, Bethesda, MD 20892. The octamer DNA motif, ATTTGCAT, is critical for the lymphoid-specific activity of immunoglobulin promoters and the immunoglobulin heavy chain enhancer. A family of transcription factors with distinct tissue distributions bind specifically and indistinguishably to the octamer motif. All B lymphocytes express a transcription factor, Oct-2, which is able to transactivate immunoglobulin promoters. Oct-2 has two regions of amino acid sequence homology with developmentally important proteins from lower eukaryotes, the homeobox and the POUbox. Oct-2 is expressed in lymphoid-myeloid progenitor cells that are capable of differentiating into either pre-B cells or mature macrophages in vitro, suggesting a role for Oct-2 very early in lymphoid-development. Oct-2 expression is not, however, confined to the lymphoid lineage. Oct-2 expression is maintained when the lymphoid-myeloid precursor cells are differentiated into mature macrophages. Some but not all T cells express Oct-2 and treatment of T cells with phorbol ester and calcium ionophore induces Oct-2 expression. Outside of the hematopoietic system, Oct-2 is expressed in the developing spinal cord. Thus, Oct-2 most likely performs multiple regulatory functions in multiple lineages. Oct-2 cannot, therefore, be solely responsible for B cell-specific gene expression but must act in concert with other B cell-restricted transcription factors to fully define the B cell phenotype.

Mechanisms of Gene Rearrangement

M 008 CONTROL OF RECOMBINATION EVENTS DURING LYMPHOCYTE DIFFERENTIATION. Frederick W. Alt, Pierre Ferrier, Paul Rothman, Suzanne Li, Beverly Gorham, Monica Mendelsohn, and Heikyung Suh. The Howard Hughes Medical Institute and Departments of Biochemistry and Microbiology, College of Physicians and Surgeons of Columbia University, New York, New York. 10032

The ability of VDJ recombinase to assemble particular gene segments in appropriate cell types and stages within lymphoid lineages appears to be effected by modulating accessibility of substrate gene segments to the common VDJ recombinase. Accessibility has been correlated with transcription of targeted unrearranged gene segments. To elucidate controlling elements, we created transgenic mice that carry a hybrid antigen receptor gene mini-locus in which germline TCR variable region gene segments (V, D, and J) were combined with various Ig or TCR receptor transcriptional regulatory elements including downstream heavy chain, light chain, or TCR enhancer elements and upstream TCR of IgH promoter elements. Consistent with the postulates of the accessibility model, we find that such transcriptional control elements can dominantly target rearrangement of the associated gene segments in a lineage specific fashion. We also have defined transcription units that initiate upstream of class-switch recombination target sequences of four different germline H chain genes including $\gamma 1$, $\gamma 2b$, $\gamma 3$, and ϵ.

Treatment of pre-B cell lines or normal splenic B cells with LPS or LPS plus IL-4 differentially induces transcription from these germline C_H promoters followed by induction of switch recombination to the corresponding genes. These results are consistent with the possibility that heavy chain class-switching is also controlled in the context of an accessibility mechanism. We have identified DNA regions that may be involved in regulating transcription of germline C region genes and, as a result, may also be involved in regulating switching. Current experiments to further define the relationship between germline transcription and recombinational accessibility will be discussed.

M 009 LYMPHOID VDJ RECOMBINATION IN NORMAL AND MUTANT CELLS, Michael R. Lieber, Pat McCloskey, George Gauss and Greg Daniels, Department of Pathology,Stanford University School of Medicine,Stanford,CA 94305.

We are interested in the mechanism and regulation of lymphoid VDJ recombination. One aspect that we are studying is the mechanism by which the VDJ recombination activity targets sites for recombination. In those phases of early B and T cell differentiation where the VDJ recombination activity is present, it is unclear why some loci remain unrecombined while others undergo active rearrangement. Previous research of others has demonstrated transcription and DNase I sensitivity of loci that are actively recombining. This has raised the possibility that transcription is linked to VDJ recombination in some way. We have examined VDJ recombination on extrachromosomal mini-chromosome substrates. On these substrates, we are varying transcription through the heptamer/nonamer recombination signal sequences over a wide range and are examining the extent to which VDJ recombination efficiency is dependent on transcription in the vicinity of or directly through the recombination signals. These studies should establish whether or not transcription plays an essential role in VDJ recombination.

We are also interested in the process by which the recombination activity and its two target sequences, the 12- and 23-base spacer heptamer/nonamer signals, interact. Recent work of others has raised the possibility of a tracking mechanism in which the activity binds at one site and pulls the DNA through until it finds the second site. We are testing this by using plasmids that have multiple 12-signals or multiple 23-signals. We are determining if there is any bias of the recombination activity to recombine proximal signals in preference to distal ones. In additional studies with multi-site constructions, we are comparing the recombination efficiency of non-consensus recombination signals with nearby consensus ones. The magnitude of these differences will determine the extent to which signal sequence variations can be responsible for known rearrangement patterns at the endogenous loci.

We are studying the murine scid defect in an effort to further understand VDJ recombination more generally. In particular, we are trying to discern why the signal joint is formed while completion of the coding joint is reduced 100 to 1000-fold in scid cells. We know that coding ends can participate in hybrid joint formation and homologous recombination in scid lymphoid cells relatively efficiently. We are exploring why the two coding segments can not join end to end to one another.

M 010 SOMATIC DNA RECOMBINATION IN ANTIGEN RECEPTOR GENES.
Hitoshi Sakano, Div. of Immunology, U.C. Berkeley.

Somatic DNA rearrangement plays a key role in generating the vast diversity of lymphocyte antigen receptors. Two blocks of sequences, CACTGTG and GGTTTTTGT, are conserved at V-(D)-J joining sites, and are assumed to be recombination signal sequences (RSSs) for a putative recombinase. We have previously reported a nuclear protein that specifically binds to an RSS probe but not to mutated RSSs. In order to isolate cDNA clones for the RSS binding protein, we screened a cDNA library of a pre-B cell line, 38B9. Proteins made from the T160 type cDNA clones showed strong binding to the RSS probe but not to the mutated probes. The C-terminal portion of the T160 protein is hydrophilic and rich in Arg, Lys and Ser residues, and is involved in interaction with the probe DNA. Moreover, the C-terminal region shows significant homology with a cruciform DNA binding protein known as HMG1.

We have previously shown that two pairs of heptamer and nonamer sequences are sufficient substrates to cause the V-(D)-J type of recombination. Effects of base substitutions and sequence changes in the RSS region were analyzed. We also studied the minimum distance between two RSS substrates that allows V-(D)-J joining. We observed a great reduction in recombination efficiency when the distance was shortened to 60 bp and no detectable joining was seen when the distance was 30 bp.

In lymphoid tissues, e.g., thymus and spleen, extrachromosomal circular DNA has been identified as a excision product of immunoglobulin or T cell receptor gene rearrangement. We noticed that circular DNA was also present in other tissues, such as brain. Although it is not clear whether a similar joining mechanism may apply, we have decided to study how the circular DNA in brain nuclei is generated. The similarities between the immune system and the central nervous system have long been discussed, particularly with respect to their memory functions and cell surface antigens. We are seeking to find whether some type of DNA rearrangement process plays a role in the development of nerve system cells. We constructed recombination substrates with two RSS's and the hsp70-lacZ fused gene, and introduced them into Drosophila embryos.

Antibody Repertoires and Somatic Mutation

M 011 DEVELOPMENT OF B CELL SUBSETS AND ANTIBODY REPERTOIRES, J. Kearney, N. Solvason, M. Vakil, A. Lehuen, and A.M. Hamilton, Division of Developmental and Clinical Immunology, Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL 35294 USA

The early B cell repertoire in mouse and man is characterized by (i) restricted V_H (and V_L) gene usage, and (ii) antibody specificities that are multireactive and interconnected. Furthermore, the phenotype of early B cells is predominantly that of the CD5 expressing B cell subset. The conserved nature of the B cell repertoire and the mutual interactions among the early appearing $CD5^+$ B cells are necessary to prime the development of B cells resulting in the acquisition of a mature B cell repertoire. To facilitate further functional and developmental studies of the CD5 B cell subset we have analyzed various embryonic tissues for the potential to give rise to $CD5^+$ B cells in scid recipients and have shown that 13 day fetal liver and omentum reconstituted $CD5^+$ B cells in the host peritoneal cavity. Serum levels of all isotypes were found in liver transplants but mostly IgM and IgG3 were found in omentum transplanted mice. These results suggest that the embryonic omental tissue may have a different potential for B cell development than fetal liver. We propose that the characteristic reactivities of B cells appearing early in development are a reflection of this CD5 B^+ cell subset which predominates at this time and is necessary for the establishment of the adult B cell repertoire. (Supported by NIH grants CA16673, CA13148 and AI 14782.)

M 012 EVOLUTIONARY DEVELOPMENT OF IMMUNOGLOBULIN GENE ORGANIZATION AND DIVERSITY, Gary W. Litman, Laboratory of Molecular Genetics, Tampa Bay Research Institute, 10900 Roosevelt Blvd., St. Petersburg, FL 33716

Comparisons of the structures of immunoglobulin genes found in species that represent critical points in the phylogeny of jawed vertebrates have provided a general overview of the evolutionary diversification of the rearranging antibody and T cell antigen receptor gene systems. Two major patterns can be discerned and are typified by: 1) the cartilaginous fishes, which possess multiple clusters of immunoglobulin genes; each cluster contains a variable (V), diversity (D), joining (J) and constant (C) segment, i.e. $(V\text{-}D\text{-}J\text{-}C)_n$ and 2) the bony fishes and all subsequent higher vertebrate forms in which V, D, J and C segments are organized in the tandem, iterative patterns described first in mammals, i.e., $V_n\text{-}D_n\text{-}J_n\text{-}C_n$. Immunoglobulin genes exhibiting the cluster-type pattern of organization share additional, unique structural and regulatory characteristics with both immunoglobulin and T cell antigen receptors found in higher vertebrates. In addition, the complexity of V_H gene families is restricted in these species. The tandem, iterative form of gene organization is associated with a full potential for combinatorial joining of segmental elements. At the phylogenetic level of amphibians, a degree of V_H gene complexity equivalent to that seen in mammals is observed. Somatic variation associated with immunoglobulin joining as well as regionalized somatic mutation and/or gene conversion are associated with the immunoglobulin genes found in species representing distant points in the evolution of jawed vertebrates. Efforts to detect cross-hybridization between highly conserved V framework and transmembrane sequences that have been identified in higher vertebrate immunoglobulin and potentially homologous sequences in species found below the evolutionary level of the jawed vertebrates have been unsuccessful; however, immunoglobulin-like heterodimers have been detected in the serum of these species. Genes encoding immunorecognition molecules found in species representative of these distant levels of vertebrate evolution may be encoded by highly divergent gene structures. Supported by NIH AI23338 and GM38656.

M 013 B CELL GROWTH AND SELECTION IN VIVO, Irmgard Förster, Hua Gu, Daisuke Kitamura, Werner Müller, Jürgen Roes, Birgit Schittek, Paulo Vieira and Klaus Rajewsky, Institute for Genetics, University of Cologne, Weyertal 121, D-5000 Cologne 41, F.R.G.

B cell growth and selection in vivo is studied by a combination of the following approaches: assessment of cell proliferation by BrdU incorporation and flow cytometric analysis; in vivo suppression of T cell help; analysis of V region genes expressed by defined B cell populations, using cDNA amplification libraries; and C_H gene targetting in embryonic stem cells. The data indicate that 1) during ontogeny, a stable compartment of $\mu^+\delta^+$ B cells, representing the majority of peripheral B cells and expressing a restricted V gene repertoire, is selected from the $\mu^+\delta^-$ B cells generated in the bone marrow; 2) the somatically mutated memory B cells generated in T cell dependent responses are stable and long-lived and 3) a distinct set of V regions is dominantly expressed in the Ly1 B cell compartment from early ontogeny. The gene targetting experiments aim at elucidating the role of the μ and δ isotypes in the various pathways of B cell selection.

M 014 CIRCULAR DNA AS A PRODUCT OF THE HEAVY CHAIN CLASS SWITCH, Uta K. von Schwedler and Matthias Wabl, Dept. of Microbiology and Immunology, University of California, San Francisco, CA 94143.

During heavy chain class switching the constant region of the immunoglobulin μ gene ($C\mu$) is deleted and replaced by a different C gene segment. Our laboratory has previously proposed that the underlying DNA rearrangement occurs via a looping out and deletion mechanism. This model predicts the existence of large ($\leq$ 200 kb) excised circles of DNA in lymphocytes that have switched in isotype.

To prove the looping out and deletion model, we have isolated circular DNA from nuclei of lymphocytes. We showed in Southern blot analyses that the circles contain $C\mu$ sequences that could not stem from chromosomal DNA contaminations. We have constructed a library of these switch circles in EMBL4 λ phage, and sequenced the recombination points.

B Cell Tolerance and Autoimmunity

M 015 NUCLEOTIDE SEQUENCE ANALYSIS OF HUMAN ANTIBODIES. Kim Victor, Jennifer Andris, Virginia Pascual and J. Donald Capra, Department of Microbiology, University of Texas Southwestern Medical Center, Dallas, TX 75235. Our laboratory has sequenced, at the nucleotide level, the heavy and light chain cDNAs from over 25 human antibodies with defined specificities. These include a number of autoantibodies (polyreactive, SS DNA, SM, rheumatoid factors, thyrogobulin, cold agglutinins, etc.) as well as antibodies to external antigens (HIV, HBV, Hib). The striking feature of these structures is that many (particularly the autoantibodies) are direct copies of germline V_H and V_L genes, exhibiting little or no somatic mutation. One striking structure contains germline V_H, D_H and J_H gene segments that involves a D-D fusion but is otherwise a nearly total VDJ germline structure. Antibodies to external antigens, in general, do not have clear cut germline counterparts. The pattern of mutation in the hypervariable regions as well as the third framework is suggestive of an antigen driven immune response. Finally, representation of several human V_HIV, V_HV and V_HVI gene families in these antibodies is clearly disproportional to the presumed number of germline genes in each family and the distribution of V_H sequences that has been deduced from the myeloma data.

Six human rheumatoid factor structures have been completed that were isolated from immortalized B lymphocytes from the synovial tissues of patients with active rheumatoid arthritis. Comparison of these structures with the previously defined mixed cryoglobulins detail several common structural features. The results will be discussed in relationship to the human B cell repertoire and the pathogenesis of autoimmune disease.

M 016 THE INFLUENCE OF ANTIGEN-ANTIBODY AFFINITY AND ANTIGEN VALENCY ON B-LYMPHOCYTE TOLERANCE, D. A. Nemazee*, J. Allison@, B. Arnold+, G. Haemmerling+, J.F.A.P. Miller@, G. Morahan@.,*National Jewish Center for Immunology and Respiratory Medicine, 1400 Jackson St., Denver, CO 80206, +Deutches Krebsforschungszentrum, Institut fuer Immunologie und Genetik, Im Neuenheimer Feld 280, D-6900 Heidelberg 1, @Walter and Eliza Hall Institute, P.O. Royal Melbourne Hospital, Victoria 3050, Australia.

B lymphocytes may be eliminated or rendered functionally unresponsive by interaction with antigens that bind to their immunoglobulin receptors. In mice expressing anti-H-2K antibody transgenes derived from the BALB/c (H-2^d) anti-C3H (H-2^k) hybridoma 3-83, B cell tolerance by clonal elimination is observed in vivo in the presence of the H-$2K^k$ antigen, to which the 3-83 antibody reacts with high affinity (1,2). We now find clonal elimination in the presence of the H-$2K^b$ antigen, which is bound with low affinity by 3-83, in 3-83 transgenic--> H-2-d/b radiation bone marrow chimeras. By contrast 3-83 transgenic mice do not exhibit deletional or functional B cell tolerance when crossed to a transgenic mouse line producing soluble H-$2K^k$, suggesting that antigen expressed on the plasma membrane, even when bound weakly, is better able to tolerize than a monomeric high affinity ligand. In addition, soluble H-$2K^k$ does not detectably inhibit tolerance to membrane-bound H-$2K^b$. Preliminary experiments examining B cell tolerance to transgene-encoded H-$2K^b$ antigens targetted to specific organs will also be presented.

1) Nemazee, D.A. & Buerki, K. (1989) Nature 337,562.
2) Nemazee, D.A. & Buerki, K. (1989) Proc. Natl. Acad. Sci. USA 86,8039.

M 017 AN ADULT MODEL OF IMMUNOLOGIC TOLERANCE WHICH FRUSTRATES AFFINITY MATURATION IN B LYMPHOCYTES, Gustav J V Nossal, Maria Karvelas, Michael McHeyzer-Williams and Paul Lalor, The Walter and Eliza Hall Institute of Medical Research, Post Office, The Royal Melbourne Hospital, Victoria 3050, Australia.

Within the primary B cell repertoire, there are remarkably few cells which can recognise protein antigens with high affinity. This became clear when methods were worked out to switch polyclonally-stimulated B cells from IgM to IgG_1 antibody-formation through the use of IL-4. The more demanding binding conditions for a bivalent (rather than a decavalent) clonal antibody product showed very few cells that were anti-KLH or anti-HSA in unimmunised spleen. When animals were immunised with alum-precipitated antigen and B.pertussis adjuvant, there was a sudden upsurge between days 5 and 7 of large numbers of anti-immunogen IgG_1 antibody-forming cell precursors in the spleen.

The injection of soluble antigen could abrogate this appearance of higher-affinity B cells. Remarkably small quantities of antigen sufficed - the end point being some where between 1 and 10 μg of soluble antigen. Furthermore, while the lower doses worked best when soluble antigen was given before challenge immunisation, the higher doses (eg. 1 mg) worked up to 4 days after challenge immunisation, i.e. just before the upsurge of the new clonotypes.

Two issues are the focus of present research. What is the upsurge in controls due to? Is it chiefly recruitment of new specificities of the pre-existing B cell pool, or might it represent very early V gene hypermutation? Secondly, is the soluble antigen effect a direct effect on the B cell or is it mediated via regulatory T cells? Adoptive transfer studies relevant to the latter issue will be presented. Current efforts are directed at pcr technology to study V gene hypermutation at the single cell level, and if work in the next three months has progressed to a sufficient stage, these results will also be presented.

B Cell Growth Factors and Their Receptors

M 018 MOLECULAR CHARACTERIZATION OF THE INTERLEUKIN-7 RECEPTOR. Steven Gillis, Linda Park, Steven Dower, Anthony Namen and Raymond Goodwin, Departments of Biochemistry, Experimental Hematology and Molecular Biology, Immunex Research and Development Corporation, Seattle, WA 98101

During the past decade, significant research investigation has focussed on elucidation of molecules which control lymphoid cell growth and differentiation. Molecular characterization of several cytokine genes, including those which encode the Interleukins 2, 4 and 6, has provided stable sources of recombinant hormones which have been shown to influence both B and T lymphocyte proliferation and differentiation. Our laboratories recently described the purification and molecular cloning of a novel cytokine, Interleukin 7, on the basis of its ability to stimulate proliferation of pre-B cells derived from long-term murine bone marrow cultures. Since the description of murine IL-7, human IL-7 gene products have been isolated and characterized and the product of that gene shown to play a major role in the development of immature and mature T-cells in addition to its effects on stimulation of early B cell precursors. In an effort to further categorize the range of IL-7 responsive cells, our laboratories have expended considerable effort towards characterization of the IL-7 receptor. Using relatively standard radiolabeling techniques, we succeeded in producing (125)I-labeled human and murine IL-7 and have used these reagents to determine biochemical characteristics of the IL-7 receptor. In addition, we have determined the range of distribution of IL-7 receptors on an extensive library of cell lines and primary tissues. Finally, using direct expression cloning techniques, we were successful in isolating a cDNA which encodes the human IL-7 receptor. The properties of this cDNA and its expression product will be discussed.

M 019 IL-6 AND ITS RECEPTOR IN IMMUNE REGULATION, T. Kishimoto, T. Taga, M. Hibi, M. Murakami, H. Yawata, S. Natsuka, T. Sugita, M. Saito, and T. Hirano, Institute for Molecular and Cellular Biology, Osaka University, 1-3, Yamada-oka, Suita, Osaka 565, Japan

IL-6 is a multifunctional cytokine active on B cells, T cells, plasmacytomas, hepatocytes and several other types of cells. In accordance with multifunctional properties of IL-6, the specific receptor for IL-6 was found to be expressed on a variety of cells. The cDNA for human IL-6R was cloned and the nucleotide sequence showed that IL-6R consists of 449 amino acids with an intracytoplasmic portion of ~82 amino acid residues. Extracellular portion of the IL-6R belongs to a newly identified cytokine receptor family. Although first 100 amino acids of IL-6R formed an Ig-like domain, deletion of this domain did not affect the function of the receptor. IL-6 was shown to be a potent growth factor for plasmacytoma/myeloma cells. However, the intracytoplasmic portion of IL-6R has no unique structure for the signal transduction, such as tyrosine kinase domain, suggesting the presence of another associated molecule which is responsible for the signal transduction. It was demonstrated that the binding of IL-6 with IL-6R triggers the association of this receptor with a non-ligand-binding membrane glycoprotein, gp130. Mutant IL-6R lacking the intracytoplasmic portion is functional, suggesting that the two polypeptide chains interact to involve their extracellular portion. In fact, a soluble IL-6R lacking the transmembrane and intracytoplasmic domains can associate with gp130 in the presence of IL-6 and mediate its function. These findings indicate that the IL-6R system consists of two polypeptide chains, ligand binding and signal transducing chains.This presentation will show the molecular cloning of a possible signal transducer, gp130. On the basis of its molecular structure, I will discuss about a novel mechanism of the signal transduction through cytokine receptors.

Signal Transduction Molecules in B Cells

M 020 THE MEMBRANE IMMUNOGLOBULIN ASSOCIATED "TRANSDUCER?" COMPLEX: STRUCTURE OF DISTINCT OLIGOMERIC, PHOSPHOPROTEIN COMPLEXES ASSOCIATED mIgM AND mIgD. John C. Cambier, Elizabeth Hager and Kerry S. Campbell, Division of Basic Sciences, Department of Pediatrics, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO 80206.

T and B lymphocyte antigen receptors exhibit single transmembrane spanning regions and very short, 3-5 amino acid, carboxyl terminal cytoplasmic tails. Ligation of these receptors leads, apparently through G-protein activation, to rapid stimulation of a polyphosphoinositide-specific phosphodiesterase (PPI-PDE). T lymphocyte antigen receptors ($\alpha\beta$) are coupled to PPI-PPE via a receptor-associated complex of membrane proteins, designated CD3. Although an analogous transducer complex is presumed to exist in B cells, no such structure has been defined. We utilized in vitro [^{32}P]-phosphorylation to identify and characterize a mIg-associated phosphoprotein complex consists of three N-glycosylated polypeptides which occur as disulfide-linked dimers, non-covalently associated with mIg. The complex associated with mIgM (pp32, pp34, and pp37 subunits) differs from that associated with mIgD (pp33, pp34, and pp37 subunits), and the isotype-specific phosphoprotein (pp32 or pp33) appears to exist as a disulfide-linked heterodimer with either pp34 or pp37. The lowest molecular weight mIgM associated protein appears to be identical to previously described B34 and MB1 (1). Aluminum fluoride stimulates phosphorylation of all of the subunits, and at least one of the proteins is phosphorylated on a tyrosine residue(s).

(1) Hombach et al, EMBO J. 7: 3451 and Sakaguchi et al EMBO J. 7: 3457.

M 021 ANTIGEN RECEPTOR SIGNAL TRANSDUCTION IN AN IMMATURE B LYMPHOMA CELL LINE, Anthony L. DeFranco, Department of Microbiology & Immunology, UCSF, San Francisco, CA 94143-0552.

During B lymphocyte development, cells that have just begun to express surface immunoglobulin (Ig) exhibit a negative response to antigen involving either death or anergy. Certain transformed B cell lines appear to also reside in this transitional immature differentiation state. They express cell surface markers characteristic of that stage, and they exhibit a negative growth response to anti-Ig antibodies, used as a surrogate for antigen. In addition to growth inhibition, anti-Ig also stimulates the phosphoinositide signaling pathway. To assess the importance of this early biochemical event, we have examined the roles of the phosphoinositide second messengers diacylglycerol and calcium in mediating the anti-Ig induced growth inhibition of WEHI-231 cells. Mimicking experiments with calcium ionophore and phorbol diester indicate that diacylglycerol and calcium are important mediators, but that there is probably a third important mediator as well. This mediator could be another second messenger resulting from phosphoinositide breakdown or it could be generated by a second signaling pathway also triggered by the B cell antigen receptor. In addition, we have selected mutants of WEHI-231 that are resistant to the growth inhibiting action of anti-Ig. Of seven independent mutants, two had defects in the synthesis or intracellular transport of membrane IgM. Four of the other five mutants had decreased phosphoinositide breakdown, apparently due to an altered phospholipase C. These mutants demonstrate the importance of the phosphoinositide signaling pathway for the negative response of these cells. Anti-Ig still induced some phosphoinositide signaling in these mutants, and calcium appeared to be the limiting second messenger. The final mutant was resistant to the growth inhibiting action of the second messenger mimicking agents, as well as that of anti-Ig. This mutant may be defective in a component that mediates the action of diacylglycerol and calcium or in a component of the growth regulatory machinery. The nature of these WEHI-231 mutants adds further support to the idea that phosphoinositide breakdown is an important signal transduction event induced by the antigen receptor of B cells.

M 022 CHLORIDE CHANNELS IN NORMAL AND CF-DERIVED B LYMPHOCYTES, Phyllis Gardner, Paul Nghiem, Jennifer Chen, Muhammad Schumann, and Howard Schulman, Departments of Medicine and Pharmacology, Stanford University, Stanford, CA. 94305.

A defect in regulation of a chloride channel appears to be the molecular basis for cystic fibrosis (CF), a common lethal genetic disease. We have used the patch clamp technique to demonstrate that a chloride channel with kinetic and regulatory properties similar to those described for secretory epithelial cells is present in EBV-transformed B cell lines. Cl^- channels can be activated by cAMP-dependent phosphorylation, as demonstrated in cell-attached patches of cells exposed to cAMP analogues or in excised patches exposed to purified subunit of cAMP-dependent protein kinase plus ATP. Importantly, activation of the channels by cAMP-dependent phosphorylation is defective in CF-derived cells (Chen et al., 1989). Cl^- channels can also be activated in cell-attached patches after exposure of the cell to Ca^{2+} ionophore; this pathway is preserved in CF-derived B cells. The Ca^{2+}-dependent pathway of channel activation was investigated with whole cell patch clamp studies. Perfusion of lymphocytes with peptide inhibitors of multifunctional Ca^{2+}/calmodulin-dependent protein kinase (CaMKII) or of calmodulin completely eliminated the Cl^- current induced by Ca^{2+} ionophore, while peptide inhibitor of protein kinase C had no effect. This data combined with preliminary studies of Cl^- channel activation in excised patches exposed to purified catalytic subunit of CaMKII, calmodulin, Ca^{2+}, and ATP suggest that CaMKII also activates channels by phosphorylation. We noted that Cl^- channel density varies greatly in unsynchronized lymphocyte populations. I^{125} efflux studies were performed to better assess the underlying reasons for channel density variation. Preliminary studies suggest that both cAMP- and Ca^{2+}-stimulated I^{125} efflux can be detected in normal lymphocytes. This I^{125} efflux is enhanced when cells are synchronized to G1, suggesting that channel expression varies with cell cycle. Finally, studies are underway to assess the relationship between the affected Cl^- channel and the recently cloned CF gene.

Chen JH, Schulman H, and Gardner P. A cAMP-regulated chloride channel in lumphocytes that is affected in cystic fibrosis. *Science* 243:657-660, 1989.

M 023 THE CD20 SURFACE MOLECULE OF B LYMPHOCYTES FUNCTIONS AS A CALCIUM CHANNEL, Thomas F. Tedder, Liang Ji Zhou, P. Darwin Bell, Raymond A. Frizzell, and James K. Bubien, Dept. of Pathology, Harvard Medical School, Boston, MA 02115 and Dept. of Physiology and Biophysics, University of Alabama at Birmingham, Birmingham, AL 35294.

CD20 is a B cell-specific, Mr 33,000 phosphoprotein expressed during most stages of B cell development. CD20 is directly involved in signal transduction since monoclonal antibody binding to CD20 can provide an activation signal and can also inhibit B cell proliferation and differentiation in response to mitogens. CD20 is not phosphorylated in resting B cells but becomes heavily phosphorylated following activation. The cloning of CD20 reveals that it is unique among lymphocyte cell-surface proteins with four membrane-spanning domains and with both ends located within the cytoplasm. In addition, immunoprecipitation of CD20 from B cells treated with DSP under mild conditions revealed the specific cross-linking of CD20 molecules with aggregate Mr of 70,000 and 140,000 composed of two and four cross-linked CD20 molecules. Thus, CD20 may form a multi-molecular complex on the cell surface with 8 to 16 transmembrane spanning domains. This putative structure is similar to that of other membrane-embedded proteins that can form transmembrane ion channels. Therefore, the role of CD20 in transmembrane ion transport was examined using whole-cell patch-clamp analysis to measure ionic currents. The binding of antibodies to CD20 on B cells led to a significant increase in instantaneous inward currents, while the binding of isotype matched antibodies to CD21 did not alter transmembrane ion currents.. Further studies showed that Ca++ was the charge carrier for the CD20-associated current and that culture with CD20 antibody resulted in higher intracellular free Ca++ levels. These studies were extended using CD20 cDNA-transfected Jurkat T cells. Similar analysis of the CD20 transfected cells showed that instantaneous inward currents were both larger and activated faster in the CD20+ cells than in cells transfected with vector alone. In addition, the CD20 transfected cells had two to three fold higher levels of free intracellular Ca++ than the vector transfected controls. These data indicate that CD20 contributes directly to the transmembrane ion flow in B lymphocytes and that CD20 antibody binding may affect B cell function by altering the regulation of intracellular Ca ion concentrations. These functional studies, coupled with the structural features of the protein, suggest that CD20 is either a Ca++ channel itself or regulates a membrane-associated Ca++ channel.

Developmental Abnormalities of B Cells

M 024 VDJ RECOMBINATION IN LEAKY SCID MICE, Melvin Bosma, Debra Kotloff, Norman Ruetsch, John Petrini and Ann Carroll. Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, PA 19111

Successful recombination of gene segments (V, D and J) that code for Ig and TCR variable regions cannot occur at appreciable frequency in developing scid lymphocytes because they express a defective VDJ recombinase activity. Most rearranged Ig or TCR genes in scid cells appear grossly abnormal and show deletions of one or both participating gene segments in a given attempted recombination (e.g., D to J or V to J). Successful V(D)J rearrangements occasionally do occur, however, as reflected by the appearance of pauciclonal populations of functional B and T cells in ~15% of young adult scid mice and in virtually all old scid mice (≥1 year). We refer to these mice as leaky scid mice. To investigate the basis for leakiness, alloreactive T cell lines were established from immunized leaky scid mice, and more recently, $C\mu^+$ pre-B cell lines were obtained by Abelson murine leukemia virus transformation of bone marrow cells from old leaky scid mice. All of these cell lines have been cloned. TCR γ and β loci of four T cell clones have been studied in detail. Southern blot analysis shows that three of the clones have apparently normal rearrangements at both expressed and nonexpressed TCR loci; moreover, the recombination junctions of seven of the nonexpressed loci have been cloned and sequenced and are normal. The remaining T cell clone, which contains four apparently normal TCR gene rearrangements, also contains two grossly abnormal rearrangements. All but one of the eight rearranged Ig heavy chain loci in four $C\mu^+$ pre-B cell clones examined to date appear normal by Southern blot analysis. The VDJ recombinase activity in one of these clones has been analyzed and found to be indistinguishable from that of normal cells. We conclude that reversion of the scid recombinase phenotype in one or more rare lymphoid progenitor cells may account for the few functional B and T cell clones in leaky scid mice.

M 025 X-LINKED AGAMMAGLOBULINEMIA: GETTING CLOSER TO THE GENE DEFECT, Mary Ellen Conley, Department of Pediatrics, University of Tennessee College of Medicine and Department of Immunology, St. Jude Children's Research Hospital, Memphis, TN. 38101.
Although X-linked agammaglobulinemia (XLA) was one of the first immunodeficiencies described, the defective gene product has not yet been identified. The disorder is characterized by severe hypogammaglobulinemia and markedly reduced numbers of B cells, but near normal numbers of pre-B cells in the bone marrow. In the last 4 years, we have taken a variety of approaches to exploring the failure of B cell differentiation in this disorder. First, to determine whether the gene defect was intrinsic to the B cell lineage or whether it might be the result of an abnormality of the bone marrow microenvironment, patterns of X-chromosome inactivation in B cells, T cells, platelets and neutrophils from obligate carriers of XLA were evaluated. The results of these studies demonstrated preferential use of the normal, non-mutant X as the active X in B cells, but in no other cell lineages from these women. This finding indicates that the XLA gene defect is intrinsic to the B cell lineage and that the gene product is not transportable between cells. Because the production of a functional immunoglobulin molecule is the hallmark of normal B cell differentiation, we then studied immunoglobulin gene rearrangements in B cell lines from XLA patients. Eighteen EBV transformed cell lines from 5 patients showed production of a variety of isotypes including IgM, IgD, IgG and IgA. The 12 IgM producing cell lines studied in greater detail showed heavy and light chain mRNA transcripts of native size with normal VDJ rearrangements. Southern blots demonstrated rearrangements of both alleles in most cell lines with clonally unrelated rearrangements in multiple cell lines isolated from the same individual. These studies indicate that the gene defect is unlikely to be related to immunoglobulin gene rearrangement. Further, the small number of B cells that could be identified in patients with XLA demonstrated an immature phenotype, with high density surface IgM, low density Ia and decreased expression of EBV receptors. The immature phenotype of the B cells suggests that the XLA gene defect may be expressed throughout B cell differentiation rather than at the single point of pre-B cell to B cell maturation. Finally, mapping studies by others have shown linkage of typical XLA to the long arm of the X at Xq21.3-22. To extend the definition of XLA, we have performed linkage analysis and X chromosome inactivation studies in families with atypical XLA; that is, XLA with growth hormone deficiency and XLA with higher than expected concentrations of serum immunoglobulins. These studies have suggested that the atypical forms may represent allelic variants of the typical forms.

M 026 ANTIBODY DEFICIENCY DISEASES IN HUMANS, Max D. Cooper, Division of Developmental and Clinical Immunology, University of Alabama at Birmingham and the Howard Hughes Medical Institute, Birmingham, AL 35294 USA
The normal development of B lineage cells begins in the fetal liver and continues thereafter in the bone marrow. Stromal cells that produce interleukin 7 (IL-7) and other cytokines provide important microenvironmental cues for pre-B cell growth and differentiation. Two cell surface metallopeptidases, CALLA/NEP and BP-1/6C3, are expressed on early B lineage cells in the bone marrow. These may protect the immature population of dividing B cell progenitors from premature exposure to various differentiation factors. During the pre-B cell phase of development, rearrangements of heavy chain genes usually, but not always, preceed light chain gene rearrangement. A small fraction of the μ chains synthesized by pre-B cells can reach the cell surface, escaping the endoplasmic reticulum retention protein (BiP) by associating with a surrogate light chain complex. However, both heavy and conventional light chains are required for IgM expression on newly-formed B cells that can migrate via the blood stream to peripheral lymphoid tissues. Given the complexity of this early differentiation process, it is perhaps surprising that more defects specifically associated with this stage of development have not been described. Even when all goes well during B cell generation and subsequent seeding to the periphery, arrests in the the equally complex program of clonal selection and terminal plasma cell differentiation may preclude normal antibody production. Two such immunodeficiency diseases, IgA deficiency and common variable hypogammaglobulinemia, appear to be associated with a gene defect in the MHC class III region. (Supported by NIH grants CA 16673 and CA 13148.)

B Cell Neoplasia

M 027 THE BCL-2 GENE ENCODES A NOVEL G PROTEIN, Subrata Haldar, Christine Beatty, Yoshihide Tsujimoto* & Carlo M. Croce. The Fels Institute for Cancer Research and Molecular Biology, Temple University School of Medicine, 3420 N. Broad Street, Philadelphia,, Pennsylvania 19140, USA, *The Wistar Institute, 3601 Spruce Street, Philadelphia, Pennsylvania 19103, USA.

Little is known about the biochemical or functional nature of the proteins encoded by the bcl-2 gene, which undergoes chromosomal translocation in B cell neoplasia. Translocation of bcl-2 sequences from chromosome 18 to the J_H segment of the immunoglobulin gene at chromosome band 14q32 in B cells results in deregulated expression of this gene, causing high steady state levels of bcl-2 messenger RNA. DNA sequences data indicate the bcl-2 encodes two proteins by virtue of alternative splicing, designated as Bcl-2α and Bcl-2β, with relative molecular masses of 26,000 and 22,000 respectively. Cell fractionation experiments indicate that the bcl-2α gene product is located at the inner surface of the cell membrane, suggesting a possible role in mitogenic signal transduction. We report here that Bcl-2α has GTP-binding activity and a protein sequence that suggests it belongs to the small molecular weight GTP-binding protein (G protein) family.

M 028 ONCOGENES IN HUMAN B-CELL MALIGNANCIES: DETECTION AND BIOLOGICAL ROLE. Riccardo Dalla-Favera, Department of Pathology, Columbia University, New York, NY 10032, USA.

B-cell malignancies in humans represent an heterogeneous group of neoplasia characterized by phenotypically distinct neoplasia such as acute lymphoblastic leukemia (ALL), non-Hodgkin lymphoma (NHL), chronic lymphocytic leukemia (CLL) and multiple myeloma (MM). These tumor subtypes have been found associated with different types of oncogenes including: i) H-, K- and N-ras. Point mutations at specific codons (12, 13, 61) of these genes were detectable in ALL (18% of cases) and multiple myeloma (30%), while no ras mutations were found in CLL and NHL (1-2); ii) c-myc. Activation by chromosomal translocation of the c-myc oncogene are found in high-grade non Hodgkin lymphoma (NHL), mainly Burkitt-type, in L3-type acute lymphoblastic leukemia (ALL) and in 70% of AIDS-associated non-Hodgkin lymphoma (AIDS-NHL) (3); iii) bcl-2. Activation of the bcl-2 oncogene by the t(14;18) chromosomal translocation is detectable in 90 % of follicular lymphoma and in 25% of diffuse lymphoma (4).

To gain insight into the role of these oncogenes in B-cell transformation, we studied their biological effects in an in vitro transformation assay in which EBV-immortalized B lymphoblastoid cells (LCL) are used as targets (5). In this assay, various oncogenes are introduced into LCL using plasmid or retroviral vectors and the cell phenotype is then analyzed for changes in transformation-related properties (serum-dependency, clonogenicity in semi-solid media and tumorigenicity in mice) as well as for changes in the expression of activation- or differentiation-associated markers detected by specific monoclonal antibodies. In this assay, all oncogenes induced malignant transformation, although at different degrees, but striking differences were detectable in the phenotype of the transformed LCL. In particular: i) H- and N-ras oncogenes induced malignant transformation and plasmacytoid differentiation leading to a phenotype analogous to MM cells (6); ii) the bcl-2 oncogene induced a weakly transformed phenotype with no apparent changes in the differentiation stage, but was very effective in potentiating c-myc induced transformation and tumorigenicity (7); iii) the c-myc oncogene induced transformation characterized by the down-regulation of the adhesion-receptor LFA-1 and by the ability of escape autologous T-cell mediated cytotoxicity, two features typical of Burkitt lymphoma cells.

We will discuss the implications of these findings for the role of the various oncogenes in different lymphoid malignancies as well as for the role of the corresponding proto-oncogenes in normal B-cell development.

1). Neri, A. et al. Proc. Natl. Acad. Sci. USA, 85, 9268-9272, 1988.
2). Neri, A. et al. J. Exp. Med., in press.
3). Subar, M., et al. Blood, 72, 667-671, 1988..
4). Bakshy, et al. Cell 41, 899-906, 1985; Tsujimoto, Y., et al. Science, 229, 1390-1393.
5). Lombardi, L., Newcomb, E.W., and Dalla-Favera, R. Cell, 49, 161-170, 1987.
6). Seremetis, S. et al. Science, 243, 660-663, 1989.
7). Nunez, G. et al. Proc. Natl. Acad. Sci. USA, 86, 4589-4593, 1989.

M 029 THE BCL-2 PROTO-ONCOGENE IN NEOPLASIA, S.J. Korsmeyer, T.J. McDonnell, G. Nunez, U. Jaeger, D.Hockenberry, and R. Young. Howard Hughes Med. Inst., Dept. Medicine, Mol. Microbiol., Washington Univ. Sch. Med., St. Louis, MO 63110.

Despite the mature B cell phenotype of follicular lymphoma the t(14;18) appear to occur early in pre B cell development juxtaposing a new gene from chromosome 18, Bcl-2, with the immunoglobulin (Ig) heavy chain gene locus at 14q32. Normal expression of Bcl-2 is associated with the activation of normal B cells. Bcl-2 is an enormous gene with an approximately 350 Kb intron and encodes a 25 KD intracellular membrane associated protein. The molecular consequence of translocation generates a Bcl-2-Ig fusion gene and chimeric RNAs. The Bcl-2-Ig allele is deregulated resulting in elevated RNA and elevated 25 KD Bcl-2 protein. Retroviral vectors introduced a deregulated Bcl-2 into B lymphoblastoid cells which improved their clonogenicity and complemented c-myc in promoting tumorigenesis. Moreover, deregulated Bcl-2 provided a death sparing effect to IL-3 dependent pro B lymphocytes, promyelocytes, and mast cells when deprived of IL-3. Bcl-2 did not influence cell cycle progression, but instead delayed the onset of death. This effect was not limited to the IL-3 pathway, however no effects were seen in IL-2-dependent T cells. Transgenic mice bearing a deregulated Bcl-2-Ig minigene produce excess 25 KD protein and develop a polyclonal follicular lymphoproliferation. Transgenics selectively accumulate a 4-fold excess of small IgM/IgD B cells that are resting in Go, but capable of normal proliferative responses. This select population demonstrates an extended survival. Moreover, Bcl-2-Ig transgenics show a progression from indolent to monoclonal high grade lymphoma providing a model to identify the 2° complimenting oncogenes.

B Cell Subsets; Microenvironment and Stem Cells

M 100 HUMAN LYMPHOID CELL LINES IMMORTALIZED BY TRANSFECTION WITH EXTRACHROMOSOMAL MOUSE DNA SECRETE THE CYTOKINES IL-1ALPHA, IL-6, AND TNF, Hinrich Abken, Christoph Bützler, Juliane Fluck, Gisela Wolff and Klaus Willecke. Institut für Genetik, Abt. Molekulargenetik, Universität Bonn Römerstr. 164, D-5300 Bonn 1, Fed. Rep. Germany
Human lymphocytes from peripheral blood were incubated with pokeweed mitogen and, subsequently, transfected with DNA isolated from cytoplasts of mouse L929 cells leading to formation of lymphoid cell lines of B- and T-cell origin with apparently unlimited growth potential. Using centrifugation of the cytoplast lysate in a CsCl density gradient, DNA with immortalizing activity could be isolated from a mitochondria depleted fraction of the cytoplasm. Two cell lines tested were found to secrete the cytokines IL-1alpha, IL-6, and TNF, whereas no activity of IL-1ß, IL-2, IL-4, IFN-γ, BCGF, or CSF could be detected in the conditioned medium (collaboration Dr. J. Banchereau). Since the immortalized cells grow in chemically defined, serumfree media without addition of cytokines, we suggest that these cytokines - by themselves or in addition to other host secreted factors - sustain continuous proliferation of these cells as part of a putative autocrine loop in growth control. Mature B-cell derived clones were immortalized that stably secrete immunoglobulins (IgM, IgG, or IgA) with monoclonally restricted heavy and light chain classes. T-cell clones were obtained that express the T-cell receptor complex (TCR), or the antigens CD1, CD3, CD4, or CD5.

M 101 IL-4 REGULATES LYMPHOPOIETIC STROMAL CELL FUNCTION, Linda G. Billips, Debra Petitte, and Kenneth S. Landreth. Department of Microbiology and Immunology, West Virginia University Health Sciences Center, Morgantown, WV 26506. IL-4 has been shown to have differential effects on myelopoiesis and lymphopoiesis. Although later stages of myelopoiesis are accelerated by increased concentrations of IL-4, earlier events in the same lineage are retarded by the same molecule. Our experiments have shown that IL-4 also affects pre-B generation by altering stromal cell function. Bone marrow stromal cell line S17 was exposed to varying concentrations of IL-4, washed to remove lymphokine, and tested for their ability to support pre-B cell generation compared to untreated cells. IL-4 treated cells failed to support pre-B cell generation. Conditioned medium from IL-4 treated S17 cells could be used to replace the stromal cell layer with similar levels of inhibition. The soluble mediator of this effect was not IL-4 and appeared to involve another regulatory cell in the bone marrow target population. This additional regulatory component could be removed from the marrow by depletion with anti-L3T4 antibody and was absent from marrow of nude mice, however, addition of syngeneic splenic T cells to Nu/Nu marrow resulted in reconstitution of IL-4 induced inhibition. These data suggest that T cells play a regulatory role in primary B lymphopoisis in the marrow. The identity of soluble mediators released by IL-4 treated stromal cells is currently under study.

M 102 THE MAB FMC7 EXCLUSIVELY RECOGNISES CD20 DETERMINANTS EXPRESSED ON MATURE B CELLS, Andries Bloem[1], Frank Staal[2], Maico van Dijk[1], Leonora Herzenberg[2], Gerhard Moldenhauer[3] and Frits Gmelig Meyling[1]. 1.Dept. of Clin. Immunol., AZU, Utrecht, Holland, 2.Dept. of Genetics, Stanford, USA, 3.Dept. of Immunol., DFKZ, Heidelberg, FRG.
The determinants recognised by the mAb's FMC7 (FMC7D) and B1 (CD20) are both B cell lineage specific markers. CD20 is a cell surface phosphoprotein expressed from the pre-B until the plasmacell stage of differentiation. Recent studies suggest that CD20 molecules function as Ca^{2+} ion-channels. FMC7D is expressed on mature blood B cells. Within this cell population the B cells capable of producing Ig after stimulation in vitro are present. Binding inhibition studies (FACS and RIA) and binding of FMC7 to CD20 cDNA transfected COS cells, revealed that FMC7 recognises CD20. B1 (IgGk) and FMC7 (IgMk) have comparable binding constants. The number of bindingsites per B cell however, differs for both mAb's (200,000 and 3,000 for B1 and FMC7, respectively). Immunohistochemical analysis of tonsil tissue sections shows that FMC7, in contrast to B1, exclusively binds to germinal centre B cells. We hypothesize that FMC7 recognises multimeric CD20 complexes which function as ion-channels in mature B cells.

M 103 THE MOLECULAR BIOLOGY OF LY1B CELLS. Condie E. Carmack, Kyoko Hayakawa, and Richard R. Hardy, Institute for Cancer Research, FCCC, Philadelphia, PA 19111

CD5+ (Ly1+ in mice, Leu1+ in human) B cells are a subpopulation of B lymphocytes whose role in normal and autoimmune immunologic processes remains unclear. They comprise the majority of B cells in fetal and neonatal life but in adult mice they are a relatively infrequent population of cells found at elevated levels in atypical cellular compartments (ie. peritoneal cavity). CD5+ B cells appear to contribute to spontaneous tumors in both mice and man at rates disproportionate to their numbers. These data suggest that CD5+ B cells are uniquely susceptible to malignant transformation, but the reason for this is not understood.

We have been interested in studying the biology and function of Ly1+ B cells in mice and have begun to study the molecular biology of the immunoglobulin genes of these cells, to examine cell numbers, VH repertoire and utilization, clonality, and the difference between these cells and conventional B cells. We have previously reported two new V gene families VH11 and VH12 found in these cells. We have extended these studies using PCR to quantitate the VH11 and VH12 repertoires in Ly1+ B cells and conventional B cells. We also report on the germline sequences of the VH11 family members.

M 104 CONSTRUCTION OF RETROVIRUS PACKAGING BONE MARROW STROMAL CELL LINES Hee Yong Chung and Henry H. Wortis, Program in Immunology, Department of Pathology, Sackler School of Biomedical Sciences, Tufts University, Boston, MA 02111.

In order to introduce retroviruses into selected hematopoietic precursors, we developed retrovirus packaging bone marrow stromal cell lines. We put the pMAV(psi-negative) plasmid into stromal cell lines by DNA cotransfection with pSV2gpt. Subsequently, these cell lines were used to produce helper-free amphotropic derivatives of HSGneo42 (ecotropic) viruses. These retrovirus producing stromal cell lines were used to establish both Dexter and Whitlock-Witte long term bone marrow cultures in which the hematopoietic cells were marked with retrovirus. The efficiency of retrovirus infection by these stromal cell lines and conventional methods using NIH3T3 cell lines will be compared.

M 105 EXTRACELLULAR NUCLEOTIDE CATABOLIC ENZYMES IN HUMAN B LYMPHOCYTE DEVELOPMENT, Amos Cohen and Jerry Barankiewicz, Department of Immunology and Rheumatology, Research Institute, Hospital for Sick Children, Toronto, Ontario, M5G 1X8.

The expression of extracellular nucleotide degrading enzymes were studied during B cell development. Cells of B lyhmphocyte lineage showed high nucleotide degrading activity, whereas T lymphocytes were unable to degrade extracellular nucleotides. The external surface of B cells contained active sites of ecto-triphosponucleotidase (ecto-ATPase), etco-disphosphonucleotidase (ecto-ADPase), and ecto-monophosphonucleotidase (ecto-AMPase). The expression of all three ectoenzyme activities seemed closely associated with B cell development. ATPase and ADPase activities increase continuously during B cell maturation, ecto-AMPase activity, on the other hand, reaches maximal activity in late pre-B cells. The activity of ATPase correlated well with the expression of cell surface ATP receptors in B cells suggesting a role for this enzyme in ensuring transient action of ATP in B-cells.

M 106 CONDITIONS REQUIRED FOR B LYMPHOPOIESIS IN VITRO

A. Cumano, K. Dorshkind, S. Gillis and C. Paige The Ontario Cancer Institute, Toronto, University of California, Riverside and the Immunex Corporation, Seattle.

We studied the different requirements for the generation of mature B lymphocytes from B220+ precursors present in fetal liver cell populations.

B lymphopoiesis occurs in the bone marrow or fetal liver as a result of precursor interactions with stromal cells and defined growth factors. Interleukin 7 was isolated from bone marrow stromal cells and previously shown to be a growth factor for B220+ pre-B cells. Using a clonal assay, we found that IL-7 can support the growth of pre-B cell colonies, although most of them do not mature to immunoglobulin secretion. Since fresh adherent cells from primary lymphoid organs can support the growth of colonies secreting immunoglobulin, we decided to test stromal cell lines for their ability to substitute heterogeneous cell populations in B lymphopoiesis.

S17 is a stromal cell line, previously described which can support myelopoiesis as well as lymphopoiesis. We demonstrate that S17 supports mature B cell development under conditions where the B cell precursor is the limiting element. This result suggests that S17 acts directly on the precursor cell. In order to define the stage at which S17 cells affect B cell maturation we used a two step culture system where B-cell precursors were incubated for 4 days in liquid with S17 cells, IL-7 or medium alone then cloned in agar under different conditions. The results show that S17 is not producing functional amounts of IL-7. In addition, we observed that this line plays a fundamental role in the late stages of culture increasing by two orders of magnitude the plaque forming colonies of pre-B cells expanded by IL-7. We therefore evaluated the effect of S17 in the cloning efficiency of mature B cells. We found that S17, as opposed to several other stromal cell lines, can increase the cloning efficiency of spleen cells from C57BL/6 mice to a frequency of 1:1 B cell.

M 107 $Ly\text{-}1^+$ B CELL PRECURSOR LINES THAT DIFFERENTIATE INTO MACROPHAGES: ONCOGENE EXPRESSION, LYMPHOKINE PRODUCTION AND ACCELERATION OF DIFFERENTIATION. Wendy F. Davidson, Jacalyn H. Pierce and Stuart Rudikoff, NCI and NIAID, National Institutes of Health, Bethesda, MD 20892 Three cell lines, BAMC1, HAFTL-3 and HAFTL-1, with the hallmarks of B cell progenitors, spontaneously differentiate with low frequency (<10%) into Ia^+, $Mac\text{-}1^+$ macrophages. To better understand the mechanisms promoting this differentiation process, progenitors and their differentiated progeny were examined for differences in levels of mRNA transcripts of oncogenes and lymphokines and for lymphokine secretion. In addition, progenitors were treated with a panel of lymphokines and differentiation inducing agents in an attempt to increase the proportions of differentiating cells. The studies revealed that the progenitors uniquely expressed high levels of *myb* and the macrophages high levels of *fos*, *fms* and *bcl-2* mRNA. The progenitor cells spontaneously secreted very low levels of GM-CSF (<20 pg/ml) and no detectable IL-6, IL-1, IL-3 or TNF. Following stimulation with LPS, GM-CSF production was increased 5-10 fold and low levels of IL-6 (10-80 pg/ml) were detected. To determine if lymphokines could accelerate the differentiation process, progenitors were treated with rIL-6, rGM-CSF, rM-CSF and WEHI-3 supernatant alone or in combination. These treatments, as well as antibodies to IL-6, had no effects on growth, differentiation or lymphokine production. In contrast to lymphokines, treatment with 5-aza-cytidine PMA and ionophore and to a lesser extent LPS increased the rate of differentiation of the progenitors. Treatment with these agents may facilitate the study of genes regulating early events in the differentiation of progenitors as well as genes that lead to committment to the macrophage lineage.

M 108 INTERLEUKIN 4 INDUCES CELLULAR ADHESION AMONG B LYMPHOCYTES.

Carina Elenström,and Eva Severinson, Dept. of Immunology, Stockholm University, S-106 91 Stockholm,Sweden. Murine interleukin 4 (IL-4) is able to induce cellular adhesion among small, resting B lymphocytes and together with lipopolysaccharide (LPS) it increases the adhesion induced by LPS. Antibodies against the adhesion molecule lymphocyte function associated antigen 1 (LFA-1) could partially inhibit the LPS-induced adhesion, whereas antibodies against other cell surface molecules where without inhibitory effect. In contrast to LPS induced adhesion, IL-4 induced adhesion was not affected by anti-LFA-1 antibodies. Furthermore, LPS stimulation of B cells resulted in an increased expression of one LFA-1 ligand, intercellular adhesion molecule 1 (ICAM-1). Stimulation with IL-4 or LPS plus IL-4 did not further increase the expression of ICAM-1, indicating that IL-4 activates an adhesion mechanism other than LFA-1-ICAM-1.

M 109 MODULATION OF HEMATOPOIESIS IN HUMAN LONG-TERM BONE MARROW (LTBM) CULTURES BY HYDROCORTISONE, Lydia A. Falk, James Kasper, Maria Sparks, and Francis W. Ruscetti, Laboratory of Molecular Immunoregulation, NCI/FCRF, Frederick, MD 21701. LTBM cultures have been utilized as an in vitro culture system to mimic hematopoiesis as it occurs in vivo. The culture conditions include fetal calf serum, horse serum, hydrocortisone (HC; 10^{-6} M), and growth at 33° C. To examine the role stromal cells play in regulating progenitor cell growth in these cultures, stromal cells were initiated and maintained with and without HC. Morphologically , LTBM cultures established in the presence of HC showed a time-dependent increase in stromal layer formation. In contrast, cultures not receiving HC showed little stromal layer formation by 3 wks. Differences were also observed in the corresponding proportions of myeloid progenitors in the non-adherent fraction of the cultures. Cultures with HC showed significant reductions in this fraction by 2-3 wks (60% and 90%, respectively). Since transforming growth factor-beta (TGF-B) has been shown to inhibit lymphoid and myeloid progenitor cell growth in vitro, these cultures were examined for differences in endogenous TGF-B production. Only cultures grown with HC showed a time-dependent increase in TGF-B mRNA expression. Thus, the growth/release of progenitors from the stromal cell layers in LTBM cultures may be regulated by differential endogenous TGF-B expression as shown in these studies using HC-containing culture conditions.

M 110 THE FcγR II IS EXPRESSED ON ALL NORMAL MURINE PRE-B CELLS, Teresa Foy, Richard Lynch, Thomas Waldschmidt, University of Iowa College of Medicine, Iowa City, Iowa 52242

Our laboratory has previously demonstrated the expression of the FcγR on all IgM-bearing B cells in neonatal spleens. The present study examines the expression of the FcγR on pre-B cells in the maturing spleen and adult bone marrow. Flow cytometric analysis of spleen cells from neonates of increasing age revealed a large number of pre-B cells, phenotypically defined as 6B2+, IgM-, between 2 and 3 weeks of age. Additional phenotypic analysis confirmed that the 6B2+, IgM- cells were pre-B cells owing to their lack of Thy 1 and CD3. Examination of these splenic pre-B cells with the monoclonal anti-FcγR antibody 2.4G2 demonstrated that they expressed levels of the FcγR similar to IgM+ B cells. Analysis of resident pre-B cells from adult bone marrow yielded similar results. Finally, in order to test whether the FcγR expressed on pre-B cells was functional, the cells were stained with aggregated IgG. Although both pre-B cells and IgM+ cells expressed similar levels of the 2.4G2 epitope, the IgM+ cells bound the IgG 10-fold greater than the pre-B cells. In summary, the results demonstrate that the FcγR receptor is present very early in B cell development. In addition, the data indicates that a large number of pre-B cells are present in neonatal spleens between 2 and 3 weeks of age.

M 111 **CHARACTERIZATION OF THE CDw75 ANTIGEN**
Steinar Funderud, Bjørn Erikstein, Heidi Kiil Blomhoff and Erlend B. Smeland, Immunology laboratory, Institute for Cancer Research, The Norwegian Radium Hospital, Oslo, Norway.

We have developed a monoclonal antibody HH2 which recognize a formol/ paraffin resistant epitope on the CDw75 antigen. This antigen is expressed on sIg+ peripheral blood B cells, but is not found on pre B-cells. Corresponding to the expression seen in normal B cells, CDw75 is also detected on cells from patients with CLL or lymphoma, but not on cells from ALL patients. When resting peripheral blood B cells are stimulated to enter S-phase, the expression of CDw75 increases 8-10 fold reaching an antigen density of 1.5 x 10^6 per cell. What is the functional role of this enormous quantity of antigen on the cell surface? When tested in highly purified B cells, HH2 show some synergy with anti-μ at low doses of anti-μ, as measured by ^{3}H-TdR uptake, but not by ^{3}H-uridine uptake. Experiments with cell lines have on the other hand shown that HH2 inhibit the growth of Ramos and U698 cells, but not Daudi cells.
The CDw75 have recently been cloned (I. Stamenkovic) and we are currently sequencing the cDNA clone, looking for hints to understand the role of this antigen.

M 112 INHIBITION OF BONE MARROW B-CELL RESPONSE BY PHYSIOLOGICAL LEVELS OF GLUCOCORTICOIDS. Beth A. Garvy and Pamela J. Fraker, Department of Biochemistry, Michigan State University, E. Lansing, MI 48824

Glucocorticoids (Gc) have a generally suppressive effect on immune function. It had been shown that pre B-cells were more sensitive to glucocorticoids than mature B-cells. However, whether physiological levels of Gc also affected immature B-cells remained to be demonstrated. Bone marrow (BM) from several strains of mice was examined to determine if the response to TNP-LPS in short term culture could be altered by glucocorticoids. Using concentrations which correspond to the serum levels found in stressed and malnourished mice, BM B-cell response was reduced 50-80% in vitro in the presence of 10^{-8} to 10^{-6} M dexamethasone (Dx). Cortisol and corticosterone also reduced the response by greater than 50% at physiological concentrations. The same pattern of inhibition was noticed regardless of whether Dx was added prior to (24 hrs.) or after (up to 48 hrs.) addition of antigen to culture. Addition of Dx 72 or 96 hrs after stimulation no longer inhibited plaque formation. These effects were found to be specific for Gc since neither testosterone nor progesterone inhibited the response to TNP-LPS. The glucocorticoid receptor antagonist RU 38 486 (Roussel-Uclaf) protected bone marrow from the inhibitory effects of Dx when used at ten-fold higher concentrations. These data indicate the Gc significantly reduced the capacity of immature bone marrow B-cells to respond to TNP-LPS at physiological Gc concentrations. The site(s) of suppression by Gc and whether or not it had a direct effect on immature B-cells remains to be determined.

(Supported by N.I.H.)

M 113 CORRELATION OF VpreB AND λ5 EXPRESSION AND STROMAL ADHERENCE IN MURINE CELL LINES, Michele A. Glozak, Richard L. Riley and Bonnie B. Blomberg, Dept. of Microbiology and Immunology, University of Miami School of Medicine, Miami, FL 33101

VpreB and λ5 have previously been described by Melchers' group (Sakaguchi and Melchers, Nature 324:579-82; Kudo and Melchers, EMBO J. 6:2267-72, 1987) as encoding V-like and JC-like regions which are expressed exclusively in pre-B cells. We have previously described a phage clone, IgS3, from a BALB/c genomic library (Blomberg et al, PNAS 78:3765-69, 1981) which we have further characterized and found to encode the VpreB and λ5 genes. This clone and another, IgS9, are quite similar to those described in BDF1 mice, but do contain some restriction fragment length polymorphisms (RFLPs). We have made probes specific for each of these genes and have used them to examine their expression in previously established pre-B and B cell lines. These cell lines have already been studied for their ability to bind to bone marrow stromal cell lines. We will present data correlating the expression of these genes with the ability to adhere to stromal cells.

M 114 GERMINAL CENTERS IN THE SPLEEN OF NON-RADIATION RAT CHIMERAS DEVELOP OLIGOCLONALLY Mirjam Hermans, Davine Opstelten, Frans Kroese, Department of Histology and Cell Biology, Immunology section, University of Groningen, Oostersingel 69/1, 9713 EZ Groningen, The Netherlands.

In lethally irradiated and TDL reconstituted rats individual germinal centers (GC's) in the spleen develop from 1-3 germinal center precursor cells (GCPC's, Kroese et al., 1987 Eur J Immunol 17:1069).

To investigate the number of GCPC's involved in the generation of GC's under more physiological conditions, we constructed non-radiation hemopoietic chimeras by i.p. injection of day 14-15 fetal liver cells into newborn hosts. We used congenic pairs of rats differing in RT7 allotype (leucocyte common antigen, CD45). Donor cells were RT7.2 and hosts were RT7.1. The RT7.2 allotype was recognized by the Mab HIS41. In these lympho-myelopoietic chimeras up to 20% of the bone marrow B cells was of donor origin. After 10 weeks, GC formation in the spleen was induced by i.v. injection of 10^9 sheep red blood cells. Five days later the spleens were taken for in situ analysis of cellular make-up of the GC's using Mab HIS41 specific for the RT7.2 allotype. Three types of GC were found: completely of RT7.1 type, completely of RT7.2 type or mixed. Their relative frequencies indicated that GC's in the spleen of these non-radiation chimeras develop, on average, from 1 to very few GCPC's.

These data strongly suggest that GC's are sites of antigen driven expansion of peripheral B cells to very large clones and form a basis for understanding molecular mechanisms that may occur in GC (somatic mutation and isotype switching).

M 115 IDENTIFICATION A NEW ANTIGEN DEFINED BY THE MONOCLONAL ANTIBODY, LIP-6, THAT IS EXPRESSED BY PRE-B CELLS, LY-1(CD5)$^+$ B CELLS AND MACROPHAGES, Kevin L. Holmes, Larry M. Lantz, Joon S. Lee, and Herbert C. Morse III, Biological Resources Branch and Laboratory of Immunopathology, NIAID, National Institutes of Health, Bethesda, MD 20892

The rat monoclonal antibody, LIP-6 was produced against an undifferentiated murine cell line, bh-2, derived from BXH-2 mice. FACS analysis of tumors showed that LIP-6 was restricted to B and myeloid lineage neoplasms. Analysis of normal tissues showed that LIP-6 is expressed by B cells and some Mac-1$^+$ cells in the spleen but not by thymocytes or peripheral T cells. All Ly-5(B220)$^+$ and BP-1$^+$ bone marrow cells are LIP-6$^+$, but Mac-1$^+$ bone marrow cells are LIP-6$^-$. Peritoneal Ly-1(CD5)$^+$ B cells and macrophages express high levels of LIP-6. LIP-6$^+$ Ly-5(B220)$^-$ bone marrow cells differentiate into Ly-5(B220)$^+$ cells when sorted onto Whitlock-Witte bone marrow feeder layers, suggesting that LIP-6 may precede Ly-5(B220) in B cell differentiation. In vivo reconstitution experiments are in progress to determine the differentiation potential of LIP-6$^+$ Ly-5(B220)$^-$ bone marrow cells.

M 116 EXTINCTION OF AN Ig KAPPA PROMOTER IN B-CELL x FIBROBLAST HYBRIDS IS MEDIATED BY THE OCTAMER MOTIF AND CORRELATES WITH SUPPRESSION OF Oct-2 EXPRESSION, Steffen Junker, Søren Pedersen, Edgar Schreiber, and Patrick Matthias, Institute of Human Genetics, University of Aarhus, Denmark; Institut für Molekularbiologie II der Universität Zürich, Switzerland; Whitehead Institute for Biomedical Research, Cambridge, Mass. 02142.

When immunoglobulin expressing B cells are fused with fibroblasts, Ig expression is rapidly suppressed at the level of transcription, a phenomenon termed "extinction". Here we show that the conserved octamer motif, which is found in Ig gene promoters as well as in the Ig heavy chain enhancer, plays a crucial part in mediating extinction. Thus, replacement of the octamer site of the kappa light chain promoter by an Sp1 or an NF1 binding site is sufficient to bypass extinction. Moreover, in early hybrids between B cells and fibroblasts extinction is correlated with absence of the cell-specific transcription factor Oct-2 and of its transcripts. Thus it appears that in B-cell x fibroblast hybrids the lack of a necessary cell-specific transcription factor is involved in the extinction of Ig expression.

M 117 RETROVIRAL LINEAGE MARKING OF MEMORY B CELLS IN VIVO, Wanda Krall and Jonathan Braun, Department of Pathology, UCLA School of Medicine, and Molecular Biology Institute, UCLA, Los Angeles, CA 90024

Immunological memory in the humoral response is operationally understood as the brisk, high avidity, and generally mature isotype response which occurs upon rechallenge with a previously encountered antigen. This response is attributed to a limited number of long-lived memory B cell clones which emerge during the primary response from a specialized germinal center microenvironment. The correlation between B cell memory and germinal center development is well known; in particular, the retention of native antigen on the surface of follicular dendritic cells in contact with B cell blasts in germinal centers suggests that these structures may provide a selective environment in which a repertoire of affinity-matured memory clones is generated. However, direct characterization of these cells has been hindered by the lack of distinctive phenotypic and clonal markers, presumably due to the changing differentiative state of the cells during this developmental stage. As a new approach, we have employed a *lacZ* retroviral marker to label germinal center B cells residing in an intact lymph node in vivo. Surprisingly, during the quiescent period following the primary response to antigen, *lacZ*$^+$ cells undergo a period of expansion in the perivascular medullary interstitium. *LacZ*$^+$ cells then wane, and by 2 weeks post-retroviral injection, these cells are undetectable in lymph nodes. Following rechallenge with antigen, long-lived *lacZ*$^+$ B cell emigrants can be found in the spleen, a distant location from their site of origin in the popliteal lymph node. In addition, the low frequency of these cells suggests that memory cells may be subject to additional processes of selection during the secondary response. These and other findings prompt reconsideration of the clonal origin of the memory immune response.

M 118 THE RAT B CELL SYSTEM: ANATOMICAL LOCALIZATION OF FACS-DEFINED B CELL SUBSETS.

Frans G.M. Kroese*/**, Mirjam Hermans**, Paul A. Lalor***, Alan M. Stall***, Eugene C. Butcher* and Lee Herzenberg***. Dept. Pathology* and Genetics***, Stanford Univ., CA and Dept. Histology and Cell Biology**, Univ. Groningen, The Netherlands.

FACS analysis of rat peripheral lymphoid organs shows two distinct IgM/IgD defined subpopulations, similar to the mouse: a major population of cells expressing little IgM and much IgD (population I) and a minor population of cells expressing much IgM but little IgD (population III), mainly found in spleen. We have developed Mab to B cell associated determinants that label either population I cells or population III cells. These Mab are called HIS22 and HIS50 respectively. Furthermore, a third Mab, named HIS24, reacts with all population I cells and subdivides population III into two subsets: about one-third of splenic population III cells are brightly stained with this Mab and the remaining two-third only dull. The HIS24-bright population III cells likely are newly formed B cells since cells with this phenotype are the predominant sIg population found in adult bone marrow (BM) and neonatal spleen. Combining immunohistological analysis of tissue sections with these FACS data, we conclude that the small (resting) follicular B cells are in population I and marginal zone B cells are found in the HIS24-dull population III. The in situ localization of the HIS24-bright population III cells (newly formed B cells) in spleen is not clear, but their phenotype indicates that they may well be found in lymphoid follicles. Based upon the relative expression of surface markers, we propose a model of B cell differentiation in the rat in which a common precursor cell (the HIS24-bright population III cells), produced in the BM, enters the spleen and leads to two distinct B cell pathways: one leading to marginal zone B cells and one (through an intermediate celltype) to small resting follicular B cells.

M 119 MOLECULAR ANALYSIS OF LYMPHOKINE INDUCED IgA B CELL DIFFERENTIATION, D. Y. Kunimoto, J. L. Claflin and W. Strober, Department of Medical Microbiology, University of Alberta, Edmonton, Alberta, Canada T6G 2H7, Department of Microbiology and Immunology, University of Michigan, LCI, NIAID, NIH, Bethesda,Maryland.

We have previously shown at a cellular level that CH12LX B cell lymphoma is a model of IgA differentiation. IL-4 induces some membrane IgA, while IL-5 increases the secretion of IgA. Most recently, we have shown that TGF-β also induces membrane IgA expression. Molecular analysis of the subclones of this line shows that the IgM-bearing phenotype is in germ line configuration, whereas the IgA-bearing phenotype has deleted both μ genes and rearranged the α genes. In addition, a dual-bearing subclone, having both IgM and IgA on its surface, shows an intermediate DNA pattern. Flow cytometry analysis of propidium iodide stained cells indicates that these are diploid. Furthermore, the VDJ sequence for μ and for α mRNA are identical, implying that this is a true switch event. This model provides a tool for dissecting the lymphokine regulation of IgA B cell differentiation at both a cellular and molecular level.

M 120 BIASED VH GENE UTILIZATION IN HUMAN CD5+ (LEU1+) EBV-TRANSFORMED B CELL LINES. Ton Logtenberg, Mieke E.M. Schutte and Frits H.J. Gmelig-Meyling. Department of Clinical Immunology, University Hospital Utrecht, Postbus 85500, Utrecht, The Netherlands.

CD5 is a 67 K glycoprotein expressed on all T cells and a subpopulation of B cells. Recently, we have demonstrated that CD5+ chronic lymphocytic leukemia B cells (B-CLL) express a restricted repertoire of immunoglobulin VH gene segments (1). In the present study, we adressed the question whether the restriction in VH gene utilization in CLL cells reflects the repertoire of normal CD5 B cells that have undergone neoplastic transformation. To that end, we analyzed the VH genes expressed in CD5+ EBV-transformed cell lines derived from healthy individuals. The results of these experiments show that EBV-transformed CD5+ B cells from healthy individuals express a similarly restricted VH gene repertoire. Furthermore, comparison of nucleotide sequences of these VH gene segments (including members of the VH3, VH4, VH5, and VH6 families) with published VH sequences revealed 100% sequence homology. Together, these results suggest that human CD5+ B cells utilize a restricted set of highly conserved VH gene segments.

T. Logtenberg et al. International Immunology, 1, 361 (1989).

M 121 ONTOGENY OF COELOMIC-ASSOCIATED B-CELL POPULATIONS.

Miguel A.R. Marcos, François Huetz, Pablo Pereira, Carlos Martinez-A. and Antonio Coutinho. Institut Pasteur, Paris, France. Previous works have shown that CD5+ B lymphocytes are preferentially clustered in gut-related mesenchymal areas, such as peritoneum, thymus and tonsils. We have found that the mouse pleuropericardial exudate contains a homogeneous population of large-sized, noncycling, nonsecretory B cells, expressing very high levels of surface IgM, little or no IgD, Mac-1 and low levels of B220. This phenotype and the over-representation of some Ab clonotypes (anti-BMRC, anti-PC) suggest a pure "CD5 B cell population", although in all mouse strains tested many of the pleural B lymphocytes are CD5-. The common origin of peritoneum and pleural layers from the primitive coelomic cavity, as well as our recent finding in these areas of B220+IgM- cells in adult stages, suggest that such populations differentiate locally from intraembryonic precursors. We propose to designate them as "coelomic" to distinguish them from the "stromal" bone marrow-derived B cells.

M 122 **ALKALINE PHOSPHATASE AS A MARKER IN B-CELL DEVELOPMENT**

C. Martinez-A., C.E. Marquez, E. Leonardo and L. Pezzi.
Centro de Biologia Molecular, CSIC. Universidad Autonoma, Campus de Cantoblanco, 28049, Madrid.

ALPase comprises a group of enzymes catalizing the hydrolisis of a wide variety of phosphate ester at an alkaline pH. In mammalians they are encoded by at least two loci that determine the structure of a set of isoenzymes which have very similar catalytic properties. Previuos work in our laboratory has shown that the ALPase is induced in mouse spleen cells upon treatment with B-cell mitogens. Further studies revealed that the ALPase is selectively expressed in activated B-cells and that the expression of the activity correlates well with the differentiation into antibody secreting cells. The expression does not occur when thje activation leads solely to cell proliferation. We have recently identified a mAb (G5.2) recognizing the ALPase which allowed us to study the expression of this enzyme during B-cell development. The results show that the ALPase is already expressed in pre B-cells (day 16 in fetal liver and bone marrow). However, the activity was absent in most resting B-cells. Based on this observations and the known protein phosphatase activity of ALPase, we have analyzed the dephosphorylation of specific proteins by this enzyme. We found that in the course of B lymphocyte differentiation the ALPase shifts from a soluble to a cytoskeleton-bound form, correlating with changes in the phosphorylation of proteins with Mr 120, 100, 90 and 75. In the light of these findings, the possible role that ALPase could play in the development of B-cells is discussed.

M 123 B-CLL CELLS EXPRESS MYELO-MONOCYTIC ANTIGENS. H. Merle-Beral, E. Legac, A. Michel, P. Debre, J.L. Binet. Département d'Hématologie and Laboratoire d'Immunologie. Hôpital Pitié Salpétrière, Paris, France. Sixty B-CLL cases were studied by flow cytometry for the expression of : a) B antigens (CD19, CD20, CD21, CD22, CD9, CD10, CD37, FMC7, IgS, HLA-DR, DQ, DP) ; b) T antigens (CD1a, b, c, CD2, CD5) ; c) activation markers (CD23, CD25, CD38, CD71, B5) ; d) myelo monocytic markers (CD11a, b, c, CD14b, c, CD16, CD32. Our results have permitted to subdivide three groups of patients : group 1 corresponding to "common CLL" : positive for CD19, CD20, CD21, CD9, CD24, CD37, CD5 ; negative for myeloid markers other than CD32 and activation markers other than CD23 and B5 ; group 2 : identical to group 1 but also presence of one or several CD11 molecules ; group 3 : same than group 2 plus myelo-monocytic antigens other than CD11 and/or activation markers (CD25, CD38, CD71). Statistical analysis and correlation tests between these three groups and several clinical and biological parameters showed that the expression of myelo-monocytic and activation antigens at the B-CLL cell surface seem to be correlated with the severity of the disease.

M 124 A CLONED BONE MARROW-DERIVED STROMAL CELL LINE ELABORATES A DIALYZABLE LYMPHOPOIETIC CO-FACTOR. Michael J. Muirhead, Randall T. Davis, Department of Internal Medicine, University of Iowa College of Medicine, Iowa City, IA 52242, and Richard C. Schwartz, Department of Microbiology and Public Health, Michigan State University, Lansing, MI 48824

A cloned stromal cell line (3E) from mouse bone marrow secretes a protease resistant molecule with an apparent molecular weight of 400 which we call Abelson Growth Promoter (AGP). AGP directly promotes the growth of an Abelson virus-transformed pre-B cell line which is IL7-unresponsive. It has no proliferative activity on normal pre-B cells as a single agent but augments the proliferative effect of IL-7. Medium conditioned by 3E cells contains no IL7-like activity in the Immunex bioassay, and yet 3E cells support the growth of feeder layer-dependent pre-B cell lines such as the Witte-Whitlock clone 3 or a ras-transformed pre-B cell line (R1). NIH 3T3 cells make trace amounts of AGP-like activity and an endothelial cell line from mouse brain makes an intermediate level of AGP compared to 3E cells. AGP is dialyzable and anionic. It is retained in the aqueous phase of a chloroform-methanol extract of 3E-conditioned medium. AGP is probably a ubiquitous product of the bone marrow microenvironment and of certain extra-medullary sites but is present in critically low amounts in standard tissue culture systems lacking an adherent cell layer.

M 125 RECONSTITUTION OF B CELL POPULATIONS FOLLOWING BONE MARROW TRANSPLANTATION. Carol Nottenburg and David Hasenstab, Division of Clinical Research, Fred Hutchinson Cancer Research Center, Seattle, WA 98104.

The reconstitution kinetics of B cells post-marrow transplant have been determined. B cells are not detectable in most recipients until day 60. The percentage of peripheral blood lymphocytes that are B cells attains normal levels by day 90. In most recipients the percentage of CD19+ B cells was higher than the percentage of Ig+ or CD20+ B cells. In contrast to other investigations, the percentage of CD5+ B cells is only slightly higher than for normal individuals (approximately 50% in patients compared to 30% in normals) and does not reconsitute before conventional B cells. We are currently examining whether the CD5+ B cells found post-transplant are derived from the hematopoeitic stem cell or from donor-derived committed progenitors.

M 126 RESPONSES OF Ly1$^+$ AND CONVENTIONAL B CELLS IN VITRO. Anne O'Garra, Ning Go, James Cupp, Josephine Polakoff & Maureen Howard Department of Immunology, DNAX Research Institute, Palo Alto, CA 94304

Ly-1$^+$ and conventional (B220$^+$, Ly-1$^-$) B cells were obtained from the peritoneum of CBA/J mice by FACS sorting. The purified populations were incubated, at low cell density, in the presence of various B-cell mitogens, for 24 or 72 hours, after which DNA synthesis was measured. Ly-1$^+$ B cells were relatively inert in response to goat anti-mouse Ig plus IL-4, although conventional B cells gave a very significant response to this costimulus. However, both populations of B cells failed to respond to high doses of goat anti-mouse Ig (previously shown to be mitogenic for B cells). These data could be explained by the fact that the B cells were not only highly purified, but also set up at low cell density. Thus, it is possible that conventional B cells require accessory cells for induction of DNA synthesis by high doses of anti-Ig, but not by the costimulatory action of anti-Ig and IL-4. Both Ly-1$^+$ and conventional B cells responded significantly to LPS, but preliminary data suggested that they were unresponsive to IL-1, IL-2, IL-4 and IL-5 alone. The lack of responsiveness to IL-5 is surprising, in light of previous findings, and again could be due to a requirement for accessory molecules. This is being investigated. Ly-1$^+$ B cells, after 24 hrs only, showed a significant response to PMA, which could be enhanced by IL-4. The response of conventional B cells to these stimuli was variable, but consistently much lower than that senn with Ly-1$^+$ B cells. These data suggest a possible difference in the regulation of conventional and Ly-1$^+$ B cells, and also support a role for accessory cells in some responses.

M 127 DYNAMICS AND LOCALIZATION OF EARLY B PROGENITOR CELLS (PRO-B CELLS) IN THE BONE MARROW OF MICE WITH SEVERE COMBINED IMMUNODEFICIENCY (SCID), Dennis G. Osmond, Robert A. Phillips*, Nancy Kim, Raffi Manoukian, Karen Jacobsen and Sergio A. Rico-Vargas, Department of Anatomy, McGill University, Montreal, Canada H3A 2B2 and *Hospital for Sick Children, Toronto. We have defined 3 subsets of early B-progenitor cells lacking μ heavy chains (pro-B cells), based on the expression of terminal deoxynucleotidyl transferase (Tdt) and B220 glycoprotein: 1) early pro-B cells (Tdt^+B220^-) 2) intermediate pro-B cells (Tdt^+B220^+) 3) late pro-B cells (Tdt^-B220^+). To test our working models of B cell genesis, pro-B cells have now been examined in 5-15 wk mutant BALB/c SCID mice. Double immunofluorescence labeling of bone marrow cell suspensions revealed normal numbers of early and intermediate pro-B cells, substantially reduced numbers of late pro-B cells and an absence of pre-B and B cells. Early and intermediate pro-B cells accumulated in metaphase in normal numbers after ip vincristine sulfate administration. $B220^+$ pro-B cells have also been localized in situ by the binding of ^{125}I-mAb 14.8, detected by light and electron microscope radioautography of bone marrow sections. Most $B220^+$ cells were located towards the periphery of SCID bone marrow, including the endosteal cell layer, surrounded by a distinctive extracellular matrix and often associated with stromal cells, including macrophages. In accord with our models, these findings in SCID mice suggest that many pro-B cells in the bone marrow occupy microenvironments near the surrounding bone; they maintain normal production rates during stages of presumptive H chain gene rearrangement apparently unaffected by the absence of a mature B cell pool, defective cells being deleted at the late pro-B cell stage, apparently by macrophages. (Supported by the NCI and MRC of Canada)

M 128 MATURE B CELL TRANSFORMATION WITH ONCOGENE-CONTAINING RETROVIRAL VECTORS. Robert W. Overell, Karen E. Weisser, Bruce Hess, and Kenneth H. Grabstein. Immunex Corporation, 51 University Street, Seattle, WA 98101

Experiments will be presented in which a large panel of oncogene-expressing retroviral vectors were assayed for their ability to promote the long-term *in vitro* growth of mature B lymphocytes isolated from murine spleen. The mature B cells were found to be readily infectable with ecotropic retroviral vectors. Infections were carried out with high-titer retroviral vectors expressing viral and cellular oncogenes alone or in combination, under the transcriptional control of the retroviral LTR or an internal SV40 early region promoter. In a minority of cases expression of the oncogenes in B lymphocytes could be readily visualized at the RNA level, and transient increases in B cell proliferation were consistently observed. Despite this, none of the vectors tested consistently induced long term proliferation (immortality) of the B cells at a significant frequency. Cell lines of other phenotypes could be obtained, however. Data will be presented on the biological activities of a second panel of retroviral vectors which have been designed to express the oncogenes to higher levels in mature B cells.

M 129 REGULATION OF B CELL DIFFERENTIATION BY CYTOKINES, Beverley L Pike and Lothar Vogel, The Walter and Eliza Hall Institute of Medical Research, Melbourne, Vic 3050, Australia

The regulation of the differentiation of B cells by a variety of cytokines has been investigated using an antigen-driven cloning system which allows dissection of the effects of cytokines on single isolated hapten-specific B cells at different stages in the differentiation pathway. Arbitrarily defined stages in the activation pathway, namely early activation, proliferation, IgM secretion and isotype switching, have been chosen for the purposes of this study. An extensive panel of cytokines, acting alone or in combinations, have been used. IL-4 plus IL-5 (and IL-2) appear to comprise the most potent combination of cytokines. Appropriately stimulated, up to 50% of splenic B cells generate clones which secrete IgM, 30% of which secrete IgG_1, with at least 10 ng of IgM and/or IgG_1 secreted per clone. Other isotypes have been examined.

The responses of B cells selected from a variety of organ sources and/or selected by markers such as IgM, IgD, B220, HSA, ThB and Ly1, have also been examined. These results as well as those obtained using B cells from primed donors and tolerant B cells will be discussed with respect to B cell differentiation.

M 130 **MONOCLONAL ANTIBODIES DEFINE SUBPOPULATIONS OF CELLS WITHIN MURINE GERMINAL CENTRES.**

Frances M. Platt*[1], Judith A. Cebra-Thomas**, Charles M. Baum**, Joseph M. Davie* and John P. McKearn*,
*Searle R&D, St.Louis, MO 63198, **Department of Microbiology and Immunology, Washington University Medical School, St Louis, MO 63110, [1]Present address, Department of Biochemistry , University of Oxford ,OX1 3QU, UK.

A series of mAbs have been identified which selectively, but not exclusively, bind to populations of cells within the germinal centres of immunised mice. All four mAbs stain B cell populations as defined by flow cytometry. Two of the mAbs, FH9.5 and CD3.5,also stain subsets of peripheral T cells ($CD4^+$ and $CD8^+$ respectively). When T cell-depleted immunised spleen cell fractions are separated by density gradient centrifugation the majority of the $FH9.5^+$ and $CD3.5^+$ B cells are found in the low density, activated fractions. The cells defined by the C6C3 and A6A2 mAbs are less frequent and from flow cytometric analysis are B cells and myeloid cells. The cell surface markers bound by these mAbs cannot be induced on mitogen stimulated small resting B cells and suggests that they are not general markers of activation. In vitro cell lines have been identified which express the four markers and have been used for immunoprecipitation studies. To date it has been demonstrated that the FH9.5 and CD3.5 mAbs immunoprecipitate proteins of approximately 26kD and 90kD repectively. These mAbs will be useful reagents for the investigation of B cell differentiation and maturation within germinal centres.

M 131 ADHESION OF HUMAN B CELL PRECURSORS TO THE BONE MARROW MICROENVIRONMENT DECREASES WITH MATURATION. Daniel H. Ryan, Bonnie L. Nucci, Camille N. Abboud, Jane L. Liesveld. University of Rochester Medical Center. Rochester, NY 14642.

Human B cell lineage populations in order of increasing maturation can be quantitated using two-color flow cytometry: CD10+/ CD34+, CD10+/CD20-, CD10+/CD20+, and CD10-/CD20+ cells in marrow and peripheral blood. The binding of blood or marrow light density cells to adherent cell layers or matrix was studied following 2 hr incubation in 24 well plates and 5 washes with medium. The absolute number of bound B lineage precursors was determined by cell counts and flow cytometry analysis. The adherence of B lineage cells to passaged human marrow fibroblasts (BM-FB) was highest in the most immature subset, the CD34+/CD10+ cells, decreasing steadily with each stage of maturation to the marrow and blood CD10-/CD20+ B cells, as shown in the table (n=14). Treatment of cells with 50 ng/ml of phorbol ester (TPA) 30 min prior to washing increased binding of mature marrow B cells by nearly sixfold, while increasing binding of marrow CD10+ cells by only 74-87% in 4 paired experiments. Treatment with azide and 2-deoxyglucose at 4° C reduced adherence of mature and immature B lineage cell to a similarly low level, indicating a significant energy-dependent component.

	CD10+/CD34+	CD10+/CD20-	CD10+/CD20+	Marrow B cells	Blood B cells
% adherence to BM-FB (SEM)	36.2 (4.3)	29.7 (3.2)	22.1 (2.4)	15.5 (1.6)	11.2 (2.4)
% adherence - TPA		61.3 (12)	44.6 (15.5)	79.6 (8.4)	
% adherence - 4° C		8.9 (2.5)	5.4 (0.8)	9.0 (1.4)	

Of all cell types tested, only monocytes were more adherent to BM-FB than the two most immature B lineage cells. Only CD10+ B precursor cells, CD10-/CD34+ precursor cells, and erythroid precursors showed significantly greater binding to BM-FB than to plastic. B lineage precursors bound equally well to primary and passaged BM-FB, but significantly less well to foreskin fibroblasts, marrow stroma, BM-FB extracellular matrix, and fibronectin. These results suggest that specific binding to marrow fibroblasts is part of the differentiation program of early B lineage precursors.

M 132 ISOLATION OF DEVELOPMENTALLY REGULATED GENES EXPRESSED BY HAEMATOPOIETIC STEM CELLS USING A RETROVIRAL ENHANCER TRAP VECTOR. Fred Sablitzky[1,2] and Robert A. Phillips[2]; [1]Max-Delbrück-Labor i. d. MPG, D-5000 Köln 30, FRG; [2]Hospital For Sick Children, Division of Haematology/Oncology, Toronto, Ontario, Canada.

Genes which are expressed by haematopoietic stem cells, but are turned off in the course of differentiation to mature blood cells are candidates to play an important role in the regulation of self-renewal and/or differentiation of stem cells. In order to identify developmentally regulated genes, a retroviral vector (pΔE/NEO) was constructed which lacks the LTR-enhancer. Since the LTR-promoter is on its own inactive, transcription of the Neomycin (neo) gene initiated at the LTR-promoter is dependent upon endogenous enhancer elements. It is therfore possible i) to select for proviral integration sites in transcriptinally active loci and ii) to identify those integration sites which result in the developmentally regulated expression of the neo gene. Bone marrow (BM) cells were infected with vΔE/NEO and selected in 2 mg/ml G418. More than 95% of the surviving CFU-GM progenitor cells contained and expressed the neo gene. Similarly, 24/24 CFU-S colonies contained at least one copy of the neo gene again indicating that only those progenitor cells survived preselection in G418 which expressed the neo gene. However, ~50% of the CFU-S contained CFU-GM progenitor cells which where no longer able to grow in the presence of G418. The expression of the neo gene was apparently turned off upon differentiation of CFU-S to CFU-GM progenitors and/or mature granulocytes and macrophages. We have cloned DNA flanking the proviral integration sites of three CFU-S colonies. Preliminary results indicate that one flanking DNA contains an endogenous enhancer element. Northern analysis are currently done to see whether the DNA flanking the proviral integration sites contain exons of developmentally regulated genes.

M 133 PHENOTYPIC CHARACTERIZATION AND RESPONSIVENESS TO IL-7 OF HUMAN CD34+ B CELL PRECURSORS (BCP), Sem Saeland[1], Valérie Duvert[1], Dominique Pandrau[1], Christophe Caux[1], Catherine Favre[1], Nicholas Wrighton[2], Frank Lee[2] and Jacques Banchereau[1], [1]UNICET, Laboratory for Immunological Research, 27 chemin des peupliers, 69570 Dardilly, France, [2]DNAX Research Institute, Palo Alto, CA 94304.

We investigated the effects of recombinant human IL-7 on the proliferation and maturation of normal adult bone marrow CD34+ cells containing BCP subpopulations and of cells from CD34+ BCP acute lymphoblastic leukemia (ALL). Among normal bone marrow CD34+ cells, a subpopulation of CD10+ cells (25.6 ± 16.5%; n = 14) was identified on the basis of low light scatter properties. Most cells within this population were CD19+ (18.8 ± 10.3%; n = 10) and a low but significant percentage of cells were found to express CD20 (10.5 ± 8.3%; n = 11), whereas neither CD21 antigen nor immunoglobulin (Ig) expression was detected.
In short-term liquid cultures of normal CD34+ cells, IL-7 induced low overall proliferative responses and cell numbers were maintained whereas a rapid decline in cell numbers ocurred in control cultures.
In contrast, no significant effect of IL-7 was observed on the proliferation of CD34+ BCP-ALL cells.
The most striking effect noted in the presence of IL-7 was a dose-dependent increase in numbers of CD20+ cells in short-term cultures of normal CD34+ cells. However, in cultures of CD34+ BCP-ALL no effect of IL-7 on CD20 expression could be demonstrated. In cultures of normal CD34+ cells, numbers of CD20+ cells recovered after 6-7 days in the presence of IL-7 were 16 to 60 times higher than in control cultures and represented a net increase by a factor 1.7 to 3.0 from input CD20+ CD34+ cells. Finally, the CD20+ cells generated in response to IL-7 were found to coexpress CD10 while lacking expression of CD21 or surface Ig, thus confirming their BCP nature.

M 134 IN VITRO DIFFERENTIATION OF EMBRYONIC STEM CELLS INTO B LINEAGE CELLS, H. Sauter, A. Cumano, M. Schilham, T. Mak and C. Paige, Institut Pasteur, Paris, France and Ontario Cancer Institute, Toronto, Canada.

Embryonic stem (ES) cells are pluripotent stem cell lines derived from the inner cell mass of 3-4 day old mouse blastocysts. In culture they undergo differentiation into a variety of cell types. We studied the lymphoid potential of these cells in vitro. In order to detect B lineage cells, a agar system has been used, in which pre-B cells are stimulated by feeder cells and mitogens to mature into IgM-secreting B cells. ES-D3 cells directly cloned under these conditions did not give rise to B cells, suggesting that additional differentiation signals are required for these cells to reach the stage of progenitors able to respond to the agar culture system. We therefore allowed ES cells to differentiate into large, round embryoid bodies surrounded by a basallamina. When these embryoid bodies were cloned under pre B cell conditions, they readily gave rise to antibody secreting B cells. In order to assure, that the B cells actually were derived from the ES-cells, a series of control experiments were done: 1) Feeder cells alone did not give rise to any B cells, 2) The B cells generated expressed the appropriate IgM-allotype. 3) Using a neo-transfected Es cell line, the embryoid bodies expressed μ-RNA-transcripts and neomycin transcripts. Experiments using replicate RNA blots to show, that, μ transcripts and neomycin transcripts are detected within identical cells are in progress now. We have not yet detected any exogenous growth factors, besides medium and serum compounds, responsible for the differentiation observed in liquid culture. This might indicate, that embryoid bodies alone provide an environment for the development of primitive lymphoid cells. The system described here should be useful to identify the growth factors involved in the transition from the blastocyst stage to early lymphoid precursor cells.

M 135 MURINE INTERLEUKIN-7 STIMULATES THE PROLIFERATION OF NORMAL PERIPHERAL BLOOD T LYMPHOCYTES IN THE PRESENCE OF PHORBOL ESTER, Christian Schmitt, Anthony E. Namen and Connie J. Eaves, Terry Fox Laboratory, B. C. Cancer Research Centre, Vancouver, B. C., Canada and Immunex Corporation, Seattle, WA 98101. Murine interleukin-7 (mIL-7) was first identified as a growth factor for murine pre-B cells, but subsequently was also shown to be a potent stimulator of murine thymocytes and to enhance the proliferative response of mature murine T cells to Con A or PMA. Although the human homologue of mIL-7 has recently been cloned, the spectrum of human cells that can respond to IL-7 has not yet been defined. In an initial series of experiments, we found that primary murine bone marrow stromal cell cultures, competent to support mIL-7-responsive pre-B cells, also supported the maintenance of human T lymphocytes for periods of ≥6 weeks, raising the possibility that mIL-7 may be able to stimulate the proliferation of normal human T lymphocytes. To test this, purified human T cells were obtained from normal blood by sedimentation over Ficoll, rosetting with AET-treated SRBC and depleted of monocytes by Percoll density centrifugation. Incubation of this >98% pure (CD3+) T lymphocyte suspension with PMA (1 ng/ml) or PHA (1 μg/ml) in the presence of 1-200 ng/ml of murine recombinant IL-7, resulted in an 18.8 ± 6.3 or 6.4 ± 2.9 -fold enhancement of the proliferative response obtained with PMA alone or PHA alone, respectively, assessed by ^{3}H-thymidine incorporation during the last 6 hr of a 3 day-culture. Murine IL-7 alone was not stimulatory. These results show that mature human T cells can respond to mIL-7.

M 136 THE GROWTH FACTOR IL-7 INDUCES EXPRESSION OF A TRANSFORMATION ASSOCIATED ANTIGEN IN NORMAL PRE-B CELLS, Pamela J. Sherwood and Irving L. Weissman, Dept. of Microbiology and Immunology and Dept. of Pathology, Stanford University School of Medicine, Stanford, Ca 94305 The B lineage antigen 6C3Ag is expressed by a number of cell types involved in lymphopoiesis. We have investigated whether there is a relationship between activation of normal pre-B cells by the growth factor IL-7 and the expression of 6C3Ag. Sorted sub-populations of bone marrow pre-B cells were cultured with IL-7, and their expression of surface antigens meas-ured. There is a rapid induction of 6C3Ag expression after exposure to IL-7 by pre-B cells, but not mature B cells. In particular, the $6C3^{lo}$ pre-B cells respond very strongly by increasing 6C3Ag expression. The expression of 6C3Ag coincides with increased DNA synthesis and formation of lymphoblasts by the responding cells. The responses seen were specific to IL-7, and to a lesser degree IL-4. We conclude from this that IL-7 specifically induces the expression of 6C3Ag.

M 137 INDUCTION OF GERMLINE MU CONSTANT REGION TRANSCRIPTS IN MURINE EMBRYONIC MAST CELL LINES, Edward J. Siden, Department of Medicine, Division of Clinical Immunology, Mount Sinai School of Medicine, New York, NY 10029

We have previously described antigen receptor gene expression in cell lines derived by Ab-MuLV infection of the murine embryo prior to colonization of the primary lymphoid organs. Germline immunoglobulin V_H transcripts and the coordinate induction of gamma T cell receptor transcripts and the B220 protein and mRNA were observed. Curiously the cell lines all have the predominant characteristics of immature or mucosal mast cells. We now present data that the immunoglobulin mu heavy chain locus can also be activated in one of these mast cell lines. Two inducers have been identified. Interleukin 6 and the anti-proliferative drug BUdR both turn on synthesis of 1.8 kb germline mu mRNA.

We also present here data suggesting that the T cell receptor gamma locus is not only susceptible to transcriptional activation in these lines but is also open for genetic rearrangement (i.e. specific deletion). We show that either maternal or paternal alleles can be deleted even though another gene on this chromosome (DHFR) is still present in normal copy number.

We believe that mast cells have a number of genetic traits that would be predicted of lymphoid stem cells and are testing this hypothesis.

M 138 *bcl*-2 ONCOGENIC-TRANSGENIC MICE, Andreas Strasser, Alan Harris, David Vaux, Sue Bath, Jerry Adams and Suzanne Cory, The Walter and Eliza Hall Institute of Medical Research, Melbourne, Victoria 3050, Australia.

Follicular B cell lymphoma, the most prevalent hematological malignancy in humans usually contains a t(14;18) chromosomal translocation that juxtaposes the putative proto-oncogene *bcl*-2 with the immunoglobulin (Ig) heavy chain locus. The normal *bcl*-2 gene encodes a 24 kD glycosylated integral membrane G-like protein. The gene is quiescent in resting B lymphocytes, expressed in proliferating B cells but downregulated in differentiated B cells. Our previous in vitro experiments indicated that *bcl*-2 may represent a new class of oncogene that promotes cell survival rather than proliferation.

To explore *bcl*-2 effects in vivo, we made transgenic mice with the *bcl*-2 gene under the control of the Ig heavy chain enhancer. Several independent lines of *bcl*-2 transgenic mice were obtained. Most display a 2-5 fold increase of the B lymphocyte compartment in spleen, lymph nodes and bone marrow and show extended survival of these cells in tissue culture, but do not show follicular hyperplasia or B cell lymphoma.

Our *bcl*-2 mice show a number of immunological abnormalities. The primary immune response to the T cell dependent antigen SRBC is greatly prolonged. Unimmunized animals display a polyclonal increase of serum Ig and develop a systemic lupus erythematosus - like disease with lymphocyte infiltrations in kidney, liver, lung and salivary glands accompanied by severe glomerulonephritis and anti-nuclear antibodies.

We are currently analyzing how *bcl*-2 collaborates with other activated oncogenes to induce malignancy, by crossing *bcl*-2 lines with mice carrying other trans-oncogenes (eg. *myc, abl, ras*). Our data suggest that the *bcl*-2 gene promotes an accumulation of non-dividing lymphocytes, but activation of at least one additional oncogene is required to produce malignant transformation.

M 139 CELLULAR INTERACTION IN THE REGULATION OF GROWTH FACTOR PRODUCTION OF STROMAL CELL CLONES, Tetsuo Sudo[1],Yasuki Ogawa[1], Masayo Ito[1], Masahiko Iizuka[1], Takahiro Kunisada[2], Minetaro Ogawa[2] and Shin-Ichi Nishikawa[2] 1. Biomaterial Res. Inst., Taya-cho, Sakae-ku, Yokohama, 244 Japan. 2. Kumamoto Univ. Medical School, 2-2-1 Honjo, Kumamoto, 860 Japan.

Two stromal cell clones ST2 and PA6 which differ in ability to support myelopoiesis and lymphopoiesis were analyzed for their capability of producing a number of growth factors upon stimulation with various signals.

In Northern blot analysis, M-CSF and TGF-β were the factors constitutively expressed in both of the stromal cell lines. mRNA of IL-1α, -2, -3, -4, -5, GM-CSF or TNF could not be detected in either of the cell lines. On the other hand, IL-1β, -6, -7 and LIF were the factors inducible in ST2, and IL-6, LIF and G-CSF were those in PA6. This difference in the ability to produce growth factors between B lymphopoiesis supporting and nonsupporting stromal cell clones may represent the actual heterogeneity of intramarrow stromal cell component.

Among these inducible factors, IL-1β, -6, LIF and G-CSF were induced more effectively by LPS/TPA than IL-1α, while IL-1α was a more potent inducer of IL-7 than LPS/TPA. We next attempted to induce these factors by co-cultivating stromal cell with cell lines of various hemopoietic lineages. Interestingly, B cell line had the equivalent effect to IL-1α, while T and myeloid cell lines were equivalent to LPS/TPA. These results suggest that stromal cell responds differentially to various cell types for producing growth factors.

M 140 ABUNDANCE AND CLONAL PATTERN OF HUMAN GERMINAL CENTER B CELLS DURING CHILDHOOD, Yadira Valles-Ayoub , Herman L.Govan,III, and Jonathan Braun
Department of Pathology University of California at Los Angeles, Los Angeles, CA 90024-1732

Germinal centers are distinct microanatomic structures which form within primary B-cell follicles after antigen and T-cell dependent stimulation, and are believed to represent a differentiative organ for the development of memory B cells. In this study, we have analyzed the abundance and clonal pattern of germinal center and mantle zone B cells in human tonsils. Flow cytometric quantitation of these B cell subpopulations revealed that germinal center cells are abundant during early childhood, but decline by early adolescence to low levels. To further explore this age-related difference in germinal center abundance, we developed a magnetic immunobead strategy to preparatively isolate germinal center (Leu-17^{hi}, IgD^{lo}) and mantle zone (leu 17 ^{lo},IgD^{hi}) cells. The clonal patterns of these fractionated subpopulations were analyzed using a novel PCR-based quantitation of VH subfamily utilization in rearranged VDJ genomic segments. Clones bearing two VH subfamilies were informative: VH1N clones were uniquely deficient in germinal center B cells at an early age period, but become abundant in later childhood; and VH3L clones were deficient in germinal center cells regardless of age. In contrast, B cell clones bearing each VH subfamily were abundant in the mantle zone subpopulation throughout childhood.

These findings suggest that the abundance and clonal pattern of germinal center B cells evolves during childhood, and may reflect a novel antigenic or ontogenic process in human germinal center B-cell development. Moreover, the distinct clonal pattern of germinal center versus mantle zone B cells suggests that a major phase of clonal selection occurs following germinal center emigration.

M 141 IDENTIFICATION OF A HUMAN PROTEIN HOMOLOGOUS TO THE MOUSE LYB-2 B CELL DIFFERENTIATION ANTIGEN, Ilka von Hoegen, Emiko Nakayama and Jane R. Parnes,
Department of Medicine, Division of Immunology & Rheumatology, Stanford University Medical Center, Stanford, CA, USA

Lyb-2 is a mouse B-cell differentiation antigen expressed on the surface of pre-B cells and B cells, but not on antibody-secreting cells. Although the function of Lyb-2 has not been adequately defined, it may be a growth factor or lymphokine receptor. Using cross-hybridization with a mouse Lyb-2 probe we have isolated and sequenced cDNA clones encoding human Lyb-2. The predicted amino acid sequence is homologous to mouse Lyb-2, and like the latter, the human protein is also related to both the asialoglycoprotein receptor and CD23, the B cell specific Fc receptor for IgE. Human Lyb-2 mRNA is expressed in normal human tonsils and bone marrow cells, in the pre-B cell line REH, some EBV-transformed B cell lines and the Burkitt lymphoma cell lines Raji and Daudi, but not in antibody-secreting myeloma cells, T cell lines or a promyelocytic leukemia cell line. We have raised a polyclonal mouse antiserum against human Lyb-2. This antiserum immunoprecipitates a M_r 42 K protein from REH, Raji and Daudi cells and from mouse L(tk$^-$) cells transfected with either a human Lyb-2 genomic clone or the human Lyb-2 cDNA in an expression vector. These data demonstrate that human B cells express a cell surface protein that is homologous to mouse Lyb-2 and has a similar pattern of expression during B cell development.

M 142 FcεR- SPLENIC B CELLS ARE ANATOMICALLY AND PHENOTYPICALLY DISTINCT FROM CONVENTIONAL B CELLS, Thomas Waldschmidt, Lorraine Tygrett, Frans Kroese, and Dan Conrad, University of Iowa College of Medicine, Iowa City, IA, University of Groningen, The Netherlands, and Medical College of Virginia, Richmond, VA

Previous studies have demonstrated that the low affinity IgE Fc receptor (FcεR) is expressed on all mature conventional B cells, but is not expressed on Ly 1 or sister B cells obtained from normal peritoneum. Additional experiments have shown that a distinct population of FcεR- B cells are also present in the spleens of normal mice. Although most of these cells are Ly 1 and Mac 1 negative, they resemble Ly 1 lineage B cells by virtue of their IgM high, IgD low phenotype. Further characterization of these FcεR- B cells by 3-color flow cytometry has revealed that when compared to conventional B cells, they express altered levels of LFA-1, pgp-1, J11d, BLA-1, BLA-2 and MEL 14. Histologic examination of splenic tissue sections demonstrated that in unimmunized mice, the marginal zones were primarily composed of FcεR- B cells, whereas the FcεR+ B cells were localized to the follicles. Taken together, the data indicates that the small population of FcεR- B cells present in the spleens of normal mice are phenotypically and anatomically distinct from the conventional B cells. Future experiments will be directed at testing whether the splenic FcεR- B cells are members of the Ly 1 lineage.
(Supported in part by NIH grants RR05372 and CA48485)

M 143 SERUM FREE CULTURE OF HUMAN LYMPHOID PRECURSORS, Martin L. Wolf and Tucker W. LeBien, Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN 55455

The dependency of in vitro B lymphopoiesis on marrow-derived adherent cells is epitomized by the murine Whitlock-Witte culture system. By restricting proliferation of human fetal bone marrow (FBM)-derived adherent cells using serum-free conditions, we have developed a supportive culture system for human lymphoid precursors. Ficoll-Hypaque-isolated FBM cells cultured in 5% fetal bovine serum (FBS) supplemented KC-2000 (Hazelton) readily form adherent cell monolayers. Transfer of adherent cells to serum-free medium (KC-2000) prevents overgrowth of adherent cells, and confers upon them lymphoid precursor supportive characteristics. Seeding of these serum-free adherent cells with fresh Ficoll-Hypaque-isolated FBM cells leads to preferential maintenance of lymphoid cells. At 21 days post-seeding, lymphoid cells are attached in aggregates on the adherent cells and can be recovered by trypsinization. Analysis of these cultures (n=3) by multiparameter flow cytometry indicates that lymphoid cells are the predominant cell type, aside from the adherent cells. The lymphoid cells are >95% $CD10^+/CD19^+$ and 12-35% $sIgM^+$, indicating that $sIgM^-$ B cell precursors and $sIgM^+$ B cells are present. Lymphoid cell yields on day 14 ranged from 63-187%, with 5-10% of lymphoid cells in cycle based on Ki-67 staining. Control (unseeded) adherent cell cultures do not yield lymphoid precursors, nor do FBM cells seeded on plastic in serum-free medium. These results indicate that human lymphoid precursors can be maintained in vitro at least 21 days when seeded onto FBM-derived adherent cells in serum-free medium.

M 144 LY-5 (B220) AND CD23 SURFACE EXPRESSION DECREASE WHILE LY-24 (PGP-1) INCREASES UPON ACTIVATION OF BOTH CONVENTIONAL AND $LY\text{-}1^+$ B CELLS. Henry H. Wortis, Cong Ying-zi and Evelyn Rabin Department of Pathology, Division of Immunology, Tufts University School of Medicine Boston MA 02111

We established that freshly isolated murine splenic B cells belong to one of two populations, low ly-5(B220), low J11d, $CD23^-$ high ly-24 (pgp-1), large, complex, low density cells, with increased levels of mRNA; and small, non-complex cells with low amounts of RNA, high CD23, high ly-5(B220), high j11d, and low ly-24. After incubation with LPS normal splenic B cells showed decreased levels of ly-5(B220), J11d and CD23. These changes were correlated with an increase in size and complexity, and an accumulation of RNA. Under these conditions there was an increase in the level of surface pgp-1. There was no change in the frequency of $ly\text{-}1^+$ cells. Thus, activation of conventional B cells induced the phenotype low ly-5(B220), low J11d, $CD23^-$ and $pgp\text{-}1^{hi}$.

Most peritoneal B cells are $ly\text{-}1^+$ and/or $MAC\text{-}1^+$. They are large cells, with low ly-5(B220) and high ly-24 (pgp-1). One explanation for this phenotype is that low ly-5 and high ly-24 expression are characteristic of a B cell lineage; alternatively, these peritoneal cells might be an activated subset of ly-1/MAC-1 cells. A small number of splenic $ly\text{-}1^+$ B cells were found to be $CD23^+$, high ly-5(B220), $pgp\text{-}1^{low}$, small, non-complex cells with a high buoyant density. When stimulated, these $ly\text{-}1^+$ $CD23^+$ B cells acquired the typical characteristics of activated cells. We propose that the phenotype low ly-5(B220), low J11d, high pgp-1, $CD23^-$, large size and low density, characteristic of peritoneal ly-1 B cells, is the result of the *in vivo* activation of $ly\text{-}1^+$ $CD23^+$ cells.

M 145 THE DEVELOPMENT OF MEMORY B CELLS IN THE MOUSE, Xiao-Ming Yin and Ellen S. Vitetta, Department of Microbiology, Immunology Graduate Program, University of Texas Southwestern Medical Center, Dallas, TX 75235

Elucidation of the events involved in the generation of memory B cells is crucial to our understanding of long-term immunity. However, our ability to study the generation of memory B cells is impeded by the lack of suitable markers on these cells. To this end, we have studied a marker detected by a monoclonal antibody (J11d) on antigen-specific virgin and memory B cells. B cells were purified by rosetting with trinitrophenyl-horse red cells and then used for staining analysis, adoptive transfer, or *in vitro* culture. After immunization *in vivo*, the J11d antigen expressed on splenic antigen-specific B cells is up-regulated for one week, after which time it decreases to normal values. It is then down-regulated and reaches a plateau 3 months later. The early increased expression of J11d was further confirmed by *in vitro* stimulation of virgin B cells with LPS. The decreased expression of this antigen after long-term immunization indicates that most antigen-specific primed B cells are $J11d^{lo}$ cells, while most virgin cells are $J11d^{hi}$ cells. Adoptive transfer of FACS-sorted $J11d^{hi}$ or $J11d^{-/lo}$ primed antigen-specific B cells showed that while both population can transfer an IgM response, only $J11d^{-/lo}$ cells can transfer an IgG response, suggesting that the latter are typical memory cells. While the expression of J11d does not depend on the expression of surface Ig, the $J11d^{lo}$ cells are also $sIgM^{lo}$, an observation consistent with earlier reports that memory B cells express lower densities of sIgM. In conclusion, $J11d^{-/lo}$ cells from primed mice may be memory cells which have the potential to produce IgM and IgG.

Gene Expression in B Cells; Control of Rearrangement

M 200 THE REMARKABLE PHENOTYPIC CHANGES IN THE MUTANT 'ALICIA' RABBIT MAY BE DUE TO A SMALL DELETION IN THE MOST 3' VH GENES. M. Allegrucci[1], B. Newman[1], A. S. Kelus[2], and R. G. Mage[1]. [1]Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health Bethesda, MD 20892, and [2]Basel Institute for Immunology, Basel, Switzerland.

The rabbit is unique in having well defined allotypes in the variable region of the heavy chain. Products of the VHa locus, (with the alleles a1, a2, and a3), account for the majority of the serum Igs. A small percentage of the serum Igs are a-negative. In 1986, Kelus and Weiss described a mutation that affected the expression of the Ig VH a2 genes in an a1/a2 rabbit. From this animal the *Alicia* rabbit strain was developed and the mutation was termed *ali*. In heterozygous *ali* rabbits the mutated chromosome was practically silent; however, homozygous *ali/ali* animals had normal levels of serum Igs resulting both from residual ability to produce Igs with the a2 allotype and from increased production of a-negative Igs. Using Southern analysis and a variety of IgH probes we found that *ali* and normal (F-I) DNA are indistinguishable; however using one pan-VH probe we saw quantitative differences. With the TAFE technique using the enzyme Sfi I and a probe for a region of DNA a few Kb downstream of the most 3' VH genes, we found that a 150 Kb fragment in normal became 135 Kb in the mutant. Studies of partial digests confirmed this finding and additional VH probing revealed that this Sfi I fragment was VH positive. The use of the enzyme Cla I which generates larger fragments, further corroborated these results. In conclusion, our extensive search using Southern analysis and the powerful TAFE technique suggests that the difference between the *ali* rabbit and normal is a relatively small deletion. However, this deletion may include some of the most 3' VH genes which, as has been described in other species, play a key role in regulation of gene expression during B-cell development.

M 201 CHROMATIN STRUCTURE AND HYPERSENSITIVE SITES OF THE IGK LOCUS DURING B-CELL DEVELOPMENT. Dieter Auch and Michael Reth; Max Planck Institut für Immunbiologie, Freiburg FRG

During B lymphocyte developement rearrangements occur at different immunoglobulin (Ig) gene loci in a controlled and ordered fashion. How rearrangement at Ig loci are specifically activated during development is not yet understood.

According to the "accessibility model" V gene rearrangements thought to be controlled via the specific opening of the Ig loci. We have tested "accessibility" of the J-Ck region using DNaseI digestion of isolated nuclei. Hypersensitive sites (HSS)were seen as early as during Vh to DJh rearrangement. We conclude that the J-Ck region is opened very early in B-cell development and that openess does most probably not account for the regulation of the rearrangement process. HSS were mapped 5'Jk and in the Jk region, at the J-Ck intron enhancer and 5' of it, at the 3' kappa enhancer and around the RS region. All HSS in this region are B cell specific as compared to T cells and liver. One site in the rs region is detectable only in kappa producing cells. This site is presently analysed in detail.

M 202 A NEW λ-LIKE LIGHT CHAIN GENE, 16.2, IS RELATED TO GENES 14.1 AND 16.1 EXPRESSED IN PRE-B CELLS, Thomas R. Bauer, Jr. and Bonnie B. Blomberg, Department of Microbiology and Immunology, University of Miami School of Medicine, Miami, FL 33101

Certain λ-like immunoglobulin genes have been found to be expressed only in pre-B cell lines. These genes, the mouse λ5 gene (Kudo et al. EMBO 6:103-7, 1987) and the human 14.1 and 16.1 genes (Hollis et al. PNAS 86:5552-6, 1989), are found expressed in addition to the μ heavy chain gene and may control B cell development. We have cloned and characterized a third human lambda related gene, 16.2. The DNA sequence of the C gene segment of the 16.2 gene showed 95.6% and 94.1% homology to the C gene segments of genes 14.1 and 16.1, respectively, and places gene 16.2 within the family of lambda-like genes. Our genomic clone of gene 16.2 has open reading frames within its J and C gene segments and also conserves the coding area immediately upstream of the J coding region. This area was shown to be expressed as mRNA by the mouse λ5 and human 14.1 genes in pre-B cell lines. In addition, an RNA acceptor splice site 84 bp 5' of the J coding regions of the mouse λ5 and human 14.1 genes was found conserved in gene 16.2. The conservation of the J and C coding regions and RNA splice sites of genes 14.1 and 16.2 provides further evidence that these genes are the human analogues of the mouse λ5 gene. We will present further data concerning the expression of the human 14.1, 16.1, and 16.2 genes in pre-B cells.

M 203 ALLELIC INHERITANCE OF RABBIT VH ALLOTYPES DETERMINED BY 3'-MOST GENE OF THE GERMLINE VH REPERTOIRE, Robert S. Becker and Katherine L. Knight, Department of Microbiology, Stritch School of Medicine, Loyola University Chicago, Maywood, IL 60153.

We have been perplexed by how rabbit VH allotypes, a1, a2 and a3, can be expressed on over 80% of serum Ig and behave as allelic genes when they are encoded by VH genes, of which there are several hundred in rabbit. Our analysis of VDJ rearrangements within leukemic rabbit B cells indicated that one or a small number of germline VH genes had been utilized. Since the leukemic cells appear to represent early B cells and early B cells in other species preferentially utilize the 3'-most VH genes, we compared the nucleotide sequences of the leukemic VDJ rearrangements with the 3'-most VH genes on the three VHa chromosomes. This analysis indicated that the 3'-most VH gene, VH1, encoded a1, a2 and a3 molecules on the a1, a2 and a3 chromosomes, respectively, and that VH1 had been preferentially utilized by the leukemic B cells. Utilization of VH1 was further confirmed by showing that the region upstream of the cloned VDJ gene from an a2 expressing leukemic B cell line was identical to the region upstream of VH1 on the germline a2 chromosome. To determine whether VH1 is responsible for the high level expression of VHa allotypes in normal rabbit B cells, we analyzed the 3'-most VH gene of a mutant a2 rabbit, Alicia, that expresses very low levels of a2 Ig. Comparison of the cosmid clones containing the 3'-most VH genes from Alicia and from a normal a2 chromosome revealed that the mutation in Alicia had deleted VH1. Based on these observations, we propose that VH1 is preferentially utilized by normal rabbit B cells in VDJ rearrangements and that VH1 is responsible for the allelic inheritance of the rabbit VH allotypes.

M 204 **MODULATION OF POLYMERASE ACTIVITY IN NORMAL B CELL ACTIVATION.** B. Beilue, K. Ariizumi, P. Tucker, and D. Yuan. Departments of Pathology and Microbiology, U.T. Southwestern Medical School. Dallas, TX. 75235. The induction of IgM secretion in LPS stimulated cells is mediated at the level of transcription in that total RNA synthesis, as well as specific transcription of the μ gene, is enhanced. Previous work in this lab has demonstrated that LPS stimulated, small resting B cells show a four-fold increase in specific μ transcription at day 4 over that of unstimulated cells. Anti-Igs have been shown to be potent inhibitors of LPS induced B cell differentiation, but not proliferation. In order to more closely examine the regulation of polymerase activity by these agents, we have transfected normal splenic B cells with both SV40 and IgH enhancer-driven CAT vectors. We found that IL-2 activated B cells can be transfected with both of the CAT plasmids. CAT activity is very low in these cells despite the fact that IL-2 stimulates extensive cell division, but not secretion. In contrast, stimulation of resting B cells by LPS before transfection dramatically enhances CAT activity of both vectors. Addition of anti-μ to the LPS stimulated cells did not reduce cell proliferation, but greatly diminished the CAT activity of transfected cells. Thus non-specific effects of LPS on the transfection efficiency can be discounted. Experiments are in progress to dissect the effects of the promoter and enhancer elements in regulating the enhanced transcription of the IgH locus induced by B cell activation.

M 205 MANIPULATION OF ANTIBODY ISOTYPE DISTRIBUTION USING ACID-TREATED SALMONELLA MINNESOTA R595, Dirk U. Bellstedt, Tersia Greeff and Kirsten J. van der Merwe, Department of Biochemistry, University of Stellenbosch, Stellenbosch 7600, R.S.A. Acid-treated Salmonella minnesota R595 can be used as a carrier for both adsorbed and covalently linked antigens. Most currently used antigenic carrier adjuvant combinations used to elicit antibodies in mice, such as keyhole limpet hemocyanin in Freund's Complete adjuvant, give rise to antibody isotype distributions consisting mainly of IgG1, smaller amounts of IgG2a and IgG2b and very little IgG3. Monoclonal antibodies resulting from such immunisation schemes also result mainly in the production of IgG1. Often, for a variety of reasons, antibodies of different isotypes are sought. The present study shows that if acid-treated Salmonella minnesota R595 are used as antigenic carriers, the isotype distribution elicited in mice changes to result in a predomination of the IgG2b and IgG3 isotypes, with smaller amounts of IgG2a and little IgG1. This is mainly the result of the lipid A component of these bacteria.

M 206 REGULATION OF IG GENE EXPRESSION IN NORMAL LYMPHOCYTES: THE HALF LIFE OF μ_s DIFFERS FROM THAT OF μ_m IN RESTING AND ACTIVATED B CELLS, Ingolf Berberich and Anneliese Schimpl, Institut für Virologie und Immunbiologie der Universität, Versbacher Str.7, 8700 Würzburg, FRG

Resting and activated B lymphocytes were used to study the stabilities of μ specific precursor and mature mRNA's. Resting cells which predominantly process the μ precursor towards μ_m do so rather slowly as reflected in a precursor half life of 1 - 2 hours. The small amount of μ_s mRNA is fairly stable (T1/2~8 hours) compared to μ_m (T1/2~4 hours). After activation the precursor processing is very fast (T1/2~10 minutes) and the stability of μ_s, which now predominates, increases (T1/2~16 hours) while the half life of μ_m stays about 4 hours. The data indicate that normal B cells regulate μ specific mRNA stability differently from tumour cells of the B cell lineage. Transfection experiments using reporter genes with the relevant μ_s and μ_m 3'sequences are being done to elucidate the underlying mechanism.

M 207 EXTINCTION OF B AND T CELL SPECIFIC GENES EXPRESSION IN SOMATIC CELL HYBRIDS, Yehudit Bergman, Ludmila Shurman, Batia Streich, Hava Sharir and Reuven Laskov, The Hebrew University-Hadassah Medical School, Jerusalem 91010, Israel.

Positive and negative regulation play an important role in the regulation of B and T cell specific gene expression. To study the negative regulation of these genes we used myeloma x fibroblast and T x fibroblast somatic cell hybrids. The expression of T-cell receptor β-chain and the Thy-1 genes was dramatically repressed in T x fibroblast hybrids. The TcR-β chain enhancer was found to confer suppression upon a heterologous gene and thus probably serves as a target for TcR-β chain repression in these hybrids.

The expression of the B lineage specific genes like Ig heavy and light chains, mouse CD20 and J chain genes was extinguished at the transcriptional level in myeloma x fibroblast hybrids. Using S1 analysis we showed that the transfected k-chain gene is also subjected to negative regulation in our hybrids. The NF-kB transcription factor known to be critical for k-chain enhancer activity, is present although in a lower amount, in the nucleus and in the cytosolic fraction of most of these hybrids (probably attached to the previously postulated I-kB inhibitor). In contrast, the expression of the NF-A2/OTF-2 transcription factor encoded by the oct-2 gene, which binds to the octameric motif located in the Ig promoters and heavy chain gene enhancer, is extinguished at the transcriptional level. Our data thus suggest that extinction of Ig genes expression occurs via an indirect mechanism in which a fibroblast factor suppresses transcription factor(s) which are critical for Ig transcription. We are currently studying the effect of expression of oct-2 gene on Ig expression in our hybrids.

M 208 ANALYSIS OF THE TRANSCRIPTIONAL EFFICIENCY OF MURINE AND HUMAN KAPPA IMMUNOGLOBULIN ENHANCERS IN PRE B AND MATURE B CELL LINES. Susan L. Christian and Brian G. Van Ness, Dept. of Labortory Medicine and Pathology and the Dept. of Biochemistry, University of Minnesota, Minneapolis, MN 55455.

Multiple sequence motifs have been identified in the enhancer region of the murine kappa immunoglobulin gene each of which contributes to full enhancer activity. Much less is known about regulatory sequences which control human kappa gene expression. Although there is a high degree of sequence identity between the mouse and human kappa enhancer regions, several potentially significant differences exist between them. The human gene possesses an inverted repeat duplication of the NFKB binding site known to be obligatory for maximal enhancer function, and another site 5' of the NFKB site binds different transcription factors in mouse and human B cell nuclear extracts. To gain an overall picture of the transcriptional efficiencies of these two enhancers, the murine 512 b.p. Hinf I fragment and the human 706 b.p. EcoRI fragment, each containing their respective enhancer regions, have been cloned into the KpCAT expresion vector containing a natural kappa promoter linked to the CAT reporter gene. Protein-DNA interactions and transient transfection assays are being performed to analyze the differences in enhancer function in both murine and human pre B and mature B cell lines.

M 209 CANDIDATE REGULATORY PROTEINS THAT BIND TO DNA SEQUENCES NEAR HEAVY CHAIN SWITCH REGIONS, John T. Collins, Laura Elenich, and Wesley Dunnick, Dept. of Microbiology and Immunology, Univ. of Michigan Medical School, Ann Arbor, MI 48109-0620.

The immunoglobulin heavy chain switch DNA rearrangement is a regulated event, and it is likely that DNA binding proteins mediate at least part of this regulation. We have searched for DNA binding by nuclear proteins from cell lines that undergo the heavy chain switch or that have characteristics that correlate with the heavy chain switch. T. Hamano and R. Asofsky have shown that the B cell hybridoma TH2.54, upon induction with anti-μ antibodies, switches from μ to γ2a. In collaboration with Dr. Asofsky, we have identified in the nuclei of TH2.54 cells treated with anti-μ antibodies a protein that binds to a DNA sequence about 1.3 kb 5' of the Sγ2a region. We have failed to find the same protein in the nuclei of untreated cells. The plasma cell hybridoma 470.25, a γ3-expressor with an Sγ1 DNA rearrangement, has a strong DNAse I hypersensitive site near the start site of the Sγ1 sterile transcripts that others have shown correlate with switching to γ1. The T-cell line EL4 has a much weaker hypersensitive site at the sterile transcript start site, but has a hypersensitive site, 3' to the start site, not found in 470.25. We have identified in the nuclei of EL4 cells a protein that binds to DNA sequences at or near this EL4-specific hypersensitive site. This EL4 nuclear protein is a candidate for a negative regulator of γ1 switch rearrangement.

M 210 TRANSLATION OF THE SIGNAL SEQUENCE PEPTIDE IS REQUIRED FOR THE STABILITY OF μ HEAVY CHAIN MRNA IN J558L CELLS, Angela Cox and J. Spencer Emtage, Celltech Ltd., 216 Bath Road, Slough, Berks., UK. It has been demonstrated by Mason *et al* (Genes and Development **2**, 1003-1011, 1988), that targetting to membrane-bound polysomes may be important for controlling the stability of immunoglobulin mRNA. We have investigated this further by transfecting plasmids containing wild-type or mutant immunoglobulin μ heavy chain constructs into J558L cells. The μ heavy chain genes were under the control of a Human heat-shock promoter, allowing a pulse of μ mRNA to be generated upon heat shock of the transfected cells. The rate of decay of this mRNA was then determined by densitometric scanning of Northern blots that had been probed with a μ-specific probe. In order to examine the fate of μ mRNA whose signal seqence codons could not be translated, a mutant μ gene was generated using *in vitro* site-directed mutagenesis, in which the normal initiating AUG codon had been removed, and an AUG had been inserted at the 5' end of the sequence coding for the mature μ polypeptide. The inserted AUG was preceded by the Kozak consensus sequence for optimal translation initiation (Kozak, M., (1987) J. Mol. Biol. **196**, 947-950). When the rates of decay of the normal and mutant mRNA were compared, it was found that the stability of the mutant mRNA was much reduced compared to the wild-type. In addition, no IgM protein was secreted from cells containing the mutant RNA, and intracellular μ heavy chain comigrated with unglycosylated μ, indicating that it had not been through the secretory pathway. Further experiments are in progress to determine the localisation of the wild-type and mutant μ mRNA in these cells.

M 211 CLONING OF TWO cDNAs WHICH RECOGNIZE NOVEL HOMEOBOX CONTAINING GENES FROM A HUMAN B LYMPHOCYTE cDNA LIBRARY, Yasuhiro Deguchi, John F. Moroney, Gaye Lynn Wilson, Anthony S. Fauci, and John H. Kehrl, Laboratory of Immunoregulation, NIAID, NIH, Bethesda, MD 20892. Homeotic proteins are sequence-specific DNA binding proteins that are implicated in the control of gene expression. We used degenerative oligonucleotides anti-sense to a conserved region in homeotic proteins to screen a cDNA library made from normal B lymphocytes. 750,000 plaques were screened and 5 positive clones isolated. Partial DNA sequence analysis revealed that 2 of them encoded for a protein identical to C1, a known homeobox containing protein. Northern blot analysis revealed that the C1 mRNA (1.1 and 1.5 kb) was expressed in 5 B cell lines. RNA protection assays are in progress to further define the expression of C1 in a variety of B cell and T cell lines. Two other clones (HB24 and HB35) encode for a previously unrecognized homeobox containing protein (based on a search of the most recent version of Genbank). Within the homeobox region the derived amino acid sequence of HB24 is 38% identical to the consensus sequence for homeobox containing proteins and 28% identical to Oct 2. Northern blot analysis revealed a single mRNA in normal B cells of approximately 2.4 kb. The last clone, HB9, also encoded for a previously unrecognized homeobox containing protein. Within the homeobox region the derived amino acid sequence of HB9 is 50% identical to a consensus sequence for homeotic proteins. Northern blot analysis of poly A+ RNA derived from the Nalm-6 pre B cell line demonstrated at least 3 distinct mRNA species of approximately 7, 5, and 2.5 kb. An RNA protection assay revealed protection of a 419 bp fragment in a variety of B and T cell lines. It is expected that each of these homeotic proteins will be important in gene regulation in human B cells.

M 212 REGULATORY ELEMENTS INVOLVED IN THE BIDIRECTIONAL ACTIVITY OF AN IMMUNOGLOBULIN PROMOTER , N. Doyen, T. Nguyen, M. Dreyfus and F. Rougeon, Unité de Génétique et Biochimie du Développement, Institut Pasteur, France. The octamer sequence ATGCAAAT or its inverse complement ATTTGCAT with an associated TATA box is both necessary and sufficient for the activity of immunoglobulin promoters in B cells. We show that the promoter from the mouse VH441 heavy immunoglobulin gene when introduced into myeloma cells is capable of directing transcription in a bidirectional manner. This is a reflection of the orientation independent activity of the octanucleotide and the presence on both strands of TATA like sequences bracketting this element. The transcriptional activity, in either orientation, correlates with the likeness of the two TATA boxes to the consensus sequence and is also influenced by an element within the distally located heavy chain enhancer. Experimental data show that bidirectional transcription from this VH promoter also occurs *in vivo* and furthermore the transcription initiation start sites in either orientation are the same *in vivo* as in the transfection experiments.

M 213 ISOTYPE SPECIFICITY AND MECHANISM OF IMMUNOGLOBULIN HEAVY CHAIN SWITCH REARRANGEMENT, Wesley Dunnick, John Collins, and Cindy Schultz*, Dept. of Microbiology and Immunology, Univ. of Michigan Medical School, Ann Arbor, MI 49109-0620, *Present address: DNAX Research Institute, Palo Alto, CA 94304.

Murine B cells have the option of six different heavy chain switch rearrangements: to Cγ3, Cγ1, Cγ2b, Cγ2a, Cε, and Cα. In collaboration with Drs. J. L. Claflin, K. A. Denis, P. Gearhart, C. Gritzmacher, T. Manser, J. Petrini, and M. Schulman, we have analyzed the switch region content of 54 hybridomas, including γ, α, and ε expressors. In 60% of the hybridomas, both heavy chain loci have rearranged to the same C gene. If the two heavy chain loci are rearranged to different C genes, most often the two genes lie next to each other in the locus. These data are consistent with the notion that switch rearrangement is regulated by chromatin accessibility. In most cases, specific "opening" would result in rearrangement to a specific gene; at times the accessibility may be propagated along the chromosome, resulting in rearrangement to a nearby gene. In collaboration with Dr. J. Stavrezer, we have obtainded DNA sequences of multiple clones which were derived from a single switch recombination event. These sequences fall into two groups, one with mutation relative to the progenitor sequence and one without mutations. These results suggest that the mechanism of switch recombination is mediated by a copy-choice mechanism in which one strand of DNA in the recombinant switch region is newly synthesized and the other is contributed by pre-existing DNA.

M 214 B CELL ABNORMALITIES INDUCED BY A μ IMMUNOGLOBULIN TRANSGENE EXTEND TO LIGHT CHAIN ISOTYPE USAGE. Jeannine M. Durdik, Departments of Medicine and Microbiology/Immunology, University of Colorado School of Medicine, Denver, CO 80262, and Alfred Nisonoff, Erik Selsing, Satyajit Rath, Department of Biology, Rosenstiel Research Center, Brandeis University, Waltham, MA 02154.

We have analyzed the phenotype of B cell populations from mice transgenic for a rearranged immunoglobulin μ heavy chain gene. We find a decrease in the number of B cells in the spleens of these mice. Transgenic B cells have decreased surface levels of both IgM and IgD. The circulating IgM in these mice is 3 to 10 fold enriched in λ light chains as opposed to non-transgenic mice although the proportion of λ chains in their serum IgG is normal.

Analysis of hybridomas from transgenic mice (which express the transgene at high levels) demonstrates that this higher λ frequency is not dependent upon that individual hybridoma expressing the transgene. A partial loss of light chain isotype exclusion is also noted in these hybridomas and is confirmed by the demonstration of a significant proportion of primary B cells expressing both κ and λ light chain on their surface. These findings suggest an ability of the Ig heavy chain to affect events in B cell ontogeny beyond the heavy chain locus.

M 215 Control of VDJ Recombinase Activity: Role of Immunoglobulin and T Cell Receptor transcriptional elements. Pierre Ferrier, Lori R. Covey, H. Suh, M. Mendelson, L. Hood, and F.W. Alt, Howard Hughes Medical Institute and the Department of Biochemistry and Microbiology, College of Physicians and Surgeons of Columbia University, New York, N.Y. 10032.

Using recombination subtrates comprising T cell receptor (TCR) *β* variable segments and Immunoglobulin heavy chain (IgH) constant region, we have previously defined "recombinational enhancer elements" that control two separate aspects of TCR*β* VDJ rearrangements within constructs integrated into the genome of transgenic mice. One lies within the DNA fragment containing the IgH enhancer and acts to initiate lymphoid specific D*β* to J*β* rearrangements; the other, associated with the V*β* region of the construct, provides T cell-specific control of complete (V*β* to DJ*β*) variable gene assembly. The recombinational activity promoted by each separate element correlates with the germline transcription of the corresponding region of the construct. Consequently, we have produced new transgenic mice using similar substrates carriing modifications (including substitution, deletion, and mutation) in the DJ*β*-associated enhancer region or V*β*-associated promoter region of the construct. The recombination potential of such modified transgenes will be presented.

M 216 THE USE OF ROBERTSONIAN (8:12) CHROMOSOME CARRYING MICE AS A MODEL FOR ALLELIC EXCLUSION OF Ig H CHAIN GENES. Jill Finlayson and Eugene B Dowdle, Department of Clinical Science and Immunology, University of Cape Town Medical School, Cape Town, South Africa.

Splenocytes from mice homozygous for the Rb(8:12) Bnr translocation, in which chromosome 8 carrying the APRT gene is translocated onto chromosome 12 which carries the IgH locus, were used for hybridoma production. The fusion partner was the HGPRT and APRT negative Fox NY myeloma line; adenine selection was used to select for hybridomas carrying the translocated chromosome. The hybridomas were screened for intracytoplasmic H chain protein and were divided into two groups - H chain producers and non-producers which carry functional and excluded alleles respectively. Karyotypic analysis confirmed the presence of a single translocated chromosome per cell. Southern Blot analysis has been performed to assay for gene rearrangements - 40 % of non-producers were in the germline configuration whereas all producers show rearrangement. It is hoped that this model will be useful for further studies in the mechanism of allelic exclusion.

M 217 **B-CELL DEVELOPMENT IN IG-TRANSGENIC MICE**

U.Fritzsche, A. Iglesias, G. Köhler, R. Lamers
MPI f. Immunbiologie, D-7800 Freiburg

Mice transgenic for heavy chain (H.C.) immunoglobulin genes show deficiencies in their mature B-cell pool. We have therefore studied the development of B-cells in these mice. First, we established the frequency of bone marrow (BM) and fetal liver pre B-cells that could be transformed with A-MuLV. The frequency for all H.C. transgenic mice was <1% of the littermate control. We conclude that either the frequency of cells that can be transformed is decreased, or that the pre B-cells are resistant to transformation. Soft agar cloning experiments showed a five times lower cloning efficiency for pre B-cells in transgenic BM and spleen. The percentage of endogenous H.C. colonies was less than 10%. Flow cytometric analysis showed a variable but significant decrease in the number of small $sIgM^-$, $B220^+$ cells in BM. Less than 20% of the $sIgM^+$ cells in BM expressed endogenous H.C. genes. Immature VDJ joining configurations were found in the few A-MuLV lines established and in splenic B-cell hybridomas. These results suggest a shorter developmental pathway of the B-cells in Ig H.C. transgenic mice.

M 218 INTERCHROMOSOMAL ISOTYPE SWITCH RECOMBINATION IN IMMUNOGLOBULIN MU HEAVY CHAIN TRANSGENIC MICE.

R. Gerstein, W. Frankel[1], J. Coffin[1], J. Durdik[2], S. Rath[3], A. Nisonoff, E. Selsing. Dept. of Biology and Rosenstiel Basic Medical Sciences Research Center, Waltham, MA. [1] Dept. of Molecular Biology, Tufts Univ. School of Medicine, Boston, MA. [2] Dept. of Allergy and Immunology, Univ. of Colorado Health Sciences Center, Denver, CO. [3] Dept. of Immunobiology, Yale University School of Medicine, New Haven, CT.

In response to immunization with p-azophenylarsonate (Ar), C57Bl/6 mice transgenic for a CRIA+, Ar-specific VDJ linked to Cμ make Ar-specific Id+ antibodies which are predominantly IgG. The mRNA sequence from Ar-specific IgG transgenic hybridomas shows the transgene VDJ linked to Cγ. We have characterized switch recombinations in transgenic hybridomas from a mouse line where the transgene has been localized to chromosome 5. In a panel of γ1 expressing hybrids, all have deleted endogenous Sγ1 and many have new bands. Several hybrids have deleted transgene Sμ, implicating the transgene in switch recombination. A set of γ2a hybrids also show deletion of Sγ2a and novel bands. One hybridoma, 2B4, has deleted all copies of a portion of transgene Sμ and shows co-migration of V(CRI-A), J-H and Sγ2a. A genomic clone of the expressed Ig heavy chain has been isolated which demonstrates that the transgene has joined to endogenous Cγ by switch recombination. We are currently analyzing other hybridomas for evidence of this interchromosomal switch recombination.

M 219 DNA SEQUENCES 3' OF THE IMMUNOGLOBULIN HEAVY CHAIN CLUSTER REARRANGE IN MOUSE B-CELL LINES, Sandra L. Giannini, Charles F. Calvo, Nancy Martinez, G.-F. Ding and Barbara K. Birshtein, Department of Cell Biology, Albert Einstein College of Medicine, Bronx, New York 10461

DNA rearrangements which take place during B cell differentiation are developmentally regulated and occur in a sequential manner, resulting in productive heavy and light chain genes. Various DNA sequence motifs composed of tracts of repetitive or repeated sequences mediate recombination events at the immunoglobulin locus. Recently, we have focused our attention on a highly polymorphic region (~50 kb) 3' of the IgA constant region gene, which is composed of repeated motifs of DNA, some of which are capable of adopting an unusual DNA conformation. In this region we have detected DNA rearrangements in a number of B-cell lines, producing Igs of different isotypes. In a variant of the mouse myeloma cell line MPC-11, there is a duplication of the expressed heavy chain gene, and sequences originating 3' of IgA interrupt one Vh gene through an inversion event. Although the functional significance of the 3' alpha rearrangements is at present unknown, evidence from other laboratories suggests that this region may contain sequences that influence IgH expression and/or contain an origin of replication. Our methylation and DNAase I sensitivity studies, in the region 3' of IgA, show patterns which differ in a tissue and developmentally specific manner, in concert with a potential regulatory or other functional role. Such control elements may be disrupted or activated by rearrangement events.

M 220 MOLECULAR REQUIREMENTS FOR CONTROL OF IMMUNOGLOBULIN GENE RECOMBINATION IN TRANSGENIC MICE, M. Goodhardt*, P. Cavelier*, S. Kallenbach*, C. Babinet+ and F. Rougeon*, *Unité de Génétique et Biochimie du Développement, +Unité de Génétique des Mammifères, Institut Pasteur, France. Immunoglobulin (Ig) and T-cell receptor (TCR) variable region genes are assembled during B and T lymphocyte development by a series of somatic DNA recombination events. Although similar recombination sequences mediate assembly of both Ig and TCR genes, formation of a variable region gene is strictly B/T lineage restricted. In order to investigate the mechanism of control of Ig gene rearrangement, we have produced transgenic mice containing an unrearranged κ light chain gene. The exogenous gene construct contained a mouse germ-line κ variable (Vκ) gene and the mouse germ-line joining (Jκ) locus including the enhancer, linked to the rabbit b9 constant (Cκ) region. A high level of V-J recombination of the κ transgene was observed. However, rearrangement occurred not only in B- but also in T-cells of the transgenic mice. Furthermore, a particularly high degree of variability in the exact site of recombination and the presence of non germ-line encoded nucleotides (N-regions) were found at the V-J junction of the rearranged κ transgene. These results show that additional sequences, other than the heptamer-nonamer signal sequences and the promoter and enhancer elements, are required to obtain stage -and lineage- specific regulation of Ig κ light chain gene rearrangement *in vivo*.

M 221 CLONING AND SEQUENCING OF THE GENE ENCODING THE NONAMER BINDING PROTEIN (NBP), Brian D. Halligan, Nadine L. N. Halligan, Rick Andrews, and Ming Teng, Department of Microbiology, Medical College of Wisconsin, Milwaukee, WI 53226
Immunoglobulin gene segment recombination is mediated by a motif of recombinational signal sequences found adjacent to all functional Ig gene segments. These recombinational signal sequences consist of a conserved seven base pair region, the heptamer, a non-conserved, defined length spacer region, and a conserved nine base pair region, the nonamer. We have previously identified a protein from lymphoid sources that specifically recognizes the nonamer region†. To better study the nonamer binding protein (NBP), we have used the site affinity screening method developed by Singh and also by McKnight, which identifies cDNA clones encoding DNA binding proteins based on their affinity for a particular DNA sequence, to screen a cDNA library prepared from a lymphoid cell line. Using this method, we have identified a cDNA clone which produces a fusion protein which is capable of specifically recognizing the nonamer sequence. We are currently sequencing the cDNA clone, analyzing the binding properties of cloned protein, and determining the levels of expression of NBP in various cell lines and tissues.

†Halligan, B. D. and Desiderio, S. V. "Identification of a DNA Binding Protein that Recognizes the Nonamer Recombinational Signal Sequences of Immunoglobulin Genes" *Proc. Natl. Acad. Sci.* (USA)., **84**, 7019-7023 (1987).

M 222 BOVINE IMMUNOGLOBULIN LIGHT CHAIN ISOTYPE REGULATION, Susan Hansal and Barbara A. Osborne, Program in Molecular and Cellular Biology and Department of Veterinary and Animal Sciences, University of Massachusetts, Amherst, MA 01003
Different species express different ratios of κ-vs-λ light chains. For example, the mouse has 95% κ/5%λ and humans have 65% κ/35% λ. However in the bovine, 90-95% of expressed light chains are of the λ isotype. The relative expression of κ-vs-λ could be due to the number of V region gene segments associated with a constant region in the germline, the organization of the light chain locus, or the presence or absence of regulatory elements. In the bovine system, we have a unique perspective to study isotype expression in a primarily λ light chain producer. By utilizing cDNA and genomic clones, the organization of the bovine light chain locus is being examined. We have isolated a bovine λ cDNA clone and are in the process of characterizing genomic λ and genomic κ clones. Preliminary southern blot data of genomic DNA indicates only a single Cλ gene in contrast to the multiple copies of Cλ found in other species such as human, mouse, rabbit and rat. The relative expression of κ-vs-λ is known to change in mouse during ontogeny. Presumably, this is due to antigenic clonal expansion of the isotype capable of generating the most diversity. Studies are underway to estimate the diversity of each type of bovine light chain by electrophoretic techniques. 2D gel electrophoresis will give an indication of the possible V region repertoire in both κ and λ light chains. We also are using anti-κ and anti-λ antibodies to immunolabel adult and fetal bovine B cells. These cells will be subjected to FACS analysis to further determine the expressed isotypic ratios. The results from these studies will presented.

M 223 MAPPING AND DELETIONAL ANALYSES OF THE DUPLICATED KAPPA LIGHT CHAIN LOCUS OF RABBITS, Nichola J.K. Hole and Rose G. Mage, Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892.

The immunoglobulin (Ig) kappa light chain locus of the rabbit has two isotypic forms, one of which (K2) is poorly expressed in the normal rabbit. The allotypic forms and restriction fragment length polymorphisms of these are inherited as linked traits. By megabase mapping using pulsed field gel and transverse alternating field electrophoresis, we have shown that the duplication which gave rise to K1 and K2 was a large one that included Vκ and Jκ regions in addition to Cκ. In Not I digests of genomic DNA we find the K1 and K2 genes on an ~2.75 Mb Not I fragment. This appears to be a partial cleavage product composed of several smaller Not I fragments. Our mapping data using combinations of infrequently cutting enzymes indicate that the Cκ1 and Cκ2 genes are ~1.25 Mb apart. The Lagomorphs are thus far unique in having two isotypes of kappa; however the human kappa locus has similar large duplications of Vκ genes. The human and mouse kappa loci have a kappa deleting element which has functioned in lambda-expressing B cells. Preliminary evidence from Southern analyses of FACS-sorted κ and λ expressing B-cells from rabbits suggest the presence of a comparable functional element. *Basilea* rabbits produce elevated levels of K2 and lambda light chains due to a defect in K1b9 mRNA splicing. Experiments are in progress to quantitate kappa deletions in normal $K1b^{9}$ compared to mutant $K1b^{bas}$ (*Basilea*) animals.

M 224 ANALYSIS OF THE GENOMIC STRUCTURE, EXPRESSION, AND PROTEIN PRODUCTS OF THE PRE-B CELL SPECIFIC GENES HOM-1 AND VpreB. Gregory F. Hollis[1], Robert E. Evans[1], Jeannine M. Stafford-Hollis[2], and John P. McKearn[2], [1] Department of Biological Sciences, Monsanto Co., [2] Molecular and Cellular Biology Department, G.D. Searle Co., St. Louis, MO 63198.

Human pre-B cells produce immunoglobulin heavy chain, but do not produce immunoglobulin light chain. We have recently isolated a pre-B cell cDNA, Hom-1, that encodes a protein that shares strong homology with immunoglobulin λ light chain joining and constant region sequences. This gene is encoded by 3 exons located on human chromosome 22 band q11 and unlike immunoglobulin genes, does not undergo rearrangement prior to expression. 26 hematopoietic cell lines were analyzed for the expression of Hom-1 and a recently isolated gene called VpreB, which encodes a protein that shares homology with an immunoglobulin light chain variable region. These studies showed that the expression of the two genes is limited to pre-B cells. Together, these two genes encode sequences homologous to an immunoglobulin light chain variable, joining and constant region and suggest that they may combine to form a surrogate immunoglobulin light chain complexed to the immunoglobulin cytoplasmic μ heavy chain. Recent western blotting and immunoprecipitation experiments confirm the presence of this multi-protein complex.

M 225 ANALYSIS OF IgA SUBCLASS SPECIFIC RNA: EVIDENCE FOR SECRETED IgA IN POLYCLONALLY ACTIVATED PERIPHERAL BLOOD LYMPHOCYTES, Khalid B. Islam, Lennart Hammarström and C. I. Edvard Smith, Center for Biotechnology, Karolinska Institute at NOVUM, S 141 52 Huddinge, Sweden and Department of Clinical Immunolgy, Karolinska Institute at Huddinge Hospital, S 141 86 Huddinge, Sweden.

Peripheral blood lymphocytes (PBL) were stimulated *in vitro* by pokeweed mitogen (PWM) and Epstein-Barr virus (EBV), and IgA subclass specific RNA was examined. In order to analyze IgA subclass specific RNA we have developed specific RNA probes for quantitation of α1 and α2 heavy chain constant region genes by solution hybridization. By this method, we can detect as little as 3 pg of specific RNA from the sample RNA. The cross-reactivity between the two IgA subclasses is less than 2%. Using this method, we found the relative proportions of IgA1 and IgA2 mRNA in the PWM-stimulated cells to be 66% and 34%, respectively, while in the EBV-stimulated cells they were 75% and 25%, respectively. By ELISA, 72% IgA1 and 18% IgA2 were found in the culture supernatant of PWM-stimulated cells while EBV-stimulation induced 85% IgA1 and 15% IgA2. In agreement with previous studies, we found an almost equal distribution of plasma cells representing the IgA subclasses, whereas, there is a preference for IgA1 both at the RNA and secreted protein level. The possible underlying mechanism(s) accounting for this preference will be discussed. In essence, we describe a method for the quantitative analysis for IgA subclass specific RNA. In addition, we report that *in vitro* stimulation of PBL by polyclonal activators results in both synthesis and secretion of both the IgA subclasses.

M 226 Isolation and Characterization of Enhancers and Genes Regulated in B Cell Differentiation.
Bill Kerr, Jeffrey Johnsen, Garry Nolan and Leonard Herzenberg. Dept. of Genetics, S-337, Stanford University, Stanford, CA, 94305.

We haved developed a series of retroviruses which can detect the induction or repression of gene expression *in situ* (Immunology **68**: 74 - 79; CSHSQB **54**, in press). These retroviruses generate transcriptionally-defective proviruses containing the bacterial reporter gene, *E. coli lacZ*. Because expression of *lacZ* is controlled in *cis* by endogenous transcription control elements these retroviruses can be used to detect developmentally-regulated chromatin and genes. Infection of 70Z/3 cells with the enhancer-search retrovirus, Enhsr1, has led to the identification of several regions of chromatin whose transcriptional activity is repressed or induced by LPS-induced differentiation of 70Z/3 cells from the pre-B to the B cell stage. In addition to the well documented induction of kappa light chain transcription by LPS, the repression of *lacZ* expression in the Enhsr1 clone, 7e17-17, indicates that LPS can also repress expression of genes in B cell differentiation.

We have recently developed a gene-search retrovirus, Gensr1, which requires transcriptional and translational fusion of *lacZ* with an endogenous gene for β-galactosidase to be expressed. We have demonstrated that this approach leads to β-galactosidase fusion proteins in clones with enzymatic activity. We have infected 70Z/3 cells with Gensr1 and have identified a β-galactosidase expressing clone, 7a291, where *lacZ* expression is repressed 5 to 10-fold during LPS-induced differentiation. We are currently characterizing this *in situ* gene fusion in greater detail. In addition, we are infecting murine hemopoietic progenitor cells and using infected cells to reconstitute lethally-irradiated allotype congenic mice. This approach may provide a stably expressed genetic marker (*lacZ*) for hemopoietic progenitor cells and their differentiated progeny as well as identify genetic changes which occur during normal hematolymphoid differentiation.

M 227 IDENTIFICATION OF NUCLEAR FACTORS BINDING TO THE MURINE IMMUNOGLOBULIN C_μ GENE SWITCH (S_μ) REGION, Moon G. Kim and Kenneth B. Marcu, Department of Biochemistry and Cell Biology, SUNY at Stony Brook, Stony Brook, NY 11794. The properties of two nuclear factors, which bind to DNA sequences within the S_μ region, were characterized by gel mobility shifts and DMS and Op-Cu nuclease footprinting techniques. One factor (designated U) is ubiquitously expressed and binds to the sequence AAAAAGCATGGCTGA which is present at multiple locations 5' of the repetitious portion of the S_μ region which consists of myriad, tandemly arranged $(GAGCT)_2GGGGTGAGCT$ motifs. The expression of the second factor (designated B) is restricted to pre-B and mature B cells; and it binds to a portion of the S_μ repeat motif GGCTGAGCTGAG which overlaps the 3' 5 bp of the U factor binding site. The B protein appears to bind with a lower affinity to the tandemly repeated portion of the S_μ region since the overlapping U + B binding site is the most efficient competitor for B factor binding and other synthetic oligonucleotides containing mutiple S_μ repeat motifs are more efficient competitors than a single motif. The U and B factors have been partially purified on Heparin-agarose, DEAE-Sepharose and Sephacryl S-300 chromatography.

M 228 GLOBAL ANALYSIS OF MEMBRANE PROTEINS FROM WEHI 279.1 SUBCLONES REVEALS CO-EXPRESSION OF GROUPS OF PROTEINS. Bernhard Kleine*, Wolfgang G. Bessler*, Antonio Coutinho+,and Jack Kettman#, *Institut für Immunbiologie, Universität Freiburg, FRG, +Unité d'Immunobiologie, Institut Pasteur Paris, France, #Southwestern Medical School, University of Texas, Dallas, TX 75235.

High-resolution two-dimensional O'Farrel gels were prepared from membrane protein extracts of subclones of the murine WEHI 279.1 B lymphoma cell line which had been characterized for reactivity to lipopolysaccharide, synthetic lipopeptide analogues of *E.coli* lipoproteins, and dextran sulfate. By visual observation we could not find individual proteins correlating which mitogen reactivity, thus be specifically expressed in a given mitogen reactive state. After scoring a subset of 45 proteins for their relative abundance in all 12 2d-gels this data set was subjected to multivariate analysis. Using two different statistical procedures, cluster analysis and principal component analysis, we grouped the 12 gels into two groups. Both groups could be distinguished by sets of surface proteins. These proteins are now studied whether they represent proteins characteristic for different stages of B cell development.

(Supported by grants from the Deutsche Forschungsgemeinschaft, Bonn, FRG)

M 229 TGF-β INDUCES ISOTYPE SWITCHING TO IgA IN LPS-STIMULATED B CELL CULTURES.
Deborah A. Lebman, Frank D. Lee, & Robert L. Coffman.
Department of Immunology, DNAX Research Institute, Palo Alto, CA. 94304
The addition of TGF-β to LPS-stimulated B cell cultures causes a 5-10 fold enhancement in secretion of IgA and an increase in the proportion of cells expressing sIgA. The increased IgA expression in the total splenic B cell population and the $sIgA^-$ population was comparable. Furthermore, TGF-β induces the appearance of two distinct sizes of α mRNA transcript. One of these is the same size as mRNA for secreted IgA whereas the other, which is 300-400 bp shorter, does not correlate in size with any form of productive α mRNA. We isolated cDNA clones corresponding to the shorter, 1.3 kb, transcript using an anchored polymerase chain reaction and a specific primer for the constant region. Analyses of these cDNA clones demonstrated that the 1.3 kb transcript consists of a 126 bp exon located approximately 1.5 kb 5' to S_α spliced to the first exon C_α. A minor fraction of the longer transcript contains the same exon. These results are consistent with a model in which TGF-β induced isotype switching to IgA is preceded by transcriptional activation of the heavy chain locus.

M 230 PURIFICATION AND CHARACTERIZATION OF NBP, A PROTEIN THAT SPECIFICALLY BINDS AN ENHANCER OF IMMUNOGLOBULIN GENE REARRANGEMENT, Min Li, Ewa Morzycka-Wroblewska and Stephen V. Desiderio, Department of Molecular Biology and Genetics and Howard Hughes Medical Institute Laboratory of Genetics, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Immunoglobulin (Ig) and T-cell receptor genes are encoded in discrete germline DNA segments that are joined by site-specific recombination during lymphocyte differentiation. These DNA rearrangements are mediated by conserved heptamer and nonamer DNA sequence elements that lie near the sites of recombination. Lymphoid cells express a protein that binds to DNA fragments containing Ig recombinational signal sequences from diverse sources. By scanning mutagenesis and binding competition assays, the recognition site for this DNA-binding protein has been shown to coincide with the conserved nonamer recombinational signal. Mutations within the nonamer sequence, but not outside of it, decrease affinity for the binding protein by 300- to greater than 1000-fold. Deletion of the binding site for the protein results in at least a 50-fold decrease in recombination frequency in vivo. By a combination of conventional and recognition site affinity chromatography, the protein has been purified to apparent homogeneity from calf thymus. A purification of greater than 20,000-fold was achieved with an overall yield of 22%. The purified protein, which we call NBP for nonamer-binding protein, has an apparent molecular weight of 63,000 by SDS-polyacrylamide gel electrophoresis. The protein exists as a globular monomer in 0.5 M NaCl. Our observations suggest that NBP is a component of the recombinational apparatus.

M 231 REGULATION OF AN ENDOGENOUS MMTV PROVIRUS IN DIFFERENTIATING B CELLS, F.E. Lund, L.B. King, and R.B. Corley. Department of Microbiology and Immunology, Box 3010, Duke Medical Center, Durham, NC 27710
We have shown that an endogenous mouse mammary tumor virus is transcriptionally upregulated as a natural consequence of murine B cell differentiation. Steady state levels of MMTV transcripts are present in normal B cells and in a wide variety of B cell lines. In B cell lines that can be induced to differentiate with LPS, including CH12-LBK, MMTV transcript levels increase from 10 to 20 fold. These transcripts are encoded by the Mtv-9 provirus. Sequence analysis of the LTR of Mtv-9 revealed no deletions or insertions throughout the U3 regulatory region, and all of the cis regulatory sites appeared normal. We are currently looking at the cis sequences that are important for the trancriptional induction of MMTV in B cells. Using a reporter construct with the entire Mtv-9 LTR as the only promoter, we transiently transfected the murine B cell line CH12-LBK. Very low constitutive levels of reporter activity were present in these cells; but upon the addition of LPS, we saw 10 fold increases in reporter activity in the LPS treated cells. Using LTR deletion constructs, we plan to distinguish the regulatory regions important for LPS induction of MMTV from those regions involved in the normal steroid regulation of the virus. In addition to upregulation of MMTV transcripts, we have shown that low levels of corticosteroids induce differentiation in CH12 cells. We are now examining the relationship between steroid and LPS activation during the late stages of B cell differentiation.

M 232 A NF-KB DEPENDANT IN VITRO TRANSCRIPTION SYSTEM

Khuzaima. Mama. and Ranjan. Sen. Rosenstiel Basic Medical Sciences Research Center and Department of Biology, Brandeis University, Waltham, Massachusetts 02254-9110

NF-KB is a B cell specific transcription factor that plays the dominant role in determining the cell type specific transcription of the K light chain gene. The inducibility of NF-KB in a variety of cell types in response to the appropriate stimuli suggested that it played a role in the inducible transcription of other genes.This has been demonstrated for IL-2R α; also NF-KB binding sequences have been found upstream of several cytokines, the MHC class 1 genes, and within several viral enhancers. We have developed a cell free system where multimerized NF-KB recognition sequences upstream of heterologous promoters will strongly stimulate transcription. This system will render mechanisms of activation amenable to experimentation and will allow us to delineate differences if any; in the factor, based on the regime of induction.

M 233 INITIATION AND PROCESSING OF KAPPA IMMUNOGLOBULIN GERM LINE TRANSCRIPTS.

Debra J. Martin and Brian G. Van Ness, Department of Biochemistry and The Institute of Human Genetics, The University of Minnesota, Minneapolis, Minnesota 55455.

Transcriptional competence of germ line immunoglobulin gene segments has been suggest to play an important role in establishing the rearrangement potential of the locus. We have determined the splicing pattern and sequences of two kappa germ line mRNAs isolated from A-MuLV transformed pre-B cells, P8 and 3-1. A 1.1 kb mRNA is derived from splicing of the previously characterized 8.4 kb germ line transcript while a 0.8 kb mRNA is the splice product of a second 4.7 kb germ line transcript that initiates 50 bp upstream of J_k1. The interaction of the two kappa germ line promoters with nuclear binding factors has also been examined. Additionally, kappa germ line mRNA has been identified in human B cells. The evolutionary maintenance of kappa germ line transcription strongly suggests it plays an important role in B cell development.

M 234 CHARACTERIZATION OF THE IMMUNOGLOBULIN K3' ENHANCER, K.B. Meyer and M.S. Neuberger, MRC Laboratory of Molecular Biology, Hills Road, UK-Cambridge CB2QH,

Cell type specific gene expression of immunoglobulin genes is regulated by at least two transcriptional activators: the V-gene promoter and the enhancer located in the J- C intron. However, in transgenic mice the Vκ promoter and κ intron enhancer are not sufficient to direct high level of κ transgene expression. We therefore searched for further transcriptional control elements in the Cκ locus, and we recently identified a strong, B-cell specific enhancer located 9kb 3' of Cκ.

We have now defined regions essential for the function of the κ3' enhancer by deletional and *mutational analysis, thereby revealing potential transcription factor binding sites. This should* help to elucidate the mechanism by which the enhancer functions. Furthermore, we are analysing at which stage in B-cell development the enhancer becomes active and whether differential induction of the κ3' enhancer and the κ intron enhancer is possible. This may further define the steps involved in B-cell maturation.

M 235 METHYLATION PATTERNS OF ENDOGENOUS RETROVIRAL ELEMENTS DURING ACTIVATION OF B-LYMPHOCYTE POPULATIONS IN BALB/cAn MICE. Judy A. Mietz and Edward L. Kuff, Laboratory of Biochemistry, NCI, NIH, Bethesda, MD 20892. Methylation is thought to have an important, perhaps determining role in maintaining specific patterns of gene expression in eukaryotic cells. We are interested in endogenous retroviral expression during B-lymphocyte activation and neoplastic transformation in BALB/cAn mice. Intracisternal A-particle proviral elements, which are abundant (1000 copies per haploid genome) and widely dispersed in mouse chromatin, may be useful indicators of changes in the level of genomic demethylation during B-cell differentiation. Using a two-dimensional agarose gel electrophoresis technique for DNA separation, we are able to detect methylation changes at many individual proviral loci. Previous studies have indicated that tissue specific genes tend to undergo a progressive demethylation as lymphocyte differentiation proceeds toward a mature phenotype. We have observed a marked decrease in proviral methylation during LPS stimulation of splenic B-lymphocyte populations. This change takes place within 16 hours of induction with LPS, prior to bulk DNA synthesis as measured by ^{3}H-Tdr incorporation. Many additional demethylated proviral copies are detected in the DNAs of primary plasma cell tumors, suggesting that genome-wide demethylation may be an early event in transformation of these cells.

M 236 SEQUENCES DOWNSTREAM OF THE IMMUNOGLOBULIN GAMMA 2b SECRETION SPECIFIC POLY A SITE CONFER B-CELL TYPE REGULATED EXPRESSION, C. Milcarek, C. R. Lassman, D. Toppmeyer, and S. Matis, Department of Microbiology, Biochemistry and Molecular Biology, University of Pittsburgh, Pittsburgh, PA 15261

Constructs were designed to determine whether the 13 nucleotide immunoglobulin, secretory-specific consensus element located downstream of the secretory-encoding poly A site plays a role in the differential regulation of processing of Ig mRNA to the secretory (sec) or membrane (mb) forms during B-cell development. Oligo-nucleotides were inserted into the Ig γ2b delta-KpnI deletion mutant which uses the mb poly A site preferentially (Kobrin, Milcarek and Morrison (1986) Mol. Cell Biol. 6:1687-1697). The constructs were stably transfected into J558L or A20, tumor lines representative of plasma cells and memory B-cells, respectively. The ratios of sec:mb mRNA forms were determined by nuclease S_1 analysis of mRNA from the transfectants. Restoration of the Ig secretion specific poly A site and downstream consensus in oligo 6 causes a significant increase in the relative amount of sec vs. mb mRNA in the plasma cell but not in the memory B-cell tumor. However, the sec:mb mRNA ratio is less than that seen in the wild type gene transfectants because, we postulate, that the CH_3 to M_1 exon splicing efficiency is increased due to the decreased IVS size. We have also demonstrated plasma cell specificity for the sec but not mb poly A site in a heterologous gene (the gpt gene of PSV_2 gpt). Splicing and polyadenylation are always competing but the 13 nt downstream consensus shifts that competition in favor of polyadenylation in the plasma cell but not the memory cell. Therefore the 13 nt consensus element plays an important, tissue specific role in regulation.

M 237 COMPLEX REGULATION OF THE IMMUNOGLOBULIN μ HEAVY CHAIN GENE ENHANCER: μB, A NEW DETERMINANT OF ENHANCER FUNCTION. Barbara Nelsen 1, Tom Kadesch 2, and Ranjan Sen 1. 1 Department of Biology, Brandeis University, Waltham MA 02254, 2 Department of Genetics, University of Pennsylvania Medical Scool, Philadelphia, PA 19104.

The B cell-specific immunoglobulin μ heavy chain gene enhancer is made up of binding sites for multiple nuclear proteins. Identification of only one B cell-specific factor (NF-A2,OCT-2) interacting with the enhancer has suggested that the tissue-specificity is governed by the octanucleotide sequence ATTTGCAT. We have used deletion and point mutagenesis to define a second element , μB (TTTGGGGAA), which is essential for enhancer function in the absence of the octamer motif in murine plasma cell. Transfection analysis in a panel of lymphoid cell lines suggests that the presence of either μB or octamer leads to a considerable enhancer activity in cell lines representing later stages of B cell differentiation, whereas both elements are needed for function in earlier cells. Furthermore, in contrast to the results in pre-B cells, both μB and octamer elements function independently in certain T cell lines in which the μ enhancer is active. Ongoing studies of the purification and in vitro characterization ot the factor binding to the μB element will be presented.

M 238 ISOLATION AND CHARACTERIZATION OF DIFFERENTIALLY EXPRESSED GENES FROM PRE-B LINES, Peter J. Nielsen, Mohamed Ayane, and Georges Koehler. Max Planck Institut fuer Immunbiologie, Freiburg i. Br. FRG.

We are interested in identifying genes involved in the regulation of B-lymphocyte differentiation. To do this we compare the expression of mRNA in pre-B and meyloma cell lines. In addition, we have used pre-B cell lines derived from SCID mice. Severe Combined Immuno Deficiency mice carry a recessive mutation which, in most pre-B cells, leads to faulty rearrangement of Ig genes and thus to a limited number of functional B cells. Since the differentiation of these cells could be arrested at the stage of immunoglobulin heavy chain rearrangement, we have looked for genes whose expression in SCID pre-B cells is altered compared with normal pre-B cells.

We have isolated one clone which shows low expression in SCID thymus and bone marrow compared to normal mice whereas spleen and testis show normal expression. We have also isolated a clone coding for a lymphoid specific protein whose expression is high in normal and SCID pre-B cells but virtually absent from plasmacytoma cells. Further characterization of these clones will be presented.

M 239 THE V(D)J RECOMBINATION ACTIVATING GENE, RAG-1 ,Marjorie Oettinger, David Schatz and David Baltimore, Whitehead Institute, Cambridge, MA 02142

We have isolated a gene that is capable of activating V(D)J recombination when introduced into NIH 3T3 fibroblasts. The mouse RAG-1 (Recombination Activating Gene-1) genomic locus was tagged with an oligonucleotide marker, allowing the gene to be follwed as it cosegregated with V(D)J recombinase activity through multiple rounds of transfection. The oligonucleotide subsequently served as the initial probe in a genomic walk that spanned 55kbp and ended with RAG-1 sequences. A RAG-1 genomic probe detected a single 6.6-7.0 kb mRNA species in transfectants and pre-B and pre-T cells, and was used to isolate mouse and human cDNA clones. The RAG-1 genomic clone and cDNA clones were biologically active when introduced into NIH 3T3 cells. Nucleotide sequencing of the human and mouse cDNA clones revealed that each contained a single long open reading frame followed by a long (3.4kb-3.8kb) 3'-untranslated region. The deduced human and mouse RAG-1 amino acid sequences are 90% identical and predict RAG-1 proteins of 1043 and 1040 amino acids respectively (molecular weight 119,000). RAG-1 has been conserved through evolution in species that carry out V(D)J recombination, and its pattern of expression correlates exactly with the pattern of expression of V(D)J recombinase activity. RAG-1 may activate the process of V(D)J recombination indirectly, or it may encode the V(D)J recombinase itself.

M 240 INFLUENCES OF IgH ENHANCER AND PROMOTER ELEMENTS ON RECOMBINATION, Eugene M. Oltz, Ami Okada, Franklin A. Wong and Frederick W. Alt, Dept. of Biochemistry, Columbia College of Physicians and Surgeons, New York, NY 10032. A PCR based assay has been developed in order to detect low level recombination in transfected substrates. This assay has been applied to unselected populations to acheive detection of events that have not been observed by Southern blotting. A series of direct- and inversion-type constructs have been prepared and introduced into various lymphoid and non-lymphoid cell lines. These substrates incorporate all combinations of heavy chain enhancer (E_μ - present/absent) and a heavy chain variable region promoter (P_{VH} - present/absent in both orientations) flanking recombinationally competent IgH - D and J segments. Previous studies support a role for transcription as an activator of the recombination process. In agreement with such a hypothesis, our preliminary evidence suggests that a pre-B cell line (38B9) rearranges substrates possessing both P_{VH} (in either orientation) and E_μ at a much greater frequency when compared with analogous constructs with only one or neither element.

M 241 IDENTIFICATION OF A SECOND STRONG ENHANCER IN THE IMMUNOGLOBULIN HEAVY CHAIN LOCUS. S. Pettersson[1], G.P. Cook[2], G.T. Williams[2], M. Bruggeman[3] and M.S. Neuberger[2], [1]Center for Biotechnology, Karolinska Institute, S-141 52 Huddinge, Sweden, [2]Laboratory of Molecular Biology, MCR, Hills Road, Cambridge, U.K. and [3]Department of Molecular Embryology, Institute of Animal Physiology and Genetics Research, Babraham, Cambridge, CB2 4AT, U.K.
The pattern of immunoglobulin (Ig) gene expression changes during B cell development. Both the activation of the Ig-gene expression and the changes in the pattern of expression that accompany B cell ontogeny are controlled at multiple levels. Two cis acting elements, a promoter and an enhancer that control Ig-heavy chain gene transcription have been identified by the use of DNA transfection assays. However, several lines of experiments suggest the existence of as yet unidentified transcriptional control elements in the Ig heavy chain locus. We have now identified second strong enhancer in the Ig heavy chain locus. This second heavy chain enhancer is not active in HeLa cells nor in T lymphocytes. Thus, this second enhancer appears to be B lymphoid specific.

M 242 EVOLUTION OF THE HUMAN AND RODENT EPSILON HEAVY CHAIN IMMUNOGLOBULIN SWITCH REGIONS, K. Yen Pham and Charles Faust, Department of Biochemistry and Molecular Biology, Texas Tech University Health Sciences Center, Lubbock, TX 79430. Although IgE is the least abundant Ig of mammals, it is, nevertheless, important in allergic reactions and parasitic immunity. In mammals IgE arises from Ig H-chain class switching, mediated by repetitive DNA elements, called switch regions, located 5' to the heavy chain constant region genes. Occasionally, aberrant translocations of the c-myc proto-oncogene utilize one of these switch regions, eventually resulting in some type of B-cell malignancy. We have previously found and characterized an aberrant switch translocation in the IgE-secreting LOU rat immunocytoma, IR162, in which the c-myc gene moved into the epsilon switch region and juxtaposed with the excluded allele of the epsilon H-chain (J. Biol. Chem. 264:1846-1853, 1989). This abnormal H-chain-myc gene rearrangement is especially interesting, because about 50% of LOU rat myelomas express IgE even though normal IgE-producing B-cells are least abundant. Since we and others have found in IgE-secreting myelomas a preference for translocation within this switch region of the LOU rat, we sought to determine if there is a unique characteristic in the epsilon switch region of the rat that might promote this translocation. Therefore, the rat epsilon switch region and its human cognate were cloned, sequenced, and compared against each other, and against the published mouse counterpart. Based on the results reported here, the evolution of the epsilon switch regions, and a potential role of the rat switch region in c-myc translocation are discussed. This work was supported in part by NIH grant, AI-23456.

M 243 TWO PHENOTYPES INDUCED BY THE SAME γ2B TRANSGENE IN INDEPENDENT MOUSE LINEAGES
P. Roth*, D. Lo+, J. Hackett*, L. Doglio*, and U. Storb*, *Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago IL 60637 and +School of Veterinary Medicine, University of Pennsylvania, Philadelphia PA 19104
Two independent lineages of transgenic mice carrying a functionally rearranged γ2b gene were established to study feedback regulation of immunoglobulin genes. Histologic sections of spleen and lymph node indicate that one lineage (343-1) has normal patterns of IgM staining while in the second lineage (348C), the majority of the B cell zones are IgM negative. In contrast, kappa staining is normal in both lines. As determined by FACS analysis, there is a reduction in the expression of B220 and surface Ig on splenic B cells from neonatal mice of both lineages. Two phenotypes emerge by 4 months of age. The 343-1 lineage expresses normal levels of surface IgM and kappa. In contrast, in the 348C line, although the percentage of kappa positive spleen cells returns to normal, there is a reduction in the level of expression. Furthermore, the number of splenic B cells expressing surface IgM remains low throughout adulthood. Although γ2b is expressed in both lineages, it appears that only 348C mice exhibit high levels of surface expression. Hybridoma analysis reveals that both lineages have retained ~30% germline heavy chain genes. Further studies are under way to determine when in fetal development the transgene is expressed in the two lineages as well as the relative levels of membrane versus secreted Ig produced. Through these studies we hope to understand the role of immunoglobulin expression in the regulation of B cell development. Supported by NIH grant HD23089. P.R. supported by NIH predoctoral training grant GM-07197 and AI-07090.

M 244 GENE EXPRESSION IN ACTIVATED, NORMAL HUMAN B CELLS, Rhiannon Sanders, Fanyi Jiang and Erik Lundgren. Unit Appl. Cell and Mol. Biol., University of Umeå, S-901 87 Umeå, Sweden.
The molecular events following activation of human B cells are being studied at the level of gene expression. Normal, human B lymphocytes were purified from peripheral blood using Lymphoprep followed by positive selection using anti-CD19-coated magnetic beads. The cells were then activated with phorbol dibutyrate (PdBU) and ionomycin for 24 hours, washed, and re-cultured in fresh medium containing low molecular weight BCGF and interleukin-2 (IL-2). As a result of the treatment with PdBU and ionomycin, the cells responded to BCGF and IL-2 by proliferating for 3 days. IL-2 receptor expression, measured both at the protein (Tac antigen) and mRNA level, was also increased in these cells. After 3 days the cells were harvested, total RNA and polyA+ RNA were prepared and a cDNA library constructed in the expression vector, λ ZAP. Total, resting lymphocytes have also been purified from buffy coats and have been used to contruct a cDNA library in the Promega vector, λGEM-4. A differential screen of the proliferating cell library is currently in progress using cDNA probes from the proliferating and from the resting cells and we have isolated a number of clones which specifically hybridise to the proliferating cell cDNA. These clones are now being analysed. We also intend to use subtractive hybridisation to facilitate the isolation of less abundant sequences. We hope to isolate "proliferation-specific", normal B cell cDNA sequences by this method, but at the same time we are working with cDNA libraries from the Burkitt´s lymphoma cell line, Daudi and an EBV-immortalised cell line, 158-B4, with the intention of detecting gene expression associated with the presence of EBV or the tumor phenotype in these cells.

M 245 **NF-μNR INTERACTS COOPERATIVELY WITH IT'S BINDING SITES FLANKING THE IGH ENHANCER.** Richard H. Scheuermann, Basel Institute for Immunology, CH-4005 Basel, Switzerland

We have described the identification of a novel nuclear protein, NF-μNR, which binds to multiple sites flanking the IgH enhancer. Several characteristics suggest that NF-μNR functions to keep IgH expression silent in non-B cells: i) the binding activity is uniquely absent from mature B cells, and ii) deletion of NF-μNR binding sites partially activates the IgH enhancer in non-B cells following transient transfection or in transgenic mice.

In order to gain a better understanding of the molecular mechanism for enhancer suppression we have analyzed the binding characteristics for NF-μNR with its cognate binding sites. We have found that the purified protein binds to a DNA fragment with high affinity only when two types of recognition sites are present. Interestingly the cooperative interaction can apparently occur even if the sites are on separate DNA molecules, a phenomenon we term 'trans-cooperativity'. This observation implies that binding to one site occurs following a change in protein structure as a result of binding to the other site. The implications of these results on models for binding protein specificity with regard to large genome sizes will be discussed.

M 246 ORGANIZATION AND EXPRESSION OF HUMAN λ-LIKE GENE FAMILY. Claudine Schiff, Mylène Bensnana, Paul Guglielmi, Michèle Milili, Marie-Paule Lefranc and Michel Fougereau, Centre d'Immunologie INSERM-CNRS de Marseille-Luminy, Case 906, 13288 Marseille Cedex 9, France.
A human liver cDNA library (<90 d) was screened with various H and L Ig probes. Several functional Ig μ or γ transcripts were identified. They use members of discrete VH families, suggesting that the repertoire is already close to that of the adult. The presence of complete γ transcripts suggests that switch may take place very early in ontogeny. Two λ-like transcripts were also identified that allowed us to characterize a new λ-like gene (Fλ1). This gene is part of the λ -like family that contains in addition 14.1 and 16.1 genes. We have mapped this gene cluster on 22q11, i.e. close to Igλ genes. The 14.1 germline gene was isolated. It contains 3 separate exons encoding for 69, 38, and 106 amino acids, respectively. Exons 2 and 3 were homologous to the normal Ig Jλ and Cλ, respectively. This gene is selectively expressed in preB cells, and represents a human equivalent of the mouse λ5 gene. It may encode a 23 kDa or 20 kDa chain (upon removal of a signal peptide) which may be S-S linked to the μ chain. The strong conservation of this gene between humans and mice points to a crucial role of the μ- λ-like complex in controling the early steps of Ig gene expression.

M 247 PRODUCTION OF STERILE TRANSCRIPTS OF Cγ GENES IN IgM PRODUCING HUMAN NEOPLASTIC B-CELL LINE THAT SWITCHES TO IgG. Sideras Paschalis, Mizuto Tatsunabu-Ryushin Kanamori Hiroshi, Suzuki. Noboru, Okamoto. Masaya, Kuze. Kogo, Ohno. Hitoshi, Doi. Shoichi, Fukuhara. Shirou, Hassan. M. Sawkat, Hammarström. Lennart, Smith. Edward, Shimizu. Akira and Honjo. Tasuku. A human neoplastic B cell line SSK41 that expressed IgM on its surface switches spontaneously to IgG. The SSK41 line contains a single immunoglobulin heavy-chain locus, whose constant region (C) genes retain the germ-line configuration. IgG-producing SSK41 cells were purified by sorting, and shown to have undergone S-S- recombination with deletion of the Cμ gene. These cells produced secretory and membrane-bound forms of γ-chain mRNA. From cDNA libraries of a mixed population of IgM^+/IgG^+ SSK41 cells, we have isolated cDNA clones encoding the mature membrane-bound and secretory forms of the μ and γ_1 heavy chains, all of which share the same variable region sequence. cDNA clones containing the mature γ_3 chain were identified as well. We also isolated cDNA clones containing $C\gamma_1$ and $C\gamma_3$ sterile transcripts. These sterile transcripts contained additional exon sequences desingated "I" which were localized upstream the $C\gamma_1$ and $C\gamma_3$ switch regions and homologous to murine counterparts. The I sequences were precisely spliced to the 5' ends of the corresponding Cγ exon sequences. Most features of germ line C_H transcripts i.e the isotype specificity to class switching, location of exons, and sequences per se are highly conserved between man and mouse. The relevance of these findings to the molecular mechanisms of the heavy chain class switch will be discussed.

M 248 DEVELOPMENTAL CONTROL OF IgM SECRETION: THE ROLE OF THE CARBOXI-TERMINAL μs TAILPIECE. Roberto Sitia°§, Michael Neuberger§., Cristina Alberini*, Paola Bet°, Anna Fra*, Caterina Valetti °, Gareth Williams§, and Cesar Milstein§.°Istituto Nazionale per la Ricerca sul Cancro, Genova,Italy, §MRC Laboratory of Molecular Biology, Cambridge,UK and *University of Brescia, Brescia,Italy. B lymphocytes do not secrete IgM and plasmacells only secrete polymeric IgM molecules. We show that the retention of secretory IgM by B cells and the failure of plasmacells to secrete monomeric IgM are both attributable to the tailpiece found at the carboxi-terminus of secretory IgM heavy chains (μs) and we specifically implicate cysteine 575 as being responsible. If cysteine 575 was replaced with alanine or serine, IgM was now secreted by B cells. Similarly, while IgG is normally secreted, a mutant IgG containing a μs tailpiece became retained within the cell: secretion was restored when the C terminal cysteine was replaced by serine. In general, retention correlated with binding to BiP, and could be mediated through either the CH1 domain, or through the μs tailpiece involving cysteine 575. Removal of cysteine 575 caused hypersecretion of monomeric IgM in plasmocytoma transfectants; following further removal of CH1, heavy chains were secreted in the absence of light chains. Thus, in B and plasma cells, cysteine 575 is involved both in polymerisation of IgM and in intracellular retention of unassembled intermediates.

M 249 SUBTRACTIVE CLONING TO IDENTIFY GENES UNIQUELY EXPRESSED IN PLASMACYTOMA CELLS, Cynthia R. Timblin and W. Michael Kuehl. NCI-Navy Medical Oncology Branch, National Naval Medical Center, Bethesda, MD 20814

Mouse plasmacytoma cell lines differ from a very mature, immunoglobulin secreting murine B cell line (A20.2J) by several notable features: 1) morphology, 2) lack of expression of certain B cell markers (e.g. surface Ia antigen), 3) lack of endogenous c-myc mRNA expression, and 4) the consistent dominance of the plasmacytoma phenotype in somatic cell hybrids formed with all B lymphoid cells. To identify genes which distinguish the plasmacytoma from the closely related B cell lymphoma, we have used PCR technology both for preparation and analysis of a subtractive cDNA library. Thus far, we have identified 32 genes which are expressed in both the parental and an unrelated plasmacytoma, but not in the subtractive B cell partner. None of these 32 clones represents a consensus clone (i.e. expressed in plasmacytoma cell lines but not in B or pre-B lymphoma cell lines). However, a number of these clones have interesting patterns of expression. Our long term goal is to identify and isolate genes which determine or are specific for the phenotype of terminally differentiated plasma cells.

M 250 THE ROLE OF PRE B SPECIFIC GENE PRODUCTS IN SURFACE EXPRESSION AND CROSS-LINKING OF IMMUNOGLOBULIN μ CHAIN, Takeshi Tsubata and Michael Reth, Max Planck Institute for Immunobiology, D-7800 Freiburg, FRG.
Several lines of evidence suggest that membrane-bound immunoglobulin-μ-chain (μm) generates a signal which controls V gene rearrangements in pre B cells. As is the case in mature B cells, this signal may be generated by cross-linking of surface μ. This study aims at answering the questions how the μ chain is expressed on the pre B cell surface without a light chain, and how sIg can be cross-linked in bone marrow microenvironment.
The products of the pre B specific genes, VpreB and λ5, have structural similarities to light chain V and C domains and, thus, have the potential to bind to μ chain. To test whether the products of the two genes bind to μ and induce its surface expression, we constructed expression vectors for λ5 and VpreB under the control of IgH enhancer and VH promoter and introduced these vectors into the μm transfectants (X63μm) of myeloma X63Ag8.653 cells. The μ chain was found on cell surface only in these X63μm transfectants which co-expressed λ5 and VpreB, suggesting that μ chain requires binding of λ5 and VpreB for its surface expression. Indeed, biochemical analysis demonstrated that, in the transfectants of X63μm, the μ chain was bound to 22kd and 18kd proteins, covalently and noncovalently, respectively. The search for the natural ligant which will cross-link the μ-λ5-VpreB complex is presently underway.

M 251 INDUCTION OF B CELL-SPECIFIC PROTEIN-DNA INTERACTIONS AT SEQUENCES UPSTREAM OF THE IgH PROMOTER WITH ANTIGEN AND IL-5. Carol F. Webb, Kiyoshi Ariizumi, Chhaya Das, Kathryn Calame, and Philip W. Tucker. Department of Microbiology, Southwestern Medical Center at Dallas, Dallas, TX 75235.

Previous studies have shown that antigen (Ag) and the cytokine interleukin-5 (IL-5) can increase Ag-specific immunoglobulin (Ig) secretion (1,2). We have recently reported that PC-KLH + IL-5 caused increased steady state μ mRNA levels in Ag (PC)-specific cell lines (3). A series of deletion constructs was used to examine the effect of sequences 5′ to the IgH promoter upon the increased μ mRNA levels. The region between -251 and -124 was shown to be necessary for this response. Further footprinting and gel retention assays have identified another B cell-specific inducible protein-DNA interaction covering a 46 base pair dyad symmetry element at -550 which may be involved in binding to the nuclear matrix. Preliminary results suggest a relationship between the sequences at -251 and -550 since the dyad symmetry element can compete for binding of proteins to the -251 sequence. Thus, DNA-protein interactions 5′ of the IgH basal promoter may play an important role in the B cell response to Ag and IL-5.

1. Alderson, M., *et al.* (1987) J. Immunol. 139: 2656.
2. Swain, S.L., *et al.* (1988) J. Immunol. 140: 4224.
3. Webb, C.F., *et al.* (1989) J. Immunol., *in press*.

M 252 A Transgenic Mouse Model of Immunoglobulin Gene Rearrangement and Expression. C.R. Wood, G.E. Morris, R. Palmer, E. Alderman, D. Wilson, B. Krippl*, F. Costantini*, F.W. Alt^{+}, and R.J. Kaufman.

Genetics Institute, Inc., 87 CambridgePark Drive, Cambridge, MA. 02140 and Depts. of Human Genetics and Development* and Biochemistry^{+}, College of Physicians and Surgeons, Columbia University, New York, NY. 10032.

Two murine, unrearranged immunoglobulin transgenes have been constructed and used to generate transgenic mouse lines. The first construct contains the V_{186-2} gene segment linked to a DJ_H2 rearrangement and $C\mu$, and the second contains the V_{186-2} gene segment linked to the germline D_{Q52}-J_H locus, C_μ and $C_{\delta 2b}$, including switch recombination sequences.
The immunoglobulin transgenes have been found to rearrange in a tissue-specific manner, and junctional variation is generated that is indistinguishable from endogenous rearranged genes. The expression of the transgene μ^a has been studied in a C57 μ^b background, and Southern blotting and PCR analyses have been employed to detect the switch recombination of the second transgene.

M 253 PARTIAL PURIFICATION OF IMMUNOGLOBULIN SWITCH REGION SPECIFIC DNA BINDING PROTEINS FROM MITOGEN STIMULATED MOUSE SPLENIC B CELLS, Robert A. Wuerffel, Asher T. Nathan, and Amy L. Kenter, Department of Microbiology and Immunology, University of Illinois at Chicago College of Medicine, 835 South Wolcott Chicago, IL 60680. We have detected nuclear proteins, termed NF-Sμ, from lipopolysaccharide (LPS) and dextran sulphate (DxS) stimulated mouse splenic B cells which bind specifically to the immunoglobulin (Ig) switch μ (Sμ) sequence. The kinetics of expression of this NF-Sμ family of proteins, in LPS/DxS stimulated B cells, parallels the induction of recombinational activity at Sμ. This suggests that NF-Sμ may be an effector of switch recombination. DNA containing the Sμ repeated sequence, GAGCTGGGGTGAGCTGAGCTGAGCT, was employed as a probe in electrophoretic mobility shift assays. Methylation interference analysis and competition assays established which residues are important for efficient binding. Heparin-agarose chromatography resulted in the separation of distinct species of Sμ-specific binding proteins indicating that the single retarded band seen with crude extract may be due to formation of a multiprotein-DNA complex. We have constructed a DNA affinity column containing multimers of the Sμ repeated sequence and have begun to purify the NF-Sμ family of proteins to enable us to more fully characterize their roles in IgH switch recombination.

M 254 **EFFECT OF IL5 ON μ CHAIN mRNA TRANSCRIPTION IN B LYMPHOCYTES.** Dorothy Yuan and Tam Dang. Department of Pathology, University of Texas Southwestern Medical Center, Dallas, Texas. 75235. Previous work from this laboratory showed that induction of μ_S mRNA production in resting B lymphocytes involves two closely related events: 1) increased polymerase loading of the IgH chain gene and 2) alterations in the site of polymerase termination which favor the production of μ_S mRNA from the primary transcript. By studying the effect of IL5 stimulation on IgM secretion we have now dissociated these two events. While small, resting B lymphocytes are not responsive to IL5, stimulation of in vivo pre-activated large B cells with IL5 in the presence of IL2 results in a 4 fold increase in μ_S mRNA accumulation in 4 days. This increase is accompanied by an alteration in the site of termination of polymerases transversing the μ gene but not by increased polymerase loading. Furthermore, the alteration can be induced even when IL5 is added 2 days after IL2 initiated cell division, suggesting that the effect of IL5 is not at the level of increasing IL2 receptor expression. These experiments confirm previous results obtained in BCL_1 tumor cells which showed that IL5 exerts its effects on B cells by altering the site of polymerase termination.

M 255 ANALYSIS OF ALTERNATIVE SPLICING IN LEUCOCYTE COMMON ANTIGENS, Suzanne L. Zebedee, Richard Tseng, Osami Kanagawa, and William C. Raschke, La Jolla Biological Laboratories of the Salk Institute, San Diego, CA 92138. The leucocyte common antigen (LCA, also referred to as CD45, Ly5, and T200/B220) is a single gene product of the murine chromosome 1 which is abundantly expressed on the surface of hematopoietic cells. In B lymphocytes the LCA molecule has a molecular weight of 220 kD, and in thymocytes the antigen is expressed as a 180 kD product. This size difference has been traced to the differential splicing of three exons located near the 5' extracellular terminus of the molecule: exons 5, 6, and 7. Analysis of LCA mRNAs by Northern blots reveals a size heterogenity among B lymphocytes, thymocytes, helper T cells, and cytotoxic T cells. To examine the LCA splicing pattern in the B and T cell lineage, we have compared the PCR products generated from various cell line mRNAs using 5' specific LCA oligonucleotides. The results indicate that PCR patterns are specific for each lineage or differentiation stage and that each cell line expresses more than one form of the LCA molecule. PCR products from selected B and T cell lines were cloned, sequenced, and found to encode many combinations of the 5' specific exons. This interesting pattern of lymphoid splicing that occurs in the LCA family of molecules can be used to further examine the tissue specific and developmental expression of genes.

M 256 FUNCTIONAL ANALYSIS OF HLA-DRA X AND Y PROMOTER ELEMENTS USING A HOMOLOGOUS IN VITRO TRANSCRIPTION SYSTEM. Nancy J. Zeleznik-Le, Jane C. Azizkhan, and Jenny P.-Y. Ting. Lineberger Cancer Research Center, Dept. of Microbiology/Immunology, Univ. of NC, Chapel Hill, NC 27599.
Several protein-binding elements in the DRA class II promoter have been defined that affect DRA gene transcription. The importance of the X and Y elements in DRA gene transcription has been demonstrated by transient transfection analyses of promoter mutants. We developed a B cell in vitro transcription system which allows transcription initiation from the DRA promoter, unlike the traditional HeLa systems. This system was developed to directly address whether purified DRA promoter-binding proteins could reconstitute DRA transcription in vitro. Using this B cell system, we observe correct transcription initiation from a plasmid containing wild type X and Y elements, whereas mutation of either X or Y elements or reversing their orientation inhibits transcription. Addition of excess oligonucleotides corresponding to X and Y elements specifically competes transcription. Furthermore, we have purified a Y-binding protein from B cell nuclear extracts by specific oligonucleotide affinity chromatography. This Y protein is capable of reconstituting correct transcription after specific Y oligonucleotide competition. This homologous B cell in vitro transcription system allows direct functional analysis of purified or cloned DRA promoter-binding proteins using an in vitro reconstituted system.

Antibody Repertoires; Tolerance

M 300 SUBSTITUTION PATTERNS CONSISTENT WITH GENE CONVERSION IN THE MURINE V_HX24 FAMILY. D. Allman*, A. Hartmann#, S.E. Ferguson*, and M. P. Cancro*. University of Pennsylvania, Phila., PA and Walter Reed Research Center, Wash., D.C.
Recent studies of V_HS107 genes from the recently derived CLA-2 strain showed that, when compared with the other known V_HS107 haplotypes, the proportion of differences consistent with gene conversion greatly exceeds that expected by random base substitution. Whether such non-random substitution patternsn are a common feature of most VH gene families remains unclear. To examine this question, the V_HX24 and V_H36-60 families of BALB/c, C57Bl/6 and CLA-2/Cn mice have been examined in detail and compared. The results show that: 1) CLA-2 and C57Bl share RFLPS for these two families; and 2) non-random substitution patterns consistent with gene conversion are present within the V_HX24 family, and sequence analyses for V_H36-60 are underway. Thus, non-random substitution patterns consistent with gene conversion are firmly established in at least two V_H families; strengthening the notion that conversion has played a role in the molecular evolution of the Vh locus.

M 301 THE EXTENT OF SOMATIC MUTATION IN THE HEAVY CHAIN VARIABLE REGIONS OF ANTIBODIES EXPRESSING MEMBERS OF THE V_HIV GENE FAMILY.
Jennifer S. Andris, Virginia Pascual, and J. Donald Capra, Department of Microbiology and Graduate Program in Immunology, U.T. Southwestern Medical Center, Dallas, Texas. During B cell ontogeny there are a number of mechanisms involved in the generation of antibody diversity, one of which is somatic mutation. We have characterized a number of human autoantibodies and antibodies to viral and bacterial proteins, among them nine of which utilize V_H gene segments from the V_HIV gene family. Sequence analysis has revealed that these expressed genes are, on the average, 85% identical, at the nucleotide level, to any of the known V_HIV germline sequences. We have utilized PCR and dot blot hybridization with allele-specific oligonucleotide probes, as well as Southern filter hybridization, to determine if any of the expressed sequences exist in the germline of normal individuals and thus represent new members of this gene family, or if the sequences result from extensive somatic mutation. From these studies we conclude that, although there are some allelic polymorphisms within the V_HIV gene family, the majority of the differences between these expressed genes and the germline genes with the closest homology are the result of somatic mutation.

M 302 DIVERSITY OF V_H AND V_L GENE USE AMONG ANTIBODIES AGAINST THE CIRCUMSPOROZOITE PROTEIN OF *PLASMODIUM FALCIPARUM*, Roberto Anker, Fidel Zavala*, Ruth S. Nussenzweig* and Brian A. Pollok, Wake Forest University Medical Center, Winston-Salem, NC 27103 and *New York University Medical Center, New York, NY 10016

The sporozoite of *Plasmodium falciparum* displays on the surface a single protein, the circumsporozoite (CS) protein, which covers the whole surface of the sporozoite. The central domain of this protein possesses a highly repetitive immunodominant epitope $(NANP)_3$; a monoclonal antibody (MoAb) that recognize this epitope can inhibit with 100% efficiency the binding of polyclonal antibodies to the sporozoite surface. Considering the highly repetitive structure of this naturally-occurring epitope and its immunodominance, we were interested in analyzing the structural diversity of the anti-CS antibody response. RNA sequencing of V_H and V_L regions was performed in five anti-CS hybridomas produced in BALB/c mice by inoculation with sporozoites. The RNA sequence showed that for heavy chain, three MoAbs used V_H elements that belong to the V_HIX family and two to the V_H J558 family. Four different V_κ subgroups were represented among the light chains. While different D and J_κ segments are utilized, four heavy chains used the J_H4 segment and one the J_H1 segment. Two MoAbs used the same recombination sites for similar $V/D/J_H$ and V/J_κ elements. Southern analysis with a J_H probe revealed a different restriction pattern between these two hybridomas, which support an independent clonal origin. Our results indicated that while the CS protein possesses a repetitive and highly immunodominant epitope, several V_H/V_L gene combinations can code for anti-CS specificity, and that one V gene pair does not dominate the anti-CS B cell response.

M 303 RESTRICTIONS IN IG-HEAVY AND LIGHT CHAIN ASSOCIATION? STUDIES IN QUADROMAS. Bert J.E.G. Bast, Kees Heye, Wim de Lau. Dept Clinical Immunology, University Hospital Utrecht, The Netherlands

During the process of production and application of bispecific monoclonal antibodies, made by cell fusion of two established hybridomas (quadromas), our yield of these antibodies was considerably less than the 50%, to be expected from an at random H-H chain pairing and an preferential homologous H-L chain pairing. We therefore analysed extensively the Ig production of eight quadromas. Due to an isotype difference in these combinations, the presence of hybrid Fc molecules could be detected in ELISA and protein A could be used to separate the IgG into the three H-H chain types. Via HPLC-hydroxylapatite chromatography antibody molecules, differing in IEP, could be separated. The various fractions were analyzed for H- and L- chain content in IEP under (non) reducing conditions and were tested for their ability to bind the respective target antigens. We concluded that different L chains can associate with H chains in all three possible ways: preferentially homologous, at random or even preferentially heterologous. This apparent unrestricted H-L chain pairing in quadromas may indiate a similar unrestricted L chain recruitment in the formation of the repertoire during B cell development. Moreover, it results in complex, undesired antibody compositions from hybrid antibody producing cells.

M 304 ORGANIZATION OF THE MOUSE VH GENE FAMILIES Peter H. Brodeur and Celine Mainville, Tufts University School of Medicine, Boston, MA 02111. We have determined the relative position of the mouse Vh gene families by deletional analyses of Abelson virus transformed pre-B cells. We reported the order of nine families for the $\underline{Igh^a}$ (BALB/c) and $\underline{Igh^b}$ (C57BL/10) haplotypes (Brodeur et al, 1988; J. Exp. Med. 168: 2261) and have recently mapped the Vh11 and Vh 12 families in these haplotypes (Pennell et al, Eur. J. Immunol. in press) as well as the Vh10 family. The Vh12 gene maps at the D-proximal end of the VhJ606 family and the Vh11 family maps near the most D-proximal Vh36-60 cluster. The two Vh11 members are separated by at least one Vh36-60 gene. The map inferred from analyses of fifty-one rearranged Igh loci is: J558 - 3609 - Vh10 - J606 - Vh12 - 36-60 - VGAM3.8 - 36-60 - S107 - VGAM3.8 - Vh11 -36-60 - Vh11 - X24 - S107 - Q52 - 7183 - DH - JH - CH. Our studies have not revealed any differences in the over-all organization of Vh gene families between $\underline{Igh^a}$ and $\underline{Igh^b}$ haplotypes. We are currently extending our studies to include additional haplotypes.

M 305 IMMUNOGLOBULIN V REGION REPERTOIRE IN THE HUMAN H. INFLUENZAE IMMUNE RESPONSE, William L. Carroll, Elisabeth A. Adderson, Penelope G. Shackelford, Department of Pediatrics, Washington University School of Medicine, St. Louis, MO 63110.
Haemophilus influenzae type b (Hib) is a major bacterial pathogen in infants and children. The restricted number of clonotypes and the demonstration of a major cross reactive idiotype associated with the antibody response to Hib polysaccharide (PS) immunization or infection suggests a restricted immunoglobulin variable (IgV) region repertoire. In an effort to determine the ontogeny and maturation of the V region repertoire to Hib PS, a panel of human anti-Hib PS hybridomas has been developed from subjects responding to two different forms of Hib PS vaccine. Immune lymphocytes were rescued by fusion to a mouse myeloma cell line, SP2/0, and to a human mouse heterohybridoma line, K6H6/B5. Hybridomas were screened for antibodies binding to Hib PS by ELISA, by Farr assay and finally, by binding inhibition with soluble Hib PS. To date 16 hybridomas have been developed. Their isotype distribution (5 IgAλ, 4 IgAκ, 4 IgGκ, 3 IgMκ) is consistent with the predominance of IgA and kappa secreting lymphocytes in the peripheral circulation 7 days after immunization with PS antigens. Preliminary V_H nucleotide sequence analysis of 3 of these hybridomas shows 85% to 95% homology of V regions with differences at the DJ segments. All are members of the human V_HIII family. The greatest homology was seen between a V_H region participating in a response against the T-independent antigen, Hib-PS, and the V region isolated from another individual in response to the T-dependent Hib PS-protein conjugate. Our preliminary data suggests a restricted immune response to Hib. Ongoing sequence analysis of Ig heavy and light chain V regions should provide insight into the spectrum of V region usage at various timepoints following antigen exposure among different age groups. Moreover the influence of T dependent forms of antigen on the molecular makeup of the immune response can be formally evaluated.

M 306 **TOLERANCE INDUCTION IN IG-TRANSGENIC MICE:DEFINITION OF ANTIGEN AND ANTIBODY DEPENDENT VARIABLES**. R. Carsetti, G. Köhler, R. Lamers MPI f. Immunbiologie, D-7800 Freiburg, FRG.
Recent studies with transgenic mice have shown that auto-antigen can cause either clonal deletion or functional silencing of self-reactive B cells. We have also exploited transgenic mice to design a flexible model for tolerance induction which could enable us to define the variables depending on the antigen (T-dependent or independent form, number of epitopes, amount,time point of exposure) and on the antibody (isotype, cell-bound and circulating form). The SP6 transgenic mice carry a μ heavy and κ light chain specific for TNP. 90% of the B lymphocytes express the transgenic μ chain,alone or with endogenous heavy chains (30%).Endogenous light chains are produced in most B cells. Our experiments show that T-dependent (TNP-BSA) and T-independent (TNP-dextran) self-antigens at high dose (100,200 μg/mouse i.p. every 3 days beginning on the day of birth) cause clonal deletion of specific B cells in the bone marrow and in the spleen.If the antigen is first administered 3 days after birth a modest effect is observed in the bone marrow. Injection of high doses (up to 2 mg) in the adult animal does not produce significant systemic modifications. Experiments are in progress to evaluate: 1) the effect of self antigen at low dosage; 2)the possible protective action of circulating antibodies against tolerance induction ; 3) the role of IgD .

M 307 BINDING SITE AND V REGION DIVERSITY IN THE REPERTOIRE OF BrMRBC-SPECIFIC ANTIBODIES, R.B. Corley, H.J. Sage, and J.D. Conger. Department of Microbiology and Immunology, Box 3010, Duke Medical Center, Durham, NC 27710
Antibodies specific for BrMRBC are of interest as models of "natural auto-antibodies" and because their primary source is $CD5^+$ B cells. In the peritoneum, most BrMRBC-specific B cells produce Ig with a single common V region combination, Vh11/Vk9. Because $CD5^+$ B cells may be positively selected in the peritoneal cavity, we prepared anti-BrMRBC hybridomas using splenic B cells to reveal the maximum available diversity in this repertoire. Data based on binding studies, Northern blot analyses, and mRNA sequence analysis indicated that there is considerable combining-site diversity. Among the BrMRBC-specific hybridomas, 4 Vh families and 4 Vk families are represented, although most of the antibodies are comprised of 2 unmutated V gene combinations, Vh11/Vk9 and Vh12/Vk4. Antibodies of these types had the highest affinities for BrMRBC, but differed greatly in their ability to bind trimethylammonium (TMA), a constituent of phosphatidyl choline. The Vh12/Vk4 antibodies bound weakly, if at all, to TMA and PC. Four distinct Vh genes were used by the other BrMRBC-specific antibodies. Three are members of the Vh558 family common to $CD5^+$ B cells and each contains a complex V-D-J join, which results in common CDR3 amino acid motifs, presumably necessary for TMA binding. Because the Vh11/Vk9-type antibodies were all efficient at lysing BrMRBC and binding TMA, we suggest that affinity considerations may determine the selective predominance of B cells with this V region configuration from an available repertoire of considerable diversity.

M 308 VH-VL PAIRING AMONG C57BL/6 HYBRIDOMAS, Lawrence A. D'Hoostelaere, Philipp Thalmann and Antonius Rolink, Basel Institute for Immunology, Postfach 4005, Basel, Switzerland.

A series of putatively random adult C57BL/6 Ig positive hybridoma RNAs were examined for Igh-V/Igk-V frequencies and pairing using a dot blot titration assay. The supernatants were tested for antigen binding activity against a panel of self and non-self antigens. Ten Igh-V probes were used at 80% stringency. Fourteen Igk-V probes were used at 80% and 90% stringency because of full-length or subregion sequence similarities among Igk-V groups. One Igh-V family and three Igk-V groups were negative in the hybridomas examined, and 9% and 17.8% of the hybridoma RNAs were negative for all of the Igh-V and Igk-V probes, respectively. Igh-VJ558 and Igk-V1 were detected at the highest frequency, but not necessarily as a VH-VL pair. Igh-V usage appeared to be random; while the Igk-V usage my not be random. The Igh-V/Igk-V pairing and antigen reactivity patterns will be discussed.

M 309 ALTERATIONS IN THE VH GENE USAGE AND FINE SPECIFICITY IN PRIMARY AND SECONDARY FITC-SPECIFC IgM.

M.R. Dalesandro, S.L. Stein, J.B. Skeath and J.A. Owen. Dept. of Biology, Haverford College, Haverford, Pa 19041.

Primary and secondary IgM antibodies specific for 5 fluorescein isothiocyanate (5FITC) have been compared by determining both their VH gene usage and their cross-reactivity with FITC homologues. We have used a panel of 20 IgM antibodies, 9 derived from fusions of SP2/0 myeloma cells with splenocytes of mice immunized once and 11 from mice immunized twice with FITC-Hemocyanin (Hy). Dot-blot hybridization of cytoplasmic RNA extracted from primary and secondary hybridomas using DNA probes for 8 of the known VH gene families revealed a dichotomy in gene usage. Primary hybrids expressed the JH proximal gene families 7183, S107 and Q52 while secondary hybrids utilized genes of the J558, 3609 and 36-60 families. Notable in the gene usage pattern of secondary antibodies is the absence of those gene families which predominated after the initial immunization. Furthermore, secondary antibodies showed greater cross-reactivity in an RIA with BSA-conjugated DTAF while primaries were more cross-reactive with BSA-XRITC or BSA-TRITC. The second FITC-Hy immunization appears to be pivotal to this shift in idiotype which is consistent with the clonal expansion of a subpopulation of cells which secrete IgM only upon secondary antigen exposure.

M 310 IG-V AND LY-1 GENE EXPRESSION IN MURINE HYBRIDOMAS SECRETING NATURAL AUTOANTIBODY Guillaume Dighiero,* Azad Kaushik+, Raoul Mayer+, Vincenzo Fidanza+, Habib Zaghouani+, Annick Lim* and Constantin Bona+, Pasteur Institute+, Paris 75015, and Mount Sinai School of Medicine, New York 10029. We have investigated the Ig-V and Ly-1 gene expression among murine hybridomas, secreting natural autoantibodies (NAAb) against DNA, cytoskeleton proteins, TNP hapten, albumin and bromelain treated isologous red blood cells (BrMRBC), from normal, immunodeficient and autoimmune mice. The detection of Ly-1 gene transcript in the hybridoma lysates by Northern blotting was used to assign hybridomas as to their origin from Ly-1 B cells or not. The data showed that multispecific NAAbs originated both from $Ly\text{-}1^+$ and $Ly\text{-}1^-$ B lymphocytes. The neonatal Xid mice also appeared to possess B cells, transcribing Ly-1 gene, that secreted multispecific NAAbs. Different Vh and Vk gene families were expressed at random both in $Ly\text{-}1^+$ and $Ly\text{-}1^-$ hybridoma clones secreting NAAbs. No particular Vh:Vk pairings were observed among NAAbs mutispecifically reactive with DNA and cytoskeleton proteins. The NAAbs reactive with BrMRBC exhibited predominant Vh 11:Vk 9 pairing, although we have for the first time noted the participation of Vk-1, -10 and -19 gene families in encoding this specificity. These observations are of direct sgnificance to B cell development and appearance of spontaneous autoimmune response during ontogeny.

M 311 ANALYSIS OF JUNCTIONAL DIVERSITY AND GENE SEGMENT USAGE IN THE PRIMARY ANTIBODY REPERTOIRE, Ann J Feeney, Medical Biology Institute, La Jolla, CA 92037
If the combinatorial association of the V, D, and J segments is random, it would generate a primary repertoire of approximately 10^7. Additional diversity arises from deletion of variable numbers of nucleotides from coding regions and addition of variable numbers of non-coded N region nucleotides Relatively little is known about the rules governing the additional diversity at the junctions of Ig gene segments, but the extent of its contribution to the primary antibody repertoire is estimated to be very large. In contrast to the size of this potential repertoire, some B cells with identical receptors and junctional sequences are present in high frequencies, even at the pre-B cell level. This implies some constraints on the randomness of junctional diversity, gene segment usage, and/or association of gene elements. To investigate this, we are analyzing hundreds of junctional sequences derived from the mRNA of LPS-stimulated spleen cells. cDNA is made, amplified by PCR, cloned and sequenced. Vh-D-Jh junctional sequences are analyzed for: (1) extent and randomness of N region additions, (2) extent and randomness of deletion of nucleotides from the coding regions of Vh, D, Jh, (3) D region usage and reading frame, (4) potential D-D joins and inverted D region sequences, (5) Jh usage, (6) association of patterns of junctional diversity with V, D, or J region usage, and (7) presence of any preferential association of sets of gene elements. Our results show that many of these parameters show non-randomness, i.e., not all Jh regions or D regions are used equally, and there appear to be preferential associations of certain Vh genes with certain Jh genes. We also show that DSP2 and DFL16 D regions are used in one reading frame 70% of the time, and that in a small percentage of D regions, inverted D segments and D-D joins are observed.

M 312 NON-STOCHASTIC VH GENE USAGE IN CBA/N MICE. **Shaw-Huey Feng and Kathryn E.Stein Office of Biologics Research, CBER, FDA, Bethesda, MD 20892.** The heavy chain variable region (VH) locus is composed of several gene families. Although initial studies of VH gene usage in adult mice indicated stochastic VH expression, proportional to the size of the family, further investigation showed that expression is not entirely random. Using probes defining 8 VH gene families, we have employed the techniques developed by Schulze and Kelsoe (J.Exp.Med. 166:163,1987) for in situ hybridization of cell colonies to determine the VH usage in LPS stimulated splenocytes from CBA/CaHN and CBA/N mice. CBA/N mice carry an X-linked immunodeficiency (xid) gene and lack a late developing B cell subset. Comparison of VH expression between the two strains revealed that CBA/N differs from CBA/CaHN most strikingly in the usage of the S107 gene family. Whereas 9.1% of B cell colonies from CBA/CaHN mice express the S107 VH gene family, 24.0% of CBA/N B cell colonies use this family, a significant overrepresentation ($p<0.001$). The S107 gene family is the predominant family expressed in CBA/N B cells in contrast to J558, the largest gene family by Southern analysis, which predominates in CBA/CaHN cells (33.3%) but is expressed in only 19.1% of CBA/N cells ($p<0.001$). These results cannot be accounted for by differences in the size of the S107 family in CBA/N mice as it has been reported to be the same as in BALB/c and CBA/J mice by Southern analysis (J.Immunol. 132:1544,1984). Our results suggest that VH gene usage may be biased in different B cell subsets.

M 313 ANALYSIS OF A NOVEL V_HS107 HAPLOTYPE IN CLA-2 AND WSA MICE: EVIDENCE FOR GENE CONVERSION IN OUTBRED POPULATIONS, Stacy E. Ferguson[*#], Barbara A. Osborne[#] and Michael P. Cancro[*]. [#]Program in Molecular and Cellular Biology, University of Massachusetts, Amherst, MA 01003 and [*]Department of Pathology, University of Pennsylvania School of Medicine, University of Pennsylvania, Philadelphia, PA 19104. A recently-derived inbred strain of Mus musculus domesticus, CLA-2/Cn, responds poorly to the hapten, phosphorylcholine (PC). Usage of the V_HS107 V1 family member predominates the response to PC in most conventional strains. The germline S107 gene segments have been characterized to 1) determine if poor-responsiveness to PC exhibited by the CLA-2/Cn strain is attributable to a defect in the appropriate germline V_H segment and to 2) evaluate the contribution of gene conversion to allelic sequence diversity evident among S107 alleles. From these studies, it was demonstrated that low-responsiveness is unlikely due to a defect in the V1 gene segment. Accumulated sequence data indicate that gene conversion contributes a great deal to allelic variation among S107 alleles. Furthermore, characterization of the WSA strain, independently inbred from the same outbred population which gave rise to CLA-2 mice, indicates that such conversion events are not an artifact of inbreeding, as these strains are of identical V_HS107 haplotypes.

M 314 EXPRESSION OF HUMAN Ig REPERTOIRE IN EBV CLONES AND NORMAL LYMPHOCYTES FROM FETAL AND ADULT ORIGIN. Michel Fougereau, Anne-Marie Cuisinier, Francis Fumoux, Véronique Guigou, Claudine Schiff, and Cécile Tonnelle. Centre d'Immunologie INSERM-CNRS de Marseille-Luminy, Case 906, 13288 Marseille Cedex 9, France.
Using different Ig probes (Cμ, Cκ, Cλ, VH1 to VH6, Vκ1 to Vκ4), human Ig expression was studied by hybridization to RNA extracted from samples representative of various stages of gestation. At 7w, fetal liver RNA hybridized exclusively with Cμ and VH5 and VH6 probes, in the absence of L chains. At 11 w, the V family expression pattern in EBV clones was already close to that of the adult with a predominance of VH3 and Vκ1. This period seems therefore crucial for the emergence of the Ig repertoire. In situ hybridization, using the same probes was utilized on EBV clones and on normal lymphocytes. A general similar pattern of VH and Vκ expression could be defined, suggesting that there was no bias of expression in EBV transformed cells. The VH subgroup distribution was VH3 > VH4 > VH1, that of Vκ was Vκ1 > Vκ3 > Vκ4. A constant proportion of EBV clones was found to be polyspecific when tested on a large panel of antigens, including autoantigens (actin, tubulin, DNA...), whatever the origin, fetal or adult, of the transformed population. The analysis of the VH and Vκ family usage revealed that polyspecific clones had the same pattern as that of the clones of undefined specificity. Therefore, the polyspecificity is not linked to a restricted repertoire.

M 315 DEPENDENCE OF B-CELL ACTIVATION ON SOMATIC MUTATION OF ANTIBODIES, Julia George, Steven J. Penner, J. Latham Claflin. Department of Microbiology and Immunology, University of Michigan, Ann Arbor, MI 48109-0260.

Our laboratory has previously shown that the M603, PC-specific response to *Proteus morganii* is clonally restricted, exhibits somatic mutations and that these mutations are characterized by specific recurring mutations. Binding studies, with ELISA, have shown that certain mutations in CDR2 are necessary to generate specificity. This observation has several possible implications. First is that mutation may predate antigenic stimulation. Second is that mutations may be induced by either environmental mitogens or cross reactive antigens. And third is that the mechanism of activation at the B-cell surface is not exclusively dependent on direct binding, or that ELISA is not accurately reproducing what is occurring at the B-cell surface. In order to determine whether B-cell activation is dependent on somatic mutation, constructs containing germline genes or appropriately mutated genes are being generated. These constructs will be transfected into B-cell lines. Following stimulation with PC-antigens or anti-isotype antibodies, stable transfectants will be assayed for activation by looking at Ca++ influx and change in expression of B-cell markers. The results will allow us to assess the functional importance of somatic mutation of antibodies in their role as surface receptors for antigen.

M 316 DIVERSITY IN THE IMMUNOGLOBULIN REPERTOIRES SPECIFIC FOR STRUCTURALLY RELATED VARIANTS OF CYTOCHROME *C*. Stephen C. Goshorn and Ronald Jemmerson. Department of Microbiology, University of Minnesota, Minneapolis, MN 55455. Epitopes on globular proteins encompass as many as 17 amino acid residues, yet a single amino acid change can result in elicitation of antibodies that differ greatly in terms of their binding specificities. We are interested in determining whether antibodies to structurally similar epitopes are related in structure and, if so, can a strategy be discerned whereby specific amino acid changes occur within antibodies in response to amino acid changes in protein epitopes. To address this question we have generated a number of monoclonal antibodies (mAbs) against several naturally occurring variants of cytochrome *c* (cyt). The mammalian cyts differ within their antigenic regions by one or two amino acids, but retain the same three-dimensional structure. The binding specificities of the mAbs were determined by reactivity with a panel of cyts in ELISA. The nucleotide sequences of the heavy and light variable (V) regions of the mAbs were determined from cloned V region cDNA, and the deduced amino acid sequences were compared. mAbs having similar binding specificities showed significant variation in V gene usage. Due to the overlapping nature of epitopes, several of these antibodies may not contact the same residues on cyt.

M 317 A S107/T15 V_H DERIVED PEPTIDE INHIBITS THE SELF-BINDING OF HUMAN ANTI-PHOSPHORYCHOLINE (PC) ANTIBODIES, Roberta Halpern*, Srinivas-Venkatesh Kaveri†, Heinz Köhler,*†, *La Jolla Cancer Research Foundation, La Jolla, CA 92037, †IDEC Pharmaceuticals Corporation, La Jolla, CA 92037

Recently, we have described the self-binding property of certain germline encoded murine antibodies. These antibodies belong to the S107/TEPC 15 (T15) variable heavy chain (V_H) family. The formation of the self-complex has been shown to involve structures of the hypervariable region of the heavy chain, because peptides derived from this sequence region are effective inhibitors of self-binding. Furthermore, the relevant hapten for the S107 or T15 antibody, phosphorylcholine (PC), can inhibit the self-binding, demonstrating that the antigen binding site is part of the self-binding region.

We have extended the investigation of the immunochemical and biophysical properties of self-binding antibodies (autobodies) to human antibodies specific for PC. We have observed that these antibodies are also self-binding, that the hapten PC is a potent inhibitor of self-binding, and that hypervariable region peptides of T15 also are inhibitors of self-binding. The finding of antibodies that are self-binding demonstrates a novel kind of antibody repertoire controlled by variable sequence structures.

M 318 THE HUMAN FETAL ANTIBODY REPERTOIRE, Jan L. Hillson, Ina R. Oppliger, and Roger M. Perlmutter, Howard Hughes Medical Institute and Departments of Medicine, Immunology, and Biochemistry, University of Washington, Seattle, WA 98195.

Pre-B and B lymphocytes developing in human fetal liver preferentially utilize a subset of the antibody heavy chain gene segment repertoire (Schroeder 1987). To determine the extent to which this restriction in reflected in fetal antibodies, sequences were obtained from heavy and light chain transcripts found in Epstein-Barr virus-transformed monoclonal B cell lines derived from fetal liver and spleen at 108 and 134 days of gestation. Among 28 fetal liver and 45 fetal spleen clones, the representation of V_H and V_κ gene families, assayed by hybridization, is indistinguishable from that in the germline. Sequences of nine fetal liver kappa light chains show preferential use of the $J_\kappa 1$ and $J_\kappa 2$ elements, but confirm that V_κ use is random to a first approximation. Heavy chain sequences reveal overrepresentation of $J_H 3$ and $J_H 4$ gene segments, the presence of two germline genes ($V_H 56p1$ and $V_H 15p1$) previously shown to be overrepresented among fetal pre-B cells and early B cells, along with previously undescribed heavy chains. Placed in the context of our previous studies, these results suggest that the restricted pre-B cell repertoire rapidly gives rise to B cells containing more diverse sequence elements, probably as a result of efficient clonal selection. Intriguingly, many of the B cells synthesize antibodies with self-specificities.

M 319 INHIBITION OF ENDOGENOUS Ig GENE REARRANGEMENT IN TRANSGENIC MICE RESULTS IN A SKEWING OF THE AVAILABLE Vh REPERTOIRE. John Iacomini, Sabita Bandyopadhay and Thereza Imanishi-Kari. Tufts University, Sackler School of Biomedical Sciences Program in Immunology. 136 Harrison Ave. Boston MA 02111.

Transgenic mice containing the 17.2.25 rearranged immunoglobulin IgM heavy chain gene (M54) were examined to determine the effect of the transgene on endogenous Ig gene rearrangement and Vh repertoire. Abelson pre B-cell lines were derived from adult bone marrow of transgenic and normal littermates. The frequency of endogenous VDJ rearrangements is greatly reduced in transgenic abelson lines. VDJ rearrangement are present in only 19% of the transgenic line analyzed (15 total). In contrast VDJ rearrangements are present in 83% of the normal lines analyzed (12 total). To determine if the inhibition of Ig gene rearrangement effects the available Vh repertoire, mRNA from LPS blasted spleen cells was analyzed for Vh expression by northern analysis. The level of expression of Jh proximal Vh families (Vh 7183, Vh Q52) is similar in transgenic and normal littermates. In contrast expression of Jh distal families (Vh J558) is reduced in transgenic mice. Therefore a increase in expression of Jh proximal Vh families most likely represents an expansion of B-cells that have undergone early rearrangement events consisting predominately of Vh 81X and Vh 7183 family members.

M 320 PREFERENTIAL REARRANGEMENT OF Vκ4 GENE SEGMENTS IN PRE-B CELL LINES, Susan L. Kalled and Peter H. Brodeur, Immunology Program, Sackler School of Graduate Biomedical Science and the Department of Pathology, Tufts University School of Medicine, Boston, MA 02111.
We have examined the in vitro Vκ gene rearrangements of murine adult bone marrow derived pre-B cell lines. We find that 27 out of 29 (93%) cell lines examined have rearranged a member of the Vκ4 family. In contrast, analysis of a Vκ cDNA library prepared from LPS-stimulated adult spleen cells indicates that only 16% of the Igκ cDNAs contain sequences belonging to the Vκ4 gene family. Half of the pre-B cell lines examined also appear to share a common reciprocal product which is readily detected in DNA from normal adult spleen cells. We suggest that, unlike the diverse Vκ repertoire expressed by mature B cells, the germline Vκ segments involved in initial rearrangements of the Igκ locus may be highly restricted, and that a frequent early event may be a common inversional rearrangement.

M 321 STOCHASTIC Vh:Vk PAIRINGS OCCUR AMONG POLYCLONALLY ACTIVATED B LYMPHOCYTES, Azad Kaushik , Dan H. Schulze, F.A. Bonilla, Constantin Bona and Garnett Kelsoe, Department of Microbiology, Mount Sinai School of Medicine, New York 10029, and Department of Microbiology, University of Texas Medical Branch, Galveston TX 77550.
We have earlier demonstrated the non-stochastic Vk gene usage by polyclonally activated B splenocytes both from adult and neonatal C57BL/6 mice. We have now examined the combinatorics of particular Vh and Vk gene family pairings by determining the frequencies at which several Vh and Vk families are expressed both independantly and in combination. Frequencies of 25 Vh:Vk pairings expressed in splenic B cell populations were determined by hybridization of Vh- and Vk family specific DNA probes to mitogen induced B cell colonies from C57BL/6 mice or hybridomas derived from Balb/c and NZB mice. The data from both of these approaches revealed that Vh and Vk gene families pair without any bias, i.e., the frequencies at which particular Vh:Vk pairs are expressed may be estimated by the product of independant Vh and Vk frequencies. Thus, we calculated the expected usage of 100 Vh:Vk pairings based upon the frequencies of 9 Vh and 12 Vk gene families. The Vh:Vk pairs representing >10% to <0.01% occur in the splenic B cell population. In the neonates, 6 Vh:Vk pairs appear to account for nearly 40% of all mitogen reactive B cells. Such a distribution of frequencies for Vh:Vk pairings becomes uniform as the neonate matures. This process may underlie the patterned acqusition of humoral immune responsiveness.

M 322 CLONING A B LYMPHOCYTE MEMBRANE PROTEIN: A PROBABLE HOMOTYPIC CELL ADHESION MOLECULE, John H. Kehrl, Gaye Lynn Wilson, Anthony S. Fauci, Laboratory of Immunoregulation, NIAID, NIH, Bethesda, MD 20892.
Using a subtractive cloning strategy cDNAs for several B lymphocyte specific proteins have been isolated. Northern blots with one of the clones, #66, revealed a 3.3 kb mRNA present in normal B cells as well as a variety of B cell lines. Clone 66 mRNA expression in the B cell lines was several fold higher than in normal B cells. In vitro activation of B cells with Staphlococcus aureus Cowan (SAC) resulted in a significant increase in expression of the 3.3 kb transcript suggesting that clone 66 recognized an activation antigen. The transcript was absent from RNA derived from normal T cells, T cell lines, Hela cells, brain, liver, and placenta. Both strands of a full length cDNA were sequenced. Sequence analysis revealed a long open reading frame of 2541 bases coding for a predicted protein of 847 aa with a molecular weight of 95 kilodaltons. The probable initiation ATG was followed by a signal peptide of 22 aa. Hydrophobicity plots revealed a single membrane spanning domain and a predicted intracytoplasmic region of 140 aa. The intracytoplasmic domain has a predicted protein kinase C phosphorylation site, a casein kinase II phosphorylation site, and 6 tyrosines. The extra-cytoplasmic portion has 12 n-linked glycosylation sites suggesting that the protein is heavily glycosylated. Search of the NBRF database revealed that clone 66 is a member of the CH_2 super Ig family and has significant homology to carcinoembryonic antigen (28% identity over 489 aa), myelin associated glycoprotein (26% identity over 425 aa), and N-CAM (21.5% over 274 aa). Each of these proteins functions as a homotypic cell adhesion protein. Transfection studies are in progress to prove that clone 66 encodes for a B lymphocyte homotypic cell adhesion molecule.

M 323 CLONAL DELETION OF PHOSPHOCHOLINE (PC)-SPECIFIC B-CELLS IN M167-TRANSGENIC MICE EXPRESSING AN X-LINKED IMMUNODEFICIENCY GENE, XID, James J. Kenny[1], Donna Sieckmann[3], and Dan L. Longo[2], Program Resources, Inc.[1], and the Biological Response Modifiers Program[2], NCI-FCRF, Frederick, MD 21701, and the Naval Medical Research Institute[3], Bethesda, MD 20814
The combined expression of the M167 μ/κ anti-PC transgenes with the xid gene in (B6.CBA/N x μ/κ 207-4) F1 mice results in an almost total failure to develop B cells in the peripheral lymphoid organs of the immune defective, F1 male progeny, whereas the phenotypically normal F1 females have large numbers of PC-specific B cells in their spleens. Immune deficient, xid male mice derived from crosses of B6.CBA/N and μ-243-4 M167 H-chain transgenic mice have large numbers of splenic B cells expressing the M167 heavy chain, but none of these B cells are PC-specific. However, the phenotypically normal F1 TG^+ female progeny from this cross have 1 to 3% of their B cells which are PC-specific. These B cells appear to express the M167 μ-transgene in association with an endogenous light chain (presumably V_κ 24) which gives rise to exclusively M167 μ/κ idiotype positive B cells. T15-idiotype positive B cells are not detectable among these PC-specific B cells. The absence of the T15-idiotype is somewhat surprising in that this is the dominant idiotype in normal mice. Transfection experiments are being conducted to determine whether or not the M167 H-chain can associate with a V_κ 22 gene product to produce a T15-idiotype positive antibody. These data suggest that: 1) TG^+, PC-specific B cells are clonally deleted in an antigen-specific manner in mice expressing the xid gene, and 2) PC-specific B cells expressing the M167 H-chain in association with the appropriate V_κ 24 endogenous light chain are specifically selected and expanded in normal M167 H-chain TG^+ mice.

M 324 A VARIABLE REGION MUTATION IN A HYBRIDOMA RESULTS IN THE LOSS OF ANTIGEN BINDING. Barry J. Kobrin, Carolyn A. Schiff, Jennifer L. Rabinowitz, Mark J. Shulman* and Matthew D. Scharff. Department of Cell Biology, Albert Einstein College of Medicine, Bronx, N.Y. and *Department of Immunology and Medical Genetics, University of Toronto, Toronto, Canada. During the course of an immune response, extensive somatic mutation, which is restricted to the variable region of the Ig molecule allows for the generation of progressively higher affinity antibodies. We studied the role of in vitro somatic mutation on antigen antibody-reactivity by characterizing a nitrosoguanidine induced antigen binding variant of a phosphorocholine (PC) binding hybridoma. Serological analysis using a panel of anti-idiotypic reagents revealed local conformational changes within the antigen binding site. mRNA sequencing indicated that a single G to A transition at position 95 of the V_H, resulted in an Asp to Asn substitution which results in total loss of Ag reactivity. These data are consistent with a computational three dimensional model of the germ line encoded (S107) PC binding site in which Asp 95 is an antigen-contacting residue. No spontaneous revertants were detected in 3 x 10^6 cells, suggesting that somatic hypermutation was no longer operative in this cell line. Reversion analysis of manipulated subclones of this cell line provides an opportunity to study somatic hypermutation in vitro.

M 325 THE MURINE V_H441 IMMUNOGLOBULIN GENE ENCODES ANTIBODIES AGAINST FIVE CARBOHYDRATE DETERMINANTS, D.M. Marcus, J. Snyder, E. Padlan and L-y. Yu-Lee, Baylor College of Medicine, Departments of Medicine, Microbiology and Immunology, Houston, TX 77030
The murine X24 V_H gene family consists of two genes, V_HX24 and V_H441. The latter gene is known to encode antibodies against levan, galactan, 3-fucosyllactosamine (3-FL) and α1-6 dextran. The V_H441 gene contains a potential N-glycosylation site at Asn58 in CDR2, and this site is retained in all V_H441-encoded antibodies sequenced previously. We recently described mAb directed against galactosyl-globoside (GG). These antibodies are directed against the side of this determinant rather than the terminal non-reducing sugar residue. Northern blotting revealed that all four anti-GG mAbs are encoded by V_H441 mRNA sequencing of two of these mAbs, 5A3 and 8A7, revealed that their glycosylation sites were eliminated by somatic mutation; sequencing of the other two mAbs is in progress. A V_KOx1 probe hybridized with mRNA from each of the four antibodies, but mRNA sequencing of 5A7 indicated that its light chain is probably encoded by V_KOx2. The use of V_H441 by antibodies against five different carbohydrate determinants provides an interesting system for studying structure-function relationships, and site-directed mutagenesis studies are in progress. A computer model of the anti-3-FL mAb PM81 will be presented.

M 326 STRUCTURE AND ORGANIZATION OF THE HUMAN IMMUNOGLOBULIN HEAVY CHAIN LOCUS

Fumihiko Matsuda, Euy Kyun Shin, Yasuhiko Hirabayashi, Hitoshi Nagaoka and Tasuku Honjo
Dept. of Medical Chemistry, Kyoto University, Kyoto, 606 Japan

The human immunoglobulin heavy chain variable region (VH) genes are located on the distal end of chromosome14 (14q32). To elucidate the molecular events underlying the generation of the antibody repertoire, it is important to examine the precise number of the VH segments, the relative orientation of the different VH genes and the overall organization of the immunoglobulin locus. We set out to construct a physical map of the entire human VH locus by cosmid cloning. We isolated cosmid clones which covered 1500 kb of this locus. Characterization of these clones as well as analysis employing pulse field gel electrophoresis established the following;

1. The human VH locus was estimated to span at least 3000 kb by PFGE.
2. VH segments belonging to different families were interspersed within the genome.
3. A 200-kb region of the VH-D-JH locus was characterized with overlapping cosmid clones. The 3' most VH segment (VH-VI) was found 20 kb upstream of the D4 segment. Four more VH segments were found in this region.
4. Multiple novel D clusters consisting of five D segments were identified among VH clusters. These duplicated regions were mapped within the VH locus of 14q32.
5. An orphan VH cluster consisting two apparently functional VH segments and two pseudogenes was found in the human genome.

M 327 GENERATION OF DIVERSITY IN THE CHICKEN IgL VARIABLE REGION BY GENE CONVERSION - A MOLECULAR MODEL, Wayne T. McCormack and Craig B. Thompson, Howard Hughes Medical Institute, Depts of Int. Med. and Microbiology/Immunology, Univ. of Michigan Medical School, Ann Arbor, MI 48109. The chicken immunoglobulin loci encode single functional VL, JL, VH, and JH gene segments, and cannot generate a complete preimmune repertoire by junctional and combinatorial diversity. Ig variable region diversity is generated in the chicken bursa of Fabricius by replacement of sequence blocks within the unique rearranged VL1 and VH1 genes with sequences from V region pseudogene segments (ψV), by a process that resembles gene conversion or double homologous recombination. No sequence modification in the ψVL region of over 30 clonal v-rel-transformed chicken B cell lines was detected by Southern blot analysis using restriction enzymes known to cut within the VL1 gene and ψVL family. Sequence polymorphisms within an F1 chicken strain allowed assignment of donor ψVL and recipient VL1 gene segments to the same allele. These results suggest that chicken VL1 gene diversification occurs by intrachromosomal gene conversion. Nucleotide sequencing of gene conversion events in 52 random IgL clones has revealed clues to a possible molecular mechanism of gene conversion. The frequency of ψVL usage and polarity in structural features of a large number of gene conversion events suggest a novel mechanism for somatic gene conversion of the chicken IgL gene. Details of this molecular model will be presented.

M 328 FUNCTIONAL AND MOLECULAR ANALYSIS OF PC-SPECIFIC B CELLS AT THE SINGLE CELL LEVEL, **Michael G. McHeyzer-Williams, Paul A. Lalor, and G.J.V. Nossal, The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia.**

Hybridoma technology has provided much information on the emergence of anti-phosphorylcholine (PC) precursors in ongoing immune responses and evidence of somatic hypermutation within their variable region genes. This approach does not allow enumeration of precursors, nor yield any information on specific precursors which do not form hybridomas. We have developed a system which can identify and isolate precursors before analysis of antibody or variable region genes. In preliminary experiments, using the FACS and multi-parameter analysis we have isolated $T15^+$ B cells from naive BALB/c mice. The results indicate that it has been possible to purify PC-specific, $T15^+$, antibody-forming cell precursors from a starting population which comprise 0.007% of spleen cells. Using the polymerase chain reaction (PCR) we have been able to amplify varible region gene mRNA from single $T15^+$ hybridoma cells to a produce sufficient double stranded DNA template for direct sequencing. We intend to track the progress of T15+ B cells following activation by antigen in vivo. The derivation of the specific anti-PC response will be followed phenotypically, functionally and at the molecular level in single cells. This approach may be used to examine B cells for mutations immediately ex vivo, independent of their ability to form hybridoma. We aim to phenotypically define the earliest precursor able to mutate their V genes and follow their progress during an immune response. We can use this strategy to compare the anti-PC to the anti-nitrophenol (NP) response, which elicit distinctly different isotype profiles following activation with antigen.

M 329 ANALYSIS OF B CELL DEVELOPMENT AND IMMUNOGLOBULIN EXPRESSION IN M167 TRANSGENIC MICE, Cathy O'Connell[1], Fred Finkelman[3], Dan L. Longo[2], and James J. Kenny[1], Program Resources Inc.[1], and the Biological Response Modifiers Program[2], NCI-FCRF, Frederick, MD 21701, and Department of Medicine[3], Uniformed Services University for the Health Sciences, Bethesda, MD 20814

The effect of immunoglobulin transgene expression on B cell development and endogenous immunoglobulin expression has been analyzed in mice expressing M167 $\mu + \kappa$, μ-only, κ-only, and $\mu + \kappa$ with a deleted (ΔM) transmembrane exon. The expression of any of these transgenes causes a reduction in the total number of spleen cells which is reflected in the reduction of $sIgM^+$ splenic B cells. The total number of cells present in the bone marrow is not affected by transgene expression, however, the number of $sIgM^+$ B cells and $B220^+$ pre-B cells is reduced by 50% in the μ-transgenic mice and by 60 to 90% in the $\mu + \kappa$ transgenic mice. Pre-B cell and B cell development is not suppressed in the κ-only and $\mu + \kappa$ ΔM transgenic mice. Expression of the μ-only transgene has very little effect on expression of endogenous IgM, thus > 90% of the splenic B cells express endogenous IgM of the μ^b allotype on their cell surface, and $\mu^{b+}B220^+$ B cells are easily detectable in their bone marrow. Endogenous μ^{b+} B cells are not detectable in the bone marrow of $\mu + \kappa$ transgenic mice and < 20% of their splenic B cells coexpress μ^b as double allotype ($\mu^a\mu^b$) positive B cells. The expression of endogenous Ig was completely suppressed in mice expressing both the M167 $\mu + \kappa$ transgenes and a γ2b transgene. Ontogenetic studies at the RNA level are in progress to determine whether these effects can be explained by differences in the timing of expression of these transgenes in fetal development.

M 330 ANTIGEN BINDING SPECIFICITIES IN THE HUMAN FETAL ANTIBODY REPERTOIRE Ina R. Oppliger, Jan L. Hillson, and Mark H. Wener, Departments of Medicine and Laboratory Medicine, University of Washington Medical Center, Seattle, WA 98195

B lymphocytes developing in human fetal liver express a small subset of the antibody variable region genes encoded in the germline (Schroeder 1987, Hillson 1989). To examine the binding specificities of this restricted repertoire, supernatants of Epstein Barr Virus (EBV) transformed B cell clones generated from fetal liver and spleen of 108 and 134 days gestation were tested for binding to a panel of self and exogenous antigens. Among 64 EBV transformed human fetal B cell lines, seven (10.9%) exhibited high level binding to ssDNA and six (9.3%) to cardiolipin (>10 X background). Reactivity to cytoskeletal intermediate filaments was detected in supernatants of two of 23 clones tested by indirect immunoflouresence. Low level binding (2-5 X background) of some supernatants was detected by ELISA to the protein antigens KLH, HSA, and Fc fragment of IgG. Cold agglutinin activity, binding to bromelain treated red blood cells and binding to thyroglobulin were absent in all samples tested by hemagglutination. Clones producing antibodies binding to self-antigens associated with autoimmune diseases are abundant among EBV transformed human fetal B lymphocytes and include both polyreactive clones and clones recognizing only one of the antigens tested.

M 331 THE BIOLOGICAL CONSEQUENCES OF IgM HEXAMER FORMATION, T.D. Randall, L.B. King, and R.B. Corley. Department of Microbiology and Immunology, Box 3010, Duke Medical Center, Durham, NC 27710

The inducible B cell lymphoma, CH12, and its in vitro adapted subclone, CH12-LBK, produce immunoglobulins of identical sequence, specificity, and isotype. Both cell lines secrete antibody at similar rates following stimulation, and the antibodies produced have equivalent (moderate) affinity for the hapten, TMA. However, antibody preparations from LPS-induced CH12 cells lyse erythrocytes six to ten times more efficiently than preparations from CH12-LBK. This higher efficiency is most evident in hemolytic plaque assays, in that CH12 cells make very large plaques when compared to CH12-LBK cells. Both cell lines secrete polymeric IgM, but while the CH12-LBK cell line secretes predominantly the expected pentameric IgM, CH12 cells secrete a mixture of pentamers and hexamers. Hexameric IgM has a specific activity twenty times that of pentameric IgM when tested by complement mediated cytolysis. Thus, an abundance of hexamers in CH12 cells is directly responsible for their high lytic capacity. J-chain is associated exclusively with pentameric IgM, and is not found in hexamers nor in any intermediate sized polymers of CH12 IgM. These experiments confirm that J-chain is not necessary either for assembly or secretion of IgM, and suggest instead that the expression of J-chain may be important in the regulation of the lytic efficiency of polymeric IgM by controlling the pentamer/hexamer ratio. The experiments further suggest a mechanism, in addition to isotype switching and somatic mutation, by which the biological efficiency of antibodies from a single clone of B cells can be regulated.

M 332 AFFINITY MATURATION BY SOMATIC MUTATION IN THE SECONDARY RESPONSE TO INFLUENZA VIRUS HEMAGGLUTININ, Robert C. Rickert, Mary Kopke Wloch and Stephen H. Clarke, Dept. of Microbiology and Immunology, University of North Carolina, Chapel Hill, NC 27514

A set of idiotypically related hybridomas, designated 68Id, specific for hemagglutinin (HA) was generated from a single Balb/c mouse following secondary (2°) immunization with influenza virus. All use the same V_H gene of the 36-60 family, V_H 1210.7, paired with one of at least three V_κ genes (V_κ21C, V_κ21E or V_κ9). The 68Id is not found among primary (1°) hybridoma antibodies and only rarely among 2° hybridoma antibodies of other mice. Sequence analysis of 2° 68Id hybridoma antibodies reveals that B cells expressing V_H1210.7 have undergone extensive somatic mutation. Analysis of V_H somatic mutations indicates a high frequency of parallel replacement mutations in CDR2 consistent with selection for higher affinity antibodies by antigen.

To further define the role of somatic mutation in the 2° response to HA vectors were constructed which allow expression of germline encoded or somatically mutated antibodies by the murine myeloma Ag8.653. Cloned variable region genes which are characteristic of the 2° response were altered from their germline sequence by site-specific mutagenesis to produce antibodies inferred to have been produced by B cells during clonal expansion *in vivo*. Competition ELISA was used to measure binding of HA by mutated antibodies relative to germline-encoded and hybridoma antibodies.

M 333 OLIGONUCLEOTIDE PROBES TO CDRs DETECT INDIVIDUAL VH GENES IN THE GERMLINE AND EXPRESSED REPERTOIRE, Daniel Rubinstein, Thierry Guillaume, Lori Tucker, Fay Young, Robert S Schwartz and Kathleen J Barrett, Division of Hematology/Oncology, New England Medical Center, Boston, MA 02111

Human VH genes have been divided into six families based on their nucleic acid homologies. Information about individual VH genes comes almost entirely from sequencing. As a result, little is known about the organization, rearrangements and expression of individual V genes. To focus on single, individual genes, we have studied the VH genes of two anti-DNA antibodies, VH18/2 and VH21/28, members of the two largest families. We have shown that oligonucleotide probes from the most variable part of the gene, the CDRs, detect a very limited number of monomorphic and polymorphic bands on Southern blotting and that combined information from both CDRs identifies a single common band for each gene. This common band contains the germline gene. In expression studies in a panel of EBV-transformed B cells, VH18/2 was found to be frequently expressed compared to VH21/29 (10% versus 1%). Cloning and sequencing of the expressed VH18/2 genes showed them to be 97% or greater homologous. We conclude that (1)Utilizing oligonucleotide probes to the CDRs, we can examine individual VH genes (2) The probes detect germline polymorphisms of the VH gene (3) Genes sharing the same (or nearly identical) CDRs also have the same (or nearly identical) frameworks. We call this subgroup of very highly-related genes of the same V gene family, a CDR family. (4)VH18/2 is overexpressed as compared to VH21/28. Bycloning and sequencing from the germline, we have examined the mechanism of the overexpression of VH18/2

M 334 EXPRESSION OF MURINE IMMUNOGLOBULIN LIGHT CHAIN λ AND ITS ROLE IN FEEDBACK ON IG GENE REARRANGEMENT. Charles Rudin, John Hackett and Ursula Storb, Dept. of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL 60637

A recent study by our laboratory of transgenic mice containing a functionally rearranged λ2 gene under the transcriptional regulation of the H chain gene enhancer (Hagman et al, 1989 J. Exp Med 169, 1911-1929) demonstrated that, whereas the expressed λ gene had prevented κ gene rearrangement in many B cells, this feedback was not ubitquitous: an initially small fraction of B cells were able to rearrange and express an endogenous κ gene despite the presence of the λ transgene, resulting in cells producing both κ and λ light chains. Preliminary results of experiments in progress suggest that these double-producing cells may have arisen from a nonconventional B cell pool (i.e. one not derived from adult bone marrow precursors): transferring bone marrow cells from transgenic donors into irradiated scid recipients leads to outgrowth of donor cells expressing only the transgene.

A search for cis-acting sequences involved in λ gene expression by DNaseI hypersensitivity mapping recently resulted in the isolation of a B cell-specific enhancer within the λ locus, approximately 15 kb downstream of the Cλ2Cλ4 cluster (Hagman et al, submitted). A second closely homologous sequence has been tentatively mapped to within 70kb of the Cλ3Cλ1 cluster. Efforts to further characterize this enhancer and to clone the second homologous region are under way.

Supported by NIH grants AI24780 and HD23089. C.R. was supported by NIH training grant GM-07281 and is the recipient of a Medical Student Research Award from the Arthritis Foundation.

M 335 ROLE OF CLASS 2 UPREGULATION IN THE INDUCTION OF TOLERANCE BY ANTI-Ig IN NORMAL AND DEFECTIVE I-E TRANSGENIC MICE, **David W. Scott, Carol Cowing* and Garvin L. Warner, Immunology Division, University of Rochester Cancer Center, Rochester, NY 14642 and *Division of Immunology, Medical Biology Institute, La Jolla, CA 92037**

We have developed a polyclonal system for tolerance in which B cells are cultured overnight with anti-Ig, washed and then challenged with specific Ag or LPS. This procedure leads to profound anergy in terms of anti-hapten antibody and total Ig synthesis. Tolerance induction is cholera toxin-insensitive and can be mimicked by reagents which cause abortive B cell exit from G_0 into G_1. Since these conditions lead to upregulation of class 2 expression, we asked whether B cell anergy could occur when this upregulation was blocked. We first examined the role of 5' upstream regulatory sequences for I-E in transgenic mice with various deletions that lead to a lack of B cell expression (5'Δ mice). Splenic B cells were incubated overnight with anti-Ig and either stained for I-E and I-A or challenged with LPS to trigger differentiation. Our results indicate that anti-Ig failed to upregulate I-E in defective 5'Δ splenic B cells while I-A induction was normal. However, anti-Ig pretreatment still led to tolerance in both normal and defective 5'Δ transgenics. In contrast, we found that cyclosporin A (CSA), which prevents class 2 upregulation in murine tissues, inhibited anti-Ig-driven B cell tolerance induction in normal splenocytes. The transgenic results show that the 5' regulatory sequences for I-E contain an anti-Ig responsive site, but that tolerance can occur in the absence of this region (perhaps via an I-A interaction.) On the other hand, our data with CSA suggest that upregulation of class 2 may be coincident with a tolerance pathway in B cells. We propose that, in the absence of a cognate T cell signal, this process is a negative signal for differentiation. (Supported by CTR grant #2493, NIH CA41363 and a BRSG award.)

M 336 QUANTITATIVE ANALYSIS OF THE PRIMARY IGH REPERTOIRE: ASSESSING RESTRICTIVE INFLUENCES ON THE UTILIZATION OF V_H GENES Kevin M. Sheehan and Peter H. Brodeur, Immunology Program, Sackler School of Graduate Biomedical Sciences and Department of Pathology, Tufts University School of Medicine, Boston, MA 02111

The generation of the primary antibody repertoire requires the somatic recombination of germline gene segments. It is not known, however, whether all functional V and J segments have an equal probability of contributing to this intitial set of antibody specificites. To address this issue, we examined the relative representation of V_H and J_H gene segments in V_H cDNA phage libraries constructed from C_μ transcripts of polyclonally activated spleen cells of adult mice. We have shown that probes specific for one, two or three functional V_H gene segments hybridize with cDNAs at frequencies directly proportional to the number of functional germline V_H gene segments detected by each probe. In contrast, the representation of 12 V_H families within each library indicates that certain families are underrepresented relative to their estimated germline gene number. These families must either have extraordinary proportions of nonfunctional genes or are influenced by as yet unidentified regulatory mechanisms or constraints on rearrangement. Subsequently, we have examined a panel of phage isolates containing cDNAs of the V_H3609 family in order to assess the utilization of V_H gene segments from one of the underrepresented families. In addition, probes specific for individual V_H genes mapping to different areas of the Igh-V region were used to determine whether the expression of a given V_H gene is influenced by its position within the Igh locus.

M 337 LIMIT-DILUTION ANALYSIS OF MURINE B CELLS SPECIFIC FOR REPEAT SEQUENCE DETERMINANTS OF PLASMODIUM FALCIPARUM, Ken Shortman, Alison Venn, Robin Anders and Beverley Pike, The Walter and Eliza Hall Institute of Medical Research, Melbourne, Victoria 3050, Australia

A culture system involving lipopolysaccharide stimulation of B cells in the presence of 3T3 fibroblast filler cells was used, with an ELISA readout for specific antibody, to determine the frequency of antibody-forming cell precursors (AFC-p) specific for the 3'-repeat epitopes of the RESA malarial antigen. One in three B cells form clones giving detectable Ig production in this system. In the spleens of unprimed mice the frequency of RESA-specific AFC-p was extremely low. After primary or secondary injection of mice with RESA peptide and various adjuvants, there was no marked increase in splenic RESA-specific AFC-p, either at the IgM or the IgG level. This was despite an increase in serum IgG titres which indicated a secondary response in the donor animals. Various interpretations of the results are being assessed, including the possibility that the secondary response to these malarial antigens arises from a distinct, LPS non-responsive B-cell subpopulation.

M 338 STRUCTURAL CHARACTERIZATION OF HUMAN MONOCLONAL COLD AGGLUTININS: EVIDENCE FOR A DISTINCT PRIMARY SEQUENCE DEFINED VH4 IDIOTYPE. Gregg J. Silverman and Dennis A. Carson. Department of Molecular and Experimental Medicine, Research Institute of Scripps Clinic, La Jolla, CA 92037

Cold agglutinins that bind the developmentally regulated I red cell determinant occur naturally among human monoclonal IgM proteins. These autoantibodies are known to use light chains that derive mainly from the minor kappa III (KIII) variable region subgroup. The KIII subgroup is also highly expressed in monoclonal rheumatoid factors. However, while most monoclonal rheumatoid factors use structurally homologous heavy chains that derive from the VH1 family, information regarding the structure of the cold agglutinin heavy chains remains fragmentary. To determine the genetic origins of these autoantibodies, we have developed anti-peptide antibodies that recognize framework and hypervariable region sequences. We found that the KIII cold agglutinin autoantibodies exclusively use heavy chains that derive from the VH4 family. Furthermore, these autoantibody heavy chains all express the same primary sequence defined idiotype, corresponding to the second hypervariable region. These data indicate that cold agglutinins use a remarkably homogeneous subset of heavy chain variable regions, which likely derive from a small VH4 subfamily, or single gene. Moreover, unique patterns of preferential VH and VL pairing clearly distinguish the anti-I cold agglutinins from all other known monoreactive autoantibodies.

M 339 AN EMBRYONIC SOURCE OF LY1+ B CELLS. *Nanette Solvason, Agnes Leheun and John Kearney. Division of Developmental and Clinical Immunology, UAB.* We have examined the potential of fetal omentum, spleen, thymus and liver to reconstitute Ly1+ B cells. Thirteen day gestation CB6F1 (H-2D^{d}XD^{b}) primordia were transplanted under the kidney capsule of SCID (H-2D^{d}) mice. Four months post transplant the host lymphoic organs were stained for the presence of donor B and T cells using a combination of anti-IgM or anti-Thy1 plus anti-H-2D^{b}. In four out of five mice with omentum transplants 25-40 % Ly1+ donor B cells were present in the peritoneal cavity while less than 1% of the splenic lymphocytes were donor derived B cells and were Ly1-. Only one out of five mice had donor derived T cells in the peripheral lymphoid organs. In comparison mice which received fetal liver transplants had B and T cells present. Mice receiving fetal spleen transplants had donor derived B cells in the peritoneal cavity which were Ly1- Thymic transplants reconstituted exclusively αβ T cells in the spleen , lymph nodes and peritoneal cavity however both αβ and γδ T cells were seen in the gut. The thymus grafts did not reconstitute B cells . Mice with omentum transplants had mostly donor derived serum IgM and some IgG3, however there was no IgG1 detected anc very little IgG2a or IgG2b. In contrast, mice with liver transplants had high levels of all gamma isotypes present in addition to IgM. Histological examination of the subcapsular omental transplants revealed the presence of loose unorganized lymphoid aggregates Hybridomas were constructed from mice with omentum transplants to analyze the repertoire of these embryo derived B cells. Southern blot analysis using a sγ1 probe has allowed us to confirm the origin (donor vs. host) of each hybridoma . V_H and V_L gene usage of each hybridoma,the extent of somatic hypermutation and evidence of V gene replacement will be sought by PCR. Suppported by NIH grant CA 16673.

M 340 IMMUNOGLOBULIN LIGHT CHAIN GENES IN *XENOPUS LAEVIS*, Lisa A. Steiner and Diane J. Zezza, Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139.

cDNA clones expressing immunoglobulin light chain epitopes were isolated from an expression library prepared from *Xenopus* splenocytes and were used to identify genes encoding light chains in this species. Southern blot analysis of *Xenopus* genomic DNA with cDNA probes corresponding to constant and variable regions suggested the presence of one or two *C* genes and many *V* genes in this light chain class. One clone from a genomic library was found to contain a single *C* gene and a single *J* segment separated by an intron approximately 3 kb in length. A classical recombination signal consisting of conserved nonamer and heptamer sequences, separated by a spacer of 23 non-conserved nucleotides, was present 5' to *J*. Many clones in the genomic library hybridized to a probe corresponding to the 3' end of the *V* cDNA. One of these clones (11 kb) contained four *V* segments separated by 2 to 3 kb. Three of the segments appear to correspond to functional genes; upstream of the coding sequences there are putative promoter elements and downstream there are recombination signals with heptamer and nonamer sequences separated by 12 bp. The fourth *V* segment appears to be a pseudogene. Differences among these sequences were found both in framework and CDR segments. The data support the assignment of this group of genes to the kappa rather than to the lambda class of light chains. This conclusion is based on: 1) the presence of many *V* gene segments and only one or two *C* gene segments; 2) the sequences of the *V*, *J*, and *C* segments; 3) the length of the *J-C* intron; 4) the lengths of heptamer-nonamer spacers 3' to *V* and 5' to *J*.

M 341 THE S107 V_H REPERTOIRE IN MUS PAHARI, Susan B. Sylvers and Barbara A. Osborne, Program in Molecular and Cellular Biology and Department of Veterinary and Animal Sciences, University of Massachusetts, Amherst, MA 01003
We have examined the S107 V_H family in a variety of species of Mus in an attempt to determine the genetic processes involved in the evolution of this important V_H family. Previous results from our lab have demonstrated that this family evolves primarily through a non-reciprocal genetic recombination process we believe to be gene conversion. In this study, we have cloned and sequenced three members of the S107 V_H gene family in Mus pahari, the most evolutionarily distant member of the genus Mus from the inbred laboratory mouse. The results from these studies demonstrate that Mus pahari has three family members, all of which are duplicates of the V1 gene. Further characterization of the Mus pahari S107 genes has revealed that two of the three genes isolated are, most probably, alleles of one another. One of these potential alleles is found in all individual mice examined while the other is found in only three of nine mice examined. The latter appears to be a pseudogene due to six transversions with in the octamer sequence. In inbred strains such as BALB/c, C57BL/10 and CBA/J, four S107 genes have been identified, V1, V11, V13 and V3. V1 has been shown to be the gene that encodes the response to phosphorylcholine, V11 has been observed to encode anti-flu antibodies and V13, while potentially functional, has not been associated with any known response. V3 has been characterized as a pseudogene in the inbred strains examined. Thus Mus pahari, is unique in that it only contains copies of the V1 gene, with V11, V13 and V3 totally absent in this species. An examination of the S107 V_H repertoire in other species of *Mus* has demonstrated that, while V11, V13 or V3 genes may be absent in any given species, in each species studied, the V1 gene is always present. These studies indicate that while other family members are expendable, the V1 gene is conserved over 8-12 million years of evolution.

M 342 FORMATION OF MEMORY B CELL LINEAGES DURING A PRIMARY IMMUNE RESPONSE, Weng Tao and Alfred Bothwell, Section of Immunobiology, Yale University School of Medicine, New Haven, CT 06510. The primary immune response to NP-CGG in C57Bl/6 mice is known to express the dominant NP^b idiotype. The majority of the antibodies use a characteristic VH gene and the λ light chain. The clonal relatedness of monoclonal antibodies isolated at various times after a primary immunization was examined. Several new features of this response have been observed 1) the response at this stage is generated from a relatively small number of progenitor clones, 2) clonal expansion of cells in the absence of somatic mutation occurs at early stages, and 3) formation of the memory lineages occurs very early.

M 343 IgG ANTI-INSULIN ANTIBODIES ARE NOT EXTENSIVELY MUTATED, James W. Thomas, U. Kevin Ewulonu and Laura J. Nell, Department of Medicine, Baylor College of Medicine, Houston, TX 77030. Our data on human EBV transformed B cells show that IgM anti-insulin V_H genes are largely in germline configuration. To avoid the bias of transformation, we sequenced the V genes from four anti-insulin IgG mAbs derived from the primary response of Balb/c mice. The four V_K genes used by these antibodies were all high homologous (98%) to known V_K germline genes, including V_K 21c, OX1, and OX2. These V_K genes preferentially rearrange to J_K4 and J_K5 and have tandem Prolines at position 95. One mAb uses the same V_H and V_L combination as a recently described anti-idiotypic antibody (A20/44) and the anti-insulin antibody uses an unmutated DFL 16.1 germline sequence. Anti-insulin V_H genes are derived from J558, V-gam 3-8 and V-gam 3-2 families, but no germline counterparts are available for comparison. To determine the role of somatic mutation in V_H, PCR was used to amplify germline genes of the V-gam 3-8 family. Four distinct members of the Balb/c V-gam 3.8 family were identified, and one isolate differs in three amino acids (one in FW2 and FW3, and one in CDR2) from the expressed anti-insulin V_H gene. While IgG anti-insulin antibodies may employ a diverse group of V_K and V_H genes, our data show that these genes are not extensively mutated. These mAb share the property of binding autologous insulin with high avidity and may reflect part of the autoimmune repertoire.

M 344 **ANALYSIS OF ISOTYPE SWITCH VARIANTS OF A MONOCLONAL AUTO-ANTI-IDIOTYPIC ANTIBODY,** *Meenal Vakil, Andreas Radbruch, and John F. Kearney, University of Alabama at Birmingham, Birmingham, AL, and Institut für Genetik, Köln, FRG.*
A hybridoma cell line MM60 (IgM λ3) was previously isolated and shown to react with a monoclonal antibody GB4-10, which in turn bound the TEPC15 idiotype. Purified antibody MM60 (anti-anti-T15) was shown to modulate the T15 anti-phosphorylcholine response in adult BALB/c mice via the expansion of an intermediate set of "GB4-10-like" B cells. To determine the role of the antibody isotype in idiotype directed modulation of B cell function, isotype switch variants were generated from this hybridoma cell line by a combination of fluorescence activated cell sorting and sequential subcloning of the sorted cells. In independent subclones heavy chains of IgG1, IgG2b and IgG2a all associate with λ3 light chains. Southern blot analysis of the immunoglobulin gene rearrangements in the parent cell and the variant cell lines suggests that generation of switch variants occurs through chromosome duplication and switch recombination on one of the two chromosomes. Further, complete nucleotide identity among the heavy chain variable region genes (Vh-D-Jh) from the parent hybridoma and all the switch variants was established following cloning and sequence analysis of the VDJ regions of the expressed allele. Surprisingly, while the secreted variant IgG2a molecules bound GB4-10 in vitro, the IgG1 and the IgG2b molecules did not. A study of the in vivo activities of the purified variant antibody molecules is currently in progress. Supported by grants AI 14782, CA 16673, and CA 13148.

M 345 NUCLEOTIDE SEQUENCE OF A HUMAN MONOCLONAL AB2 DERIVED FROM A $CD5^+$ EBV TRANSFORMED B CELL CLONE SPECIFIC FOR A RABIES VIRUS NEUTRALIZING MURINE MONOCLONAL AB1: EXTENSIVE SOMATIC VARIABILITY SUGGESTS AN ANTIGEN DRIVEN IMMUNE RESPONSE, Roger W.J. van der Heijden, Virginia Pascual*, Hans Bunschoten, Fons G.C.M. UytdeHaag, Albert D.M.E. Osterhaus and J. Donald Capra*, Dept.Immunobiology, National Institute of Public Health and Environmental Protection, P.O.Box 1, 3720 BA Bilthoven, The Netherlands, *Dept. Microbiology, The University of Texas Southwestern Medical Center, Dallas, Texas 75235.

We previously described the isolation and characterization of a human $CD5^+$ EBV transformed B cell clone that was isolated from peripheral blood lymphocytes following immunization with rabies vaccine and that produced an IgM,λ anti-idiotypic monoclonal antibody ($Ab_2$383). Though the phenotype of the B cell clone as well as the isotype of $Ab_2$383 are characteristic for multispecific autoantibodies, $Ab_2$383 seems to be monospecific for a rabies virus neutralizing murine MoAb (6-15-C4). Neither reactivity with a panel of murine MoAbs having the same isotype (IgG2b,κ), nor reactivity with ten different human autoantigens could be detected. The V_H chain of $Ab_2$383 derives from the small V_HV family of human variable region gene segments. Parallel studies on the germline V_HV gene isolated from the same donor, revealed that the expressed molecule contains 19 nucleotide differences in the V_H gene segment. The D segment of $Ab_2$383 could have arisen by a D-D fusion; the J segment is a J_H6. Extensive somatic variation evident in the V_H region of this naturally arising Ab_2 suggests that $Ab_2$383, the product of a $CD5^+$ B cell, was the consequence of an antigen driven immune response. Sequence analysis of the λ light chain and the search for the human analog of MoAb 6-15-C4 as well as the search for other '$Ab_2$383 like' Ab_2's in different unrelated donors is in progress.

M 346 ONTOGENY AND EXPRESSION OF ANTIBODIES ENCODED BY THE MOST JH-PROXIMAL HUMAN VH6 GENE SEGMENT USING A MONOCLONAL ANTIBODY. Johan H. van Es, Frits H.J. Gmelig-Meyling and Ton Logtenberg. Department of Clinical Immunology, University Hospital Utrecht, Postbus 85500, Utrecht, The Netherlands.

We have recently described properties of autoantibodies (anti-DNA, anti-cardiolipin) encoded by the most JH-proximal Human VH-6 gene segment (1). To further delineate the role of these antibodies in B cell ontogeny and in autoimmune disease we have developed a monoclonal antibody that specifically recognizes germline encoded VH6 gene products. Analysis of EBV-transformed cell lines, polyclonally activated B cells and serum samples from healthy individuals (neonates, adults) and patients with SLE with this antibody revealed that a) the germline VH6 gene segment is exclusively expressed in early B cell ontogeny and b) germline VH6-encoded antibodies contribute to the pool of natural autoantibodies in cord blood, but are not present in sera of either healthy or diseased adult individuals. These results indicate that VH6-encoded antibodies play a special role in early cell development.

1) T. Logtenberg et al. J. Exp. Med. 170, 1347 (1989).

M 347 ABSENCE OF EXPRESSION OF THE SOMATIC HYPERMUTATION MECHANISM IN THE J REGION OF UNREARRANGED HEAVY AND LIGHT CHAIN IMMUNOGLOBULIN GENES, John S. Weber and J. Latham Claflin, Department of Microbiology and Immunology, University of Michigan, Ann Arbor, MI 48109-0620. The murine immune response to phosphocholine in Proteus morganii results in B cells with somatic mutations in the V regions of immunoglobulin genes. Twenty one of the hybridomas formed from these B cells had an unrearranged K allele. One set of seven hybridomas had an unrearranged H chain allele. To determine if the hypermutation mechanism acted upon the unrearranged allele, the 5' part of the K chain JC intron and H chain JC intron of the unrearranged allele was sequenced and compared to the sequence of the same region in the productively rearranged allele. The evidence indicates that the mutation rate was significantly lower in unrearranged alleles (0/8710 bp for K and 1/2700 bp for H) than in rearranged alleles (25/6050 bp for K and 15/3300 bp for H). These results support the theory that V_K-J_K and V_H-D_H-J_H rearrangements are necessary for complete expression of the mechanism.

The functionality of the unrearranged H chain was also examined. The enhancer region and the proximal DQ52 gene segment are being sequenced to determine if they have been altered. Hybridization studies with switch region probes revealed that switching occurs in this allele indicating that heavy chain isotype switching is not dependent on any cis rearrangements at the J locus.

M 348 RESPONSE OF NORMAL HUMAN PRE-B CELLS TO INTERLEUKIN-3 AND INTERLEUKIN-4, B. Wormann, F. Steckel, Th. Buchner, W. Hiddemann, Med. Univ. Klinik A, Albert Schweitzer Str. 33, 44 Munster, W. Germany. We have studied the sensitivity of normal human pre-B cells to cytokins. Previous experiments on leukemic pre-B cells had identified interleukin-2 (IL-2), interleukin-3 (IL-3) and B cell growth factors as potential stimulators of proliferation. Using monoclonal antibodies against the cell surface antigens CD34, CD19, CD10, CD20 and sIgM, the pre-B cell development can be subdivided into at least 3 stages, i.e. CD34+/CD10+, CD34-/CD10+/CD20-/sIgM-, CD10+/CD20+. We have isolated cells from fetal and adult bone marrow by fluorescence activated cell sorting, and have incubated them with recombinant cytokines. Cells were also incubated with human stroma cells from patients with acute leukemia and with murine fibroblasts. Cells of all 3 stages showed a 2 to 3 fold increase in 3H-TdR uptake in response to IL-3. Interleukin-4 (IL-4) only stimulated the proliferation of CD10+/CD20+ or CD10+/sIgM+ cells. Incubation of cells with IL-2 induced expression of CD20, but did not stimulate proliferation. Coincubation with stroma cells and murine fibroblasts increased the viability of pre-B cells of all stages. No significant growth was observed in liquid suspension and in a methylcellulose colony formation assay. Our data support the results from patients with acute lymphoblastic leukemia, showing a sensitivity of human pre-B cells to IL-3. IL-4 only stimulated the more mature cells, while the human stroma cells from patients alone did not significantly enhance the in vitro growth of these cells.

Control of B Cell Growth; B Cell Signalling

M 400 CONSTITUTIVE SECRETION OF IL-5 BY A RAT MYELOMA CELL LINE Mark R. Alderson and Kenneth H. Grabstein, Immunex Corporation, 51 University St. Seattle, WA 98101. The YB2/0 cell line is a non Ig-secreting rat myeloma that produces a soluble factor(s) that stimulates proliferation and immunoglobulin secretion by normal murine B cells. Bioassays for various cytokines revealed that YB supernatants contained non-detectable levels of IL-1, IL-2, IL-3, IL-4, IL-6, IL-7, G-CSF, GM-CSF and IFN-γ. However, YB supernatants were able to cause the growth of eosinophils from both normal and parasitized murine bone marrow, suggesting the supernatants may contain IL-5. The ability of YB cells to secrete IL-5 was confirmed by northern blot analysis and by using a neutralizing monoclonal antibody against the murine IL-5 receptor. Furthermore, the secretion of IL-5 appeared to be constitutive and was unaffected by an array of B cell mitogens. We are currently investigating the possibility that normal B cells may produce IL-5.

M 401 INDUCTION OF IL4 RECEPTORS ON HUMAN AND MURINE B CELLS Richard J. Armitage, M. Patricia Beckmann and William C. Fanslow. Immunex Corporation, Seattle, WA 98103.

Human and murine recombinant IL4 were modified with biotin-N-hydroxysuccinimide and used, together with flow cytometry, to examine the expression of IL4 receptors (IL4R) on freshly isolated and activated B cells. Use of these labeled lymphokines previously has been found to provide a method of IL4R determination which is at least as sensitive as ^{125}I-IL4 binding.

Approximately 50% of freshly isolated tonsil B cells stained with biotinylated-human IL4, and stimulation with IL4 or phorbol ester resulted in a significant increase in IL4R expression by 48 hours. Similarly, Staphylococcus aureus Cowan I (SAC) induced an increase in IL4R expression in most experiments performed. In contrast, activation with IL2 or anti-IgM had no effect on the level of IL4R detected. EBV-transformed lymphoblastoid cell lines and Burkitts lymphoma lines showed little or no reactivity with biotinylated-IL4. Similarly, pre-B lines Nalm 6 and BMB expressed low levels of IL4R, although the pre-B line JM1 showed strong reactivity with biotinylated-IL4.

Flow cytometric analysis showed that murine splenic B cells expressed significant levels of IL4R prior to stimulation. Activation with IL4, Lipopolysaccharide (LPS) or phorbol ester induced an increase in IL4R expression to levels comparable to that seen on activated human B cells. In contrast, the murine pre-B line 70Z3 showed low levels of reactivity with biotinylated-IL4.

The results obtained by flow cytometry and ^{125}I-IL4 binding will be compared with respect to the distribution of IL4R and the sensitivity of these two methods for the detection of IL4R on both human and murine B cells

M 402 LONG TERM PROLIFERATION OF RESTING HUMAN B LYMPHOCYTES THROUGH CROSSLINKING OF CD40, Jacques Banchereau, Paolo de Paoli, Alain Vallé, Eric Garcia, Françoise Rousset, UNICET, Laboratory for Immunological Research, 27 chemin des peupliers, 69570 Dardilly, France.

Recently, it has been shown that resting T cells strongly proliferate in response to crosslinked anti-CD3 Mabs. Accordingly, a variety of Mabs specific for B cell surface antigens were crosslinked and tested for their ability to induce the proliferation of highly purified resting B cells. Two anti-CD40 Mabs (Mab 89 and G28-5) were able to induce a strong proliferation (^{3}H thymidine incorporation), whereas anti-CD19 (B4), anti-CD21 (8F1), anti-CD23 (Mab 25), anti-CD24 (IOB3) and anti-CD37 (IOB1) were ineffective. Maximal ^{3}H thymidine incorporation was obtained with as low as 30 ng/ml Mab 89 (10^{-10} M) and was long lasting as it could be measured as late as ten days after the onset of the culture. Proliferation was a result of CD40 crosslinking as Fab fragment of Mab 89 blocked the proliferation induced by the intact antibody. The [^{3}H] thymidine incorporation reflects B cell proliferation as 1-3 fold more viable B cells are recovered (at day 9) than was input at the start of the culture. In the absence of Mab 89 all input B cells die after five days of culture. IL-4, IL-6 and IFN-γ strongly enhance the anti-CD40 dependent B cell proliferation whereas IL-2 and LMW BCGF are ineffective in this system. After 9 days, 10-15 fold more B cells than input, can be recovered in the presence of IL-4 and Mab 89. Whereas B cells cultured without cytokines produce low Ig amounts, addition of IL-4 enhances IgG and IgM secretion and induces a strong production of IgE.

M 403 ESTABLISHMENT OF HUMAN LONG-TERM B-CELL CULTURES, DEVELOPMENT OF AN *IN-VITRO* PROGENITOR CELL ASSAY, AND THE ENRICHMENT FOR THE HUMAN B-CELL PROGENITOR, Charles Baum and Ann Tsukamoto. SyStemix, Inc. 3400 W. Bayshore Rd. Palo Alto, CA 94303

In the mouse, the Whitlock-Witte cultures have served a pivitol role in identifying B-cell progenitors and growth factors influencing the growth and development of these cells. The lack of the equivalent long-term culture systems for human B lymphocytes has hampered these kinds of studies. We report the successful establishment of long-term human B cell cultures. Cell suspensions from fetal and adult bone marrow have been used to initiate co-cultures which have continuously produced hematopoietic cells, including B-cells, for up to 20 weeks. Cytospin and FACS analysis show maintenance of human hematolymphoid cells. These cultures are a mixture of myeloid, monocytoid, and lymphoid lineages by morphology. Approximately 15-40% of the cells are mononuclear and have a lympnoid morphology. Approximately 10-40% of the cells staim with the CD15 antibody, 15-50% of the cells stain with the B lineage markers, CD10, CD19, and CD20. "Switch cultures" have been used in the mouse system to demonstrate the presence of a B-lymphoid progenitor in mouse Dexter cultures. Here we demonstrate that cultures that are initiated and maintained with hydrocortisone for approximately 4 weeks and show predominantly myeloid outgrowth may be switched to media without hydrocortisone, and are able to generate B-lymphoid as well as myeloid cells. Cultures initiated in the presence of hydrocortisone (Dexter-like conditions) have no detectable T or B cells (CD3,4,8,10,19 & 20 negative) and have a large percentage of granulocytes and myeloid cells. After switching to media without hydrocortisone for a minimum of 2 weeks, both CD10 and CD19 cells could be detected in the non-adherent cell population. The presence of mitotic figures and the long-term maintenance of these cultures indicates the presence of some active progenitor cell in these co-cultures.

M 404 THE EFFECT OF EPSTEIN-BARR VIRUS INFECTION ON EXPRESSION OF THE INTERLEUKIN 2 RECEPTOR α GENE IN B LYMPHOCYTES, Christine M. Bentivoglio and Douglas S. Lyles, Department of Microbiology and Immunology, Wake Forest University, Winston-Salem, NC 27103

Epstein-Barr virus (EBV) infection induces expression of the interleukin 2 receptor alpha chain (IL-2Rα) on a subclass of B lymphocytes. We have studied IL-2Rα gene expression in the EBV-negative Burkitt's lymphoma cell line, BJAB, and an EBV-infected subline, BJAB-B958, as a model for EBV induction of IL-2Rα. We have found that high levels of mRNA encoding IL-2Rα are expressed BJAB-B958 cells, while BJAB does not express levels of IL-2Rα mRNA detectable by Northern hybridization. The activity of the IL-2Rα gene promoter was assayed by transfection of a chloramphenicol acetyltransferase (CAT) expression plasmid containing the IL-2Rα promoter and 481 nucleotides of upstream sequences. Both cell lines expressed equally low levels of CAT activity compared to T cells induced with phorbol myristate acetate, indicating that sequences outside this region are responsible for the difference in IL-2Rα gene expression.

M 405 SIGNALLING MECHANISMS THROUGH MHC CLASS II MOLECULES INVOLVED IN B LYMPHOCYTE ACTIVATION. Bondada Subbarao and Arthur R. Baluyut, Center on Aging & Dept. of Microbiology & Immunology, Univ.of Kentucky, Lexington, KY 40536.

Our laboratory is investigating the role of MHC class II molecules in B cell activation. We have previously reported that anti-Ia antibodies enhanced anti-μ antibody induced B cell proliferation, thereby demonstrating that Ia antigens may function as signal transducer molecules. In correlation with this finding, we have now shown that anti-Ia antibodies can enhance the anti-μ induced transcription of c-myc protooncogene. We as well as other investigators have demonstrated that anti-Ia antibodies can induce an intracellular rise in cAMP. In this study we also report that anti-Ia antibodies, when cross-linked appropriately, can induce calcium mobilization in B cells. The cross-linking of other surface molecules such as B220 did not result in a similar calcium response. Anti-Ia antibody induced calcium mobilization appeared to be from extracellular sources. Furthermore, in preliminary studies we showed that an allo-Ia^b reactive T cell line also induced calcium mobilization in the B lymphocytes from BL/6 mice. (Supported in part by the NIH grants AI21490, AG05731 and K00422.)

M 406 TYROSINE KINASE PROTEINS EXPRESSED BY HUMAN PRE B CELL LEUKEMIA CELL LINES. Andrew W. Boyd, Evelyn Salvaris, Ian P. Wicks, Karen Welch and Jurgen Novotny,
Cellular Immunology Unit, Walter and Eliza Hall Institute, PO Royal Melbourne Hospital, 3050, Australia.

We have defined two proteins in pre B cell lines which have been shown to be tyrosine kinases. The first of these was isolated using a monoclonal antibody which defined a protein unique to one of these lines. Purification on antibody columns and sequence analysis show this to be a unique receptor-type tyrosine kinase. Gene cloning is now in progress to isolate full length cDNA clones. The second molecule was isolated by PCR of the kinase domain using cDNA from these pre B cell lines. A unique clone was identified by sequence analysis of the PCR product. Based on sequence data this molecule belongs to the SRC gene family of tyrosine kinases. On screening a panel of haemopoietic cell lines by Northern blot analysis this unique clone was restricted to the four pre B cell lines tested. This probe is now being used to clone a full length cDNA.

M 407 Lyl POSITIVE AND NEGATIVE B CELL CLONES RESPOND TO DISTINCT LYMPHONKINE COMBINATIONS AND DIFFER IN THEIR IL-2 RECEPTOR EXPRESSION, Kathryn H. Brooks, Department of Microbiology and Public Health, Michigan State University, E. Lansing, MI 48824. Two neoplastic B cell clones which differ in their expression of the Lyl molecule were evaluated for a differentiative gamma response to various lymphokines. The Lyl- 223-11 clone was not significantly stimulated by rIFNγ, rIL-2, rIL-4 or rIL-5 alone. These cells also did not respond to any combination of rIL-1, rIFNγ, and rIL-2. IgM secretion was induced by stimulation with both IL-4 and IL-5. In contrast, the Lyl+ BCL_1-3B3 clone was induced to secrete IgM in the presence of either IL-2 or IL-5. IgM secretion was not induced by rIFNγ, rIL-1, or rIL-4. The amount of IgM released by the BCL_1-3B3 cells was maximal in the presence of both IL-2 and IL-5. Thus, although both clones respond to IL-5, they differ in their ability to be costimulated by IL-2 and IL-4. Consistent with this pattern of response is the lack of high-affinity IL-2 receptors on the 225-11 cells.

M 408 RECOMBINANT INTERLEUKIN-4 UPREGULATES CD25 EXPRESSION IN QUIESCENT HUMAN TONSILLAR B LYMPHOCYTES. Robert D.J. Butcher, Gail M.McGarvie and William Cushley. Department of Biochemistry, Glasgow University, Glasgow, G12 8QQ, Scotland, U.K.

Recombinant human interleukin-4 (rhIL-4) induces CD25 (Tac) expression on up to 30% of tonsillar and adenoidal small, dense human B cells (CD19+) compared with 70-80% of the cells which upregulate mCD23 in response to IL-4 in the same cultures. Both CD25 and CD23 upregulation by IL-4 show a time and dose dependancy, with maximal expression after 48-60 hours, and both responses are inhibitable by IFN-γ. However, CD23 upregulation displays an EC50 of 5-8U/ml IL-4, whilst CD25 upregulation has an apparent EC50 of 32-40U/ml average.

Induction of CD25 on the quiescent B cell population could not be promoted by any cytokine other than IL-4. Furthermore, IL-4-driven CD25 upregulation appeared to be restricted to the B lymphocyte lineage. Thus, CD25 upregulation by IL-4 was also noted in the large bouyant B cell population, but could not be demonstrated in T cells (from the same source).

The phenotype of the B cells upregulating CD25 expression in response to IL-4 and the functional properties of IL-2 receptors on these cells will be discussed.

M 409 PATHWAYS OF HLA CLASS II MEDIATED SIGNAL TRANSDUCTION IN HUMAN B LYMPHOCYTES. **D. CHARRON, C. BRICK-GHANNAM, L.Y. JU, C. GRILLOT-COURVALIN, N. MOONEY.** Institut des Cordeliers - Paris - Aside from their role as recognition structures, we investigated the role of the MHC class II antigens in signal transduction in resting human B lymphocytes. Anti MHC class II mAbs induced a hydrolysis of PIP2. The augmentation of intracellular free calcium is classically described as an early event following PIP2 hydrolysis. We observed an augmentation of $(Ca^{++})i$ after cross-linking of soluble anti-MHC class II antibodies. Protein kinase C induced phosphorylation is part of its signal transduction pathway. New phosphoproteins were apparent on 2D SDS-PAGE after stimulation via class II molecules in addition to those observed after PMA stimulation.Proliferation of resting B lymphocytes in response to immobilized anti MHC class II mAbs was also observed. When resting B cells were incubated with anti MHC class II mAbs for up to 8 hours preceding stimulation with PMA, a significant decrease in the PMA induced stimulation was noticed providing further evidence for the use of a PKC mediated pathway of signal transduction for MHC class II mediated signaling. Recent data have demonstrated that the PKC mediated pathway represents only one route of signal transduction for α-IgM or α-IgD depending in which way the ligand is presented. This may well be the case for MHC class II antigens. We are therefore examining possible pathways used by different ligands mediating signals via MHC class II molecules.

M 410 Characterization of IgM and IgD Associated Proteins of Normal Murine B Cells and Modification of One of These Proteins in CBA/N (*xid*) Mouse, Jianzhu Chen, Leonard. A. Herzenberg and Leonore. A. Herzenberg, Department of Genetics, Stanford University, Stanford, CA 94305

We have identified proteins which noncovalently associate with IgM and/or IgD on normal murine B cells. After digitonin lysis of surface labelled cells, anti-μ immunoprecipitation reveals three proteins (Mr 34 to 42kd) which associate with IgM. Anti-δ immunoprecipitation reveals up to six proteins which associate with IgD (Mr 32 to 42kd). Both IgM and IgD share two proteins, whereas others are specific for either IgM or IgD. Furthermore, some proteins associated with IgM or IgD are shared among B cells from spleen, lymph node and peritonium, while others are tissue specific. A 42kd protein, which is present in all three tissues tested and associates with both IgM and IgD in BALB/C and CBA/Ca mice, is absent or exists in an altered form in CBA/N (*xid*) mouse. These previously undetected proteins are probably analogous to the accessory proteins in the CD3 complex of the T cell receptors and involved in signal transduction by Ig receptors. The difference between IgM and IgD associated proteins is consistent with the hypothesis that distinct antigenic signals are transduced by IgM and IgD during B cell activation.

M 411 CELL CYCLE ANALYSIS OF MOUSE B CELLS AND ES CELLS, U. Chen, Basel Institute for Immunology, Basel, Switzerland.

We have used the bivariate Brdu/Hoechst-ethidium bromide flow cytometric method to study cell cycle progression after activation of mouse B cells. This method enables us to visualize up to four consequent cell cycles. Small resting B cells were activated by bacterial lipopolysaccharide (LPS), anti-μ, and/or growth factors. Interleukin-4 (IL-4), IL-5, and Y-interferon (INF-Y) are active in this system. LPS causes ~ 15% of B cells to leave the resting stage, and the cells continue to progress through 4 cycles after 66-72 h stimulation. Some cells have already entered S phage by 18h. From 30 to 72 h, the average cell cycling time is 12 h, but a small subpopulation divides as fast as 6 h. Anti-μ alone stimulates ~ 40% of the cells to enter the cell cycle, and they are arrested at the G_1 phase in the second or third cycle. The combination of LPS + anti-μ activates 95% of resting B cells, suggesting a synergistic effect of these two stimuli. Both IL-4 and IL-5 are competence factors when used in combination with anti-μ. In addition, IL-4 and IFN-Y are S phase progressing factors. Unlike those activated with other stimuli, B cells activated with anti-μ + IL-4 were preferentially distributed in G_1 and S of the second and third cycles; few cells accumulate in G_2. This distribution is reminiscent of results obtained with embryonic fibroblasts. Thus, depending on the stimulus, adult B cells can behave either like other adult cells or like embryonic cells. We have applied the same method to study mouse embryonic stem cells (ES) grown in medium contianing growth factors. ES cells enter S phage 7-8 h after stimulation and have progressed through at least three subsequent cycles after 34 h stimulation. There are few cells in the G_2 phase. Interestingly, the preferential distribution of cycling ES cells in G_1 and S in reminiscent of the above results with anti-μ plus IL-4-stimuated B cells. Based on the above data, we propose a new model of B-cell activation. * This work was done with H. Seyschab & H. Höhn, University of Würzburg, West Germany and P. Rabinovitch, University of Washington, Seattle, USA.

M 412 SIGNAL TRANSDUCTION THROUGH THE HUMAN B-CELL ASSOCIATED SURFACE MOLECULE CD40, Edward A. Clark, Thomas B. Barrett, and Geraldine Shu, Dept of Microbiology SC-42, University of Washington, Seattle WA 98195

CD40 (Bp50) is a 43-47kDa phosphorylated glycoprotein expressed on human B lineage cells, follicular dendritic cells, normal basal epithelium, and some carcinomas and melanomas. The predicted sequence of a cDNA encoding CD40 has revealed that CD40 is closely related to human nerve growth factor (NGF) receptor. This homology and the potent growth factor-like activity of mAb to CD40 suggests that the ligand for CD40 may be a cytokine. MAb to CD40, while not triggering increases in [Ca2+]i in resting B cells, do increase phosphorylation of CD20 within min, as well as homotypic adhesion of B cells and new protein synthesis within hrs. The rapid induction of homotypic adhesion by CD40 requires new protein synthesis, is partially blocked by CD18 mAb, and is dependent on the presence of intact CD40 molecules: a murine B cell transformant expressing human CD40 can be induced to aggregate by ng/ml quantities of CD40 mAb, whereas a control transformant expressing similar levels of CD40 without its cytoplasmic tail is not affected by mAb. The effect of CD40 mAb on heterotypic adhesion is currently being studied. However, it is already clear that CD40 mAb also induce the production of IL-6 by B cells and increase the ability of resting B cells to trigger alloantigen-specific T cell activation. Furthermore, IL-6 induces increased phosphorylation of CD40 implying that CD40 may be able to transmit B cell-regulatory signals either after direct cross-linking or after signalling via IL-6 (Supported by NIH grants GM37905, DE08229, and RR00166).

M 413 **CLONING AND CHARACTERIZATION OF cDNA INVOLVED IN B CELL ACTIVATION,** Isabelle Cloutier, Thomas Leanderson, Michael Julius and Rafick-Pierre Sekaly, Laboratory of Immunology, Clinical Research Institute of Montreal, Montreal, Canada. H2W 1R7

The B lymphocytes express immunoglobulins (Ig) at their surface which are the specific receptor for an antigen (Ag). The interaction between these Ig and the specific Ag leads to the activation and differentiation of B cells. The Ig receptor share with the specific receptor of the T lymphocytes (TcR) a common evolutionary origin and structural homologies. It's possible that the receptor Ig, by analogy with the TcR, can be associated with proteins implicated in the transduction activation signals.
We have used the murine TcR/CD3-zeta probe to screen by Northern blot analysis poly A^+ RNA extracted from LPS blasts, B cells lines and hybridomas. At high stringency, we can detect, in these populations, transcripts with a different size from ζ. We have used this probe to pull out several clones from a LPS blast cDNA library. Nucleotide sequence analysis reveals that some of these clones have a high homology with the TcR associated CD3 ζ chain. Other clones, while crosshybridazing with the ζ probe show very little homology with this chain or the other CD3 chains. We are now trying to identify the protein encoded by these cDNA. With the recent demonstration that NK cells express CD3 ζ chain, it seems likely that this protein or homologous protein can also be present in B cells and exert signalling functions during B cell activation.

M 414 MECHANISMS OF SIGNAL TRANSDUCTION IN PHORBOL ESTER RESPONSIVE MURINE PERITONEAL B LYMPHOCYTES, Darrel P. Cohen and Thomas L. Rothstein, Departments of Microbiology and Medicine, Boston University School of Medicine, Boston, MA 02118

While it is well accepted that conventional murine B cells are stimulated to initiate DNA synthesis by the combination of a phorbol ester and a calcium ionophore, recent work from this laboratory has shown that Ly-1^+ B cells from the peritoneal cavities of BALB/c mice differ in that they are fully stimulated by the single signal provided by phorbol ester, acting alone (J Immunol 140:2880; J Immunol 141:4089). Several hypotheses that might explain the hyperresponsiveness of Ly-1^+ B cells to phorbol ester were tested. 1) Measurements using the Ca^{++} sensitive dye, Indo-1, showed baseline levels of intracellular Ca^{++} in peritoneal (PER) B cells are similar to those of conventional splenic (SPL) B cells, with no change resulting from phorbol ester treatment. 2) Measurements of protein kinase C (PKC) based on the in vitro phosphorylation of exogenous histone showed enzymatic activity in PER B cells to be about 2 times greater than that of SPL B cells on a per microgram protein basis. 3) The loss of PKC produced by phorbol ester treatment differed in the two cell types, so that after 4 hours, PER B cell membranes contained about 4 times as much PKC activity as SPL B cell membranes, and the same relationship held true for cytosolic fractions. These results suggest that differences in the amount and phorbol ester stimulated rate of loss of PKC contribute to the unusual phorbol ester hyperresponsiveness of Ly-1^+ B cells. Whether or not this reflects a unique intracellular distribution of PKC isoenzymes is the subject of current investigation.

M 415 DUAL CONTROL OF INTRACELLULAR pH IN HUMAN B LYMPHOCYTES BY BOTH Na^+/H^+ AND Cl^-/HCO_3^- ANTIPORTS. Isabelle Bourget, Dariush Farahifar, Nicole Grenier-Brossette and Jean-louis Cousin, INSERM U210, Faculté de Médecine (Pasteur) F06034 Nice cedex France.

Two mechanisms are involved in the regulation of the intracellular pH (pH_i) of B lymphocytes : The Na^+/H^+ antiporter and a Na^+-independent Cl^-/HCO_3^- exchanger. The Na^+/H^+ antiporter acts as an alkalinizing mechanism. It can be revealed, by pH_i recovery after acid-loading in a bicarbonate-free medium. This pH_i rise is inhibitable by amiloride or a Na^+-free medium. The Cl^-/HCO_3^- exchanger act as a cell acidifying mechanism. Bicarbonate turns on this antiporter which is inhibitable by anion transport inhibitors as disulfonic stilben derivatives or Cl^--free medium. Regulation of these two mechanisms will be discussed

M 416 INDUCTION OF ANTIBODY SYNTHESIS BY CD4+ T CELLS: IL-5 IS ESSENTIAL FOR INDUCTION OF ANTIGEN SPECIFIC ANTIBODY RESPONSES BY Th2 BUT NOT Th1 CLONES. R. H. DeKruyff, T. R. Mosmann, D. T. Umetsu. Stanford University, and the DNAX Research Institute.

It is known that the isotype of Ig produced by LPS stimulated B cells is regulated by lymphokine(s) present in the culture. However, factors regulating Ig synthesis induced in normal B cells under antigen driven MHC restricted conditions in the absence of LPS have not been fully elucidated. We and others have shown that under cognate conditions, CD4+ T cell clones of the Th1 subset, which produce IL-2 and IFN-γ, and T cell clones of the Th2 subset, which produce IL-4 and IL-5, are both capable of inducing anti-TNP IgG plaque forming cells (PFC).

We have examined in detail the lymphokine requirements for induction of Ig synthesis by B cells cultured under cognate conditions with Th1 and Th2 T cell clones. Using (1) Th2 clones that varied in the amount of IL-5 synthesized, (2) a neutralizing mAb against IL-5, and (3) T cell clones pretreated with cyclosporin A to inhibit lymphokine secretion, we found that IL-5 was essential for induction of IgG synthesis by Th2 T cells. Although we demonstrated that IL-2 could actually upregulate the synthesis of IL-5 by Th2 clones, the induction of Ig synthesis by Th2 clones was entirely independent of IL-2. In contrast, induction of IgG synthesis by Th1 clones was absolutely dependent upon the presence of IL-2, but was not affected by the presence of IL-5. With both Th1 and Th2 clones, we found that the predominant IgG subclass induced was IgG1. Thus, these studies support the idea that at least two independent pathways exist for the induction of IgG1 antibody synthesis: one IL-4/IL-5 dependent and the other IL-2 dependent.

M 417 SPECIFIC EXPRESSION OF A NOVEL TYROSINE KINASE GENE, blk, IN B LYMPHOID CELLS, Susan M. Dymecki+,#, John E. Niederhuber*,+, and Stephen V. Desiderio+,#, +Department of Molecular Biology and Genetics, #Howard Hughes Medical Institute Laboratory of Genetics and *Departments of Surgery and Oncology, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.

Several pathways of transmembrane signaling in lymphocytes involve protein-tyrosine phosphorylation. With the exception of p56lck, a T lymphoid tyrosine kinase of the Src family that specifically associates with the T cell transmembrane proteins CD4 and CD8, the kinases that function in these pathways are unknown. By screening a mixed B and T cell cDNA library with degenerate oligonucleotides derived from nucleotide sequences conserved among four tyrosine kinase genes (lck, v-abl, c-src, and v-yes), we isolated a murine lymphocyte cDNA that represents a new member of the src family. This cDNA, termed blk (for B lymphoid kinase), specifies a 55-kD polypeptide (p55blk) which is related to but distinct from previously identified products of retroviral or cellular genes. Overall, p55blk shows 61% and 63% amino acid sequence identity to the products of lck and hck, respectively. Upon its expression in bacterial cells, p55blk exhibits tyrosine kinase activity. In the normal mouse, blk is specifically expressed in the B cell lineage. Among cell lines, blk RNA was detected in pre-B cells, mature B cells, and a subset of pro-B cells, but not in non-B lineage cells. This pattern of expression distinguishes blk from all other members of the src family, and suggests that the tyrosine kinase encoded by blk functions as a B lymphoid-specific signal transduction element analogous to the T cell tyrosine kinase p56lck.

M 418 THE REGULATION OF ANTIGEN SPECIFIC ANTIBODY RESPONSE BY INTERLEUKIN 4. Kenneth H. Grabstein, Kurt Shanebeck, Timothy Sato, and Charles Maliszewski. Immunex Corporation, 51 University Street, Seattle, WA 98101

Interleukin 4 (IL-4) was found to strongly inhibit primary anti-SRBC IgM and IgA antibody production *in vitro*. Primary anti-TNP antibody responses were also inhibited by IL-4 as were secondary anti-hapten response *in vitro*. In spite of the inhibition of antigen specific antibody responses, IL-4 augmented polyclonal immunoglobulin secretion induced by IL-5. The effect of IL-4 on antigen specific and polyclonal immunoglobulin isotype expression will be discussed.

M 419 RETINOL COMPLEXED WITH SPECIFIC CARRIER PROTEINS IS REQUIRED FOR LYMPHOCYTE PROLIFERATION, Ulrich Hammerling, Annette Garbe, Sharon Abish and Jochen Buck, Department of Immunology, Memorial Sloan-Kettering Cancer Center, New York, NY 10021. Sixty years ago Wolbach and Howe (1929) described, among other effects, a severe lymphadenopathy as a consequence of dietary vitamin A withdrawal. We have discovered what might be the in-vitro correlate by showing that activated lymphocytes maintained in vitamin A-deficient medium fail to proliferate. This profound inhibition applies to both activated B and T lymphocytes of at least two species, mouse and man. The growth-promoting effect is not provoked by free retinol in the medium but is dependcent on the presence of specific proteins, i.e., prealbumin and retinol-binding protein. Among the retinoids that can form an active growth factor with the carrier are several derivatives of the alcohol and aldehyde forms of retinol, whereas retinoic acid, a morphogen and differentiation-inducing agent for a variety of cells, exhibited no growth factor activity for lymphocytes.

M 420 MECHANISM OF IL-4 MEDIATED ENHANCEMENT OF B CELL ACTIVATION BY ANTI-IGM ANTIBODIES. P.D. Hodgkin, N.F. Go, J. Cupp and M. Howard. DNAX Research Institute, Palo Alto, CA 94304

We have performed a multiparametric examination of the effects of IL-4 on B cell activation by goat anti-IgM antibodies. IL-4 has the dual effect on the anti-IgM dose response curve of reducing by 5-fold the concentration of anti-IgM required to give 50% activity, and of increasing the maximum response level by two-fold. This latter effect is completely explained by the ability of IL-4 to prevent B cell death in culture and thereby increase the number of responding cells. High dose anti-IgM (50 μg/ml) stimulates 65-70% of the viable cells into cell cycle - irrespective of whether or not IL-4 is present.

Anti-IgM stimulated B cell division, at any dose or B cell number, is limited to only a small number of division rounds. IL-4 does not alter the maximum number of cell divisions, but it does enhance the number of cells which enter the second round of cell division.

At 48 h, there is a linear relationship between the number of cells in S phase of the cell cycle and the ^{3}H-TdR uptake measured per culture. This correlation holds for cultures stimulated at different anti-IgM doses and in the presence and absence of IL-4. Thus, IL-4 does not enhance the thymidine uptake per stimulated cell, and lower concentrations of anti-IgM do not slow down the rate of passage through S phase of cycle. Also thymidine incorporation experiments can be directly interpreted as being proportional to the number of dividing cells in culture. These data are consistent with a simple model whereby the major role of IL-4 is to increase the sensitivity of resting B cells to the anti-Ig stimulus .

M 421 IMMUNOGLOBULIN CLASS SWITCH: CHARACTERISATION OF THE IL4-DEPENDENT CONTROL REGION OF Sγ1 GERMLINE TRANSCRIPTION, Harald Illges and Andreas Radbruch, Institute for Genetics, University of Cologne, Im Weyertal 121, D 5000 Köln 41, FRG The molecular basis for IL4 mediated directed class switching from murine IgM to IgG1 is probably the control, by IL4, of transcription of the "germline" sγ1 switch region. Sγ1 transcripts and a DNaseI hypersensitive site, covering the region of initiation, are rapidly induced by IL4. In addition, demethylation and DNA-binding proteins are observed at specific sites. The transcriptional control exerted by IL4 does not use any of the known immunoglobulin promotor or enhancer elements.

M 422 CHARACTERIZATION OF HUMAN INDUCING FACTOR (BIF) AND RECEPTOR EXPRESSED ON ACTIVATED HUMAN B LYMPHOCYTES AND B CELL LYMPHOMAS, Gajin Jeong, Jong Seon Choi, Do-Yong Jeon, and Sung So. Department of Microbiology, Seoul National University, Seoul 151-742, Korea. Human B cell inducing factor (BIF) was produced by normal peripheral blood T cells. BIF induces immunoglobulin secretion in purified peripheral blood B cell population that has been mitogenically stimulated by Staphylococcus aureus Cowan I strain (Sac), and in certain IgM, IgG, and IgA secreting cell lines. BIF was partially purified by FPLC system (Pharmacia) and followed gel filtration. M.W. was determined on SDS-PAGE and estimated to be about 20Kd, and it seems that BIF is highly glycosylated. When BIF was induced from activatd human T cells, tunicamycin (Sigma) blocked BIF production, so did swainsonine (Genzyme), but deoxymannojirimycin (Genzyme) could not. Glycosylation is essential for BIF activity, and it may explain the heat resistance of BIF molecule. When BIF was kept in boiling water for five minutes, at least two thirds of the activity still remained. Polyclonal antibody developed in rabbit against BIF receptor expressed on activated human B cells or cell lines such as SKW6.4 which has previously approved to constituitively express BIF receptor(s). Rabbit antisera adsorbed on normal human peripheral MNC agglutinated activated human B cells, SKW6.4, DAKIKI, and ARH77, implying B myeloma cell lines always have BIF receptor(s). Anti-IL-6 which was from Dr. Hirano of Osaka University, did not block BIF activity significantly. Receptor molecules after immunoprecipitation reveal a different pattern from that of IL-6. Neuraminidase treatment of BIF also decrease the activity. Comparison study of receptor molecule(s) from different cell lines, such as SKW6.4, DAKIKI, and ARH77, as well as from activated B cells are now in progress.

M 423 T-B CELL INTERACTIONS IN A SECONDARY IMMUNE RESPONSE: THE ROLE OF BYSTANDER ACTIVATION, Julia G. Johnson and Ronald Jemmerson, Department of Microbiology, University of Minnesota, Minneapolis, MN 55455. It is known that B cells can be activated into antibody production by two distinct pathways. One ("cognate") pathway requires direct MHC-restricted helper T cell interaction and thus linked antigenic stimulation, while the other ("bystander") pathway appears to be mediated by soluble T cell factors alone. The importance of bystander activation in the normal physiology of the immune system is unknown and therefore requires further examination. In the present study, bystander activation of secondary B cells was analyzed at the clonal level using the splenic fragment culture system. Cytochrome *c* (cyt *c*) primed B cells were adoptively transferred in limiting dilution to irradiated hemocyanin (Hy) primed recipient BALB/c mice. Splenic fragments were cultured the following day with either cyt *c* coupled to Hy, Hy alone, or no antigen. Of the cyt *c*-specific secondary B cells, 10-20% were activated to produce anti-cyt *c* antibody in the presence of the T cell antigen (Hy) alone. The antibody produced was neither isotype- nor epitope-restricted. The population of 10-20% B cells responsive to bystander activation may constitute a definable subset of secondary B cells which do not require cognate interaction, or they may be at a particular state of activation and thus competent to respond to T cell help alone. Alternatively, bystander activation could be explained by the availability of excessive T cell help such that those memory B cells which homed to fragments with the greatest number of Hy-primed T cells could be activated by the bystander pathway. The latter possibility was addressed by measuring IL-3 in each fragment culture as an indication of T cell activation and correlating this with the production of antibody. The evidence suggests that excessive T cell help is not responsible for the bystander activation of cyt *c*-primed B cells in splenic fragment cultures; rather, some characteristic of the B cell appears to determine its susceptibility to bystander activation. In support of this, preliminary results indicate that the most susceptible B cells are low density blast cells.

M 424 A NEW LYMPHOCYTE SPECIFIC PROTEIN, LSP1, CO-CAPS WITH sIg, J.Jongstra, D.P.Klein and J.Jongstra-Bilen, Dept. of Immunology, University of Toronto and the Toronto Western Hospital, 399 Bathurst Street, Toronto, Ont., Canada, M5T 2S8. We have isolated cDNA clones from mouse and human origin which code for a lymphocyte specific intracellular phosphoprotein, designated LSP1. In vitro studies show that recombinant LSP1 protein is Ca2+-binding and can serve as a substrate for protein kinase c (PKC). In vivo we have shown that TPA treatment of B-cell lines results in the rapid hyper-phosphorylation of LSP1 protein, presumably through the action of PKC. Immunofluorescence and subcellular fractionation experiments revealed that approximately 70% of the total cellular LSP1 protein is cytoplasmic, 15% is associated with the cytoskeleton and 15% is associated with the inner side of the plasma membrane. Double label immunofluorescence studies show that the intracellular LSP1 protein co-caps with sIg molecules after crosslinking of sIg with anti-Ig antibodies. The subcellular localization of LSP1 protein, in particular its co-capping with activated sIg, together with the biochemical characteristics of LSP1 such as Ca2+-binding and its role as a putative substrate for PKC, suggest strongly that this protein is part of the sIg signaling pathway.

M 425 B-CELL ACTIVATION AND DIFFERENTIATION MEDIATED BY ACTIVATED T_H-CELL MEMBRANES AND LYMPHOKINES, Marilyn R. Kehry, Leslie C. Yamashita, J. Alexander Appleman, and Philip D. Hodgkin, Department of Immunology, DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, CA 94304. Plasma membrane-enriched fractions from activated T_H1 and T_H2 cells have been found to induce activation and proliferation of dense B lymphocytes. Between 60% and 70% of small dense B cells were activated by T_H membranes, and the mode of B-cell activation was not antigen dependent or MHC restricted. Membranes from resting T_H cells, $P388D_1$, P815, or a B-cell line were ineffective in activating B cells. The appearance of B-cell stimulatory activity in the T_H membranes peaked at 6 hr after T_H cell activation and was inhibited by cycloheximide or cyclosporin A. B-cell proliferation induced by T_H membranes was not inhibited by anti-IL2, anti-IL4, anti-IL5, anti-IL6 or anti-IFNγ. B cells activated by T_H membranes were unable to synthesize immunoglobulin unless lymphokines were included in the cultures. Immunoglobulin production by B cells stimulated with T_H membranes and T_H supernatants closely resembled that induced by intact T_H cells and antigen. The pattern of isotypes produced varied according to the particular lymphokines present. This supports a model for T-B cell collaboration whereby antigen stimulation of the T_H cell induces the expression of a new T_H membrane component that drives proliferation of the B cell; concomitant lymphokine production drives B-cell differentiation and antibody production.

M 426 CROSS-TALK BETWEEN IL-4 AND SURFACE IG RECEPTORS: ROLE OF PROTEIN KINASE C, Gerry G.B. Klaus and Margaret M. Harnett, Division of Immunology, National Institute for Medical Research, Mill Hill, London NW7, Great Britain.
A classical property of IL-4 is its capacity to stimulate resting murine B cells to synthesize DNA in conjunction with submitogenic doses of anti-Ig. In order to study which of the known second messengers elicited by stimulation of sIg receptors synergizes with signals emanating from IL-4 receptors, B cells were cultured with IL-4 and protein kinase C (PKC)-activated phorbol esters (such as PDB) in the presence or absence of ionomycin. Carefully titrated combinations of PDB + IL-4 induce substantial DNA synthesis in B cells, whereas ionomycin + IL-4 do not. IL-4 substantially reverses the PDB-mediated inhibition of DNA synthesis stimulated by mitogenic doses of anti-Ig. It also overcomes the inhibition of B cell activation resulting from down-regulation of PKC by pretreatment with phorbol esters. Thus far we have been unable to detect any effects of IL-4 on the inhibition of anti-Ig-induced phosphoinositide hydrolysis by PKC activators. Nevertheless, these results point to a central role for PKC in the crosstalk between IL-4 and sIg receptors in the activation of resting B cells.

M 427 A B CELL FACTOR WHICH SIGNALS THE EXPANSION OF PRECURSOR MEMORY B CELLS. Peter H. Koo, Department of Microbiology and Immunology, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272.
An immunostimulatory factor (IST) has been isolated and purified from the ascitic fluid of X-irradiated DBA/2 mice bearing a spontaneously regressing subline of murine L1210 lymphoma. IST is found to be associated with spontaneous regression of L1210 lymphoma in $CD2F_1$ hybrids or DBA/2 mice. IST can potently stimulate the response of secondary antibodies of various immunoglobulin classes and subclasses, without affecting primary antibody response whatsoever, if it is injected to animals before primary immunization. IST injected after primary immunization has no effect on the memory response. Even those animals which were treated with IST one month before secondary immunization still produced enhanced secondary antibody response in comparison with the control animal treated with saline solution instead. IST, however, does not stimulate primary or secondary cytotoxic T cell response, natural killer activity, or contact hypersensitivity reaction. We conclude that IST may directly or indirectly signal at least a subpopulation of precursor memory B cells to expand. Once memory B cell is formed, it is no longer responsive of IST.

M 428 MOLECULAR NATURE OF AN Fcμ RECEPTOR ON HUMAN B-LINEAGE CELLS, T. Ohno, H. Kubagawa, S. K. Sanders, and M. D. Cooper, University of Alabama at Birmingham and the Howard Hughes Medical Institute, Birmingham, AL 35294.
An IgM receptor (IgM-R), a single polypeptide chain of M_r 60,000, was identified on B cells within 18 hr following activation with phorbol myristate acetate (PMA). Resting and PMA-activated T cells, monocytes and granulocytes did not express detectable amounts of the IgM-R. The IgM-R could be detected on pre-B and B cell lines, as well as their leukemic counterparts (including cytoplasmic μ^- common ALL cells and isotype-switched CLL cells), suggesting no direct association with the cell surface expression of IgM molecules. The level of IgM-R expression was increased after activation with PMA and incubation with IgM. The IgM-R molecules from both pre-B and B cell origins were identical in size, had O-linked carbohydrate moieties, an acidic pI (6.0-6.5), and appeared to be anchored to the cell membrane through a glycosylphosphatidylinositol linkage. The binding of IgM to the 60 kDa cell surface receptor was isotype specific and mediated via the Fcμ portion, probably the C_H3 and/or C_H4 domain. Sialic acid residues on the IgM-R were important for IgM binding. We conclude that this 60 kDa Fcμ receptor is a B-lineage specific activation marker that can be expressed on the cell surface as early as the pre-B cell stage in differentiation. (NIH grants CA16673 and CA 13148; MDC is a HHMI investigator.)

M 429 **ANTI-IMMUNOGLOBULIN PRETREATMENT INDUCES A CALCIUM MOBILIZATION RESPONSE TO THE CHEMOTACTIC AGENT N-FORMYL-MET-LEU-PHE IN DAUDI LYMPHOBLASTOID CELLS**, Pramod M. Lad, John S. Kaptein, Ching-Kow Lin, Cosmas I. Kalunta, and Stephen J. Scott, Regional Research Laboratory, Kaiser Permanente Medical Center, 1515 N. Vermont Avenue, Los Angeles, CA 90027.
Anti-immunoglobulin (anti-Ig) treatment of Daudi cells induces a calcium mobilization. The chemotactic agent f-met-leu-phe (FMLP) does not cause calcium mobilization, however exposure of the cells to FMLP after the first hit with anti-Ig shows a rapid, FMLP dose-dependent, calcium mobilization. The expression of the FMLP-dependent response occurs in less than two minutes and is stable. Binding of [^{3}H]-FMLP is increased in anti-Ig-treated but not control cells. The induction is specific for FMLP since another chemoattractant, platelet activating factor, did not mobilize calcium. The FMLP antagonist t-BOC-phe-leu-phe-leu-phe did not mobilize calcium on its own, either before or following anti-Ig treatment, but inhibited the calcium mobilization at low doses of FMLP. Treatment of the cells with phorbol-12-myristate-13-acetate or pertussis toxin prior to anti-Ig treatment caused a dose-dependent abolition of both the anti-Ig-mediated calcium mobilization and the subsequent FMLP-dependent calcium mobilization. Metabolic inhibitors which act predominantly by lowering the ATP levels within the cell (iodoacetate, sodium fluoride, oligomycin and 2-deoxyglucose) all produced a greater inhibition of the FMLP-mediated calcium mobilization than the anti-Ig-mediated response. Lowering the temperature from 37°C to 22°C reduced the anti-Ig response and completely inhibited the expression of the FMLP effect. Our results indicate that **activation of the calcium-mobilization pathway in B cells by crosslinking of surface immunoglobulin causes an induction of FMLP-sensitive calcium mobilization.**

M 430 THE EXPRESSION OF NF-kB DURING MURINE B LYMPHOCYTE ACTIVATION, Jialing Liu, Thomas C. Chiles, and Thomas L. Rothstein, Department of Microbiology and Medicine, Boston University School of Medicine, Boston, MA 02118
The nuclear factor NF-kB, crucial for expression of the kappa light-chain immunoglobulin gene, also plays a role in the expression of several other eucaryotic genes, but little is known about the role of NF-kB during normal murine B lymphocyte activation. By EMSA (Electrophoretic Mobility Shift Assay) and specific competition, we have observed a low level of nuclear expression of NF-kB in resting murine B cells. Nuclear NF-kB expressions can be induced to a higher level by several mitogenic stimuli including LPS, PMA, $F(ab)'_2$ fragments of anti-IgM, and PMA plus ionomycin. Time course experiments demonstrate that the kinetics and magnitude of stimulation is different among these agents. Further studies will help to understand the regulation and role of NF-kB in B cell mitogenesis.

M 431 INTERLEUKIN-4 INDUCES SYNTHESIS OF IgE AND IgG4 IN HUMAN B CELLS, Mats Lundgren, Ulla Persson, Edvard Smith, Lennart Hammarström and Eva Severinson, Dept. of Immunology, Stockholm University, 106 91 Stockholm, Sweden. Interleukin 4(IL-4) has been shown to regulate the IgG subclasses and induce IgE production in splenic mouse B cell. Here we show that IL-4 and phorbol-12-myristate 13-acetate (PMA) induce, on a per cell basis, very high IgE secretion in purified human B cells by using a mouse thymoma (EL-4) co-culture method. In addition, a marked increase in the number of IgG4 producing cells was also observed. Furthermore IL-2 could synergize with IL-4 and PMA in the production of IgE. By using limiting dilution analysis, a considerable increase in the precursor frequency for IgE was found when IL-4 and PMA were added to cultures as compared to cultures with PMA only. This indicates that IL-4 induces an isotype switch in human B cells.

M 432 THYMUS-INDEPENDENT RESPONSES TO NATIVE PROTEIN EPITOPES. Michael Mage and Bernardetta Nardelli, Laboratory of Bichemistry, National Cancer Institute, Bethesda MD 20892. We are investigating the immune response to defined polymer-protein conjugates, because complexes of proteins with cationic polymers have been reported to function as T independent antigens, and because T independent responses might be useful when protein antigens are deficient in T helper peptide sequences, or when T helper cells have been destroyed by disease. We recently introduced polyacrylamide-streptavidin (PASA) as a reagent for the construction of soluble multivalent macromolecular conjugates (Nardelli et al., J. Imm. Methods 1989, 120:233-239). We immunized nude and conventional mice with PASA, using molecules of approximately 2 million daltons, containing an average of 20 streptavidin molecules attached to each soluble linear polyacrylamide molecule of 1 million daltons. Doses ranged from 0.01 microgram to 5 micrograms, given IM with incomplete Freund's adjuvant. Both groups of mice, at all doses, developed an IgM antibody response to Streptavidin but not to polyacrylamide as measured by ELISA assays. On boosting with the same antigen by the same route, the conventional but not the nude mice also developed IgG antibodies to streptavidin.

M 433 SOLUBLE CYTOKINE RECEPTORS SPECIFICALLY INHIBIT IL-1 AND IL-4 INDUCED B CELL ACTIVITIES, Charles R. Maliszewski, Timothy A. Sato, and Kenneth H. Grabstein, Department of Immunology, Immunex Corporation, Seattle, WA 98101.

The cell surface receptors for IL-1 and IL-4 have recently been cloned and recombinant receptors have been produced that bind their respective ligand in soluble form. The ability of soluble forms of the murine IL-1 receptor (sIL-1R) and IL-4 receptor (sIL-4R) to inhibit B cell functions *in vitro* was examined. Proliferation of B cells treated with anti-immunoglobulin plus IL-1 or IL-4 was inhibited by the appropriate soluble receptor. SIL-4R also inhibited IL-4 dependent B cell differentiation, as measured by: induction of IgG1 and IgE secretion by LPS blasts; downregulation of IgG3 secretion by LPS blasts; increased Ia expression; and increased CD23 expression. The inhibitory effects of the soluble receptors were found to be highly specific in that sIL-4R had no effect on IL-1-induced B cell activity and sIL-1R had no effect on IL-4 activity. These results have prompted studies on the possible *in vivo* inhibitory effects of the soluble receptors.

M 434 SUPEROXIDE GENERATION OF B LYMPHOCYTES IS UPREGULATED IN BLASTS AND REGULATED VIA CELL SURFACE STRUCTURES. Friedrich - E. Maly, Adrian Urwyler and Alain L. de Weck, Institute of Clinical Immunology, University of Bern, Switzerland.
We showed earlier that human B lymphocytes express an NADPH oxidase - type oxidase that can generate reactive oxygen species like superoxide. Here, we evaluated the behaviour of B cell oxidase activity in normal purified B cells (over 95% B) driven to blast transformation by PMA and Ionomycin. Both agents "primed" B cells for enhanced oxidase activity in response to cross-linking of sIgM. Maximal effects (10-100 fold increases in oxidase activity) were found when B cells were cultured with intermediate doses of both agents. Such cells showed no oxidative burst when exposed to particles like opsonized zymosan or to PMA. Co-cross-linking of sIgM with HLA-DR enhanced oxidase activation further 3-fold, while co-cross-linking of sIgM with CD5 completely abolished oxidase activation. We conclude that B lymphocyte blast transformation is associated with strongly increased activity of the B cell oxidase which is subject to regulation via non-Ig surface structures. Thus, measurement of B cell oxidase activity may be useful to determine the state of activation of B cells. Particularly strong oxidase activation by cross-linking of sIgM with HLA-DR suggests the B cell oxidase may be active when B cells present antigen to T cells.

M 435 PROTEIN KINASE C INVOLVEMENT IN Th CONTACT-DEPENDENT, LYMPHOKINE-INDEPENDENT B CELL ACTIVATION. Lisa S. Marshall and Randolph J. Noelle, Ph. D. Department of Biochemistry, Dartmouth Medical School, Hanover, NH.

We have developed a system to investigate the early biochemical events induced in B cells as a consequence of direct interaction with Th. In this system, anti-T3 activated, fixed Th are used to elicit B cell biochemical events. Earlier studies showed that anti-T3 activated, fixed Th cells induced B cell RNA synthesis in a lymphokine-independent manner. Employing specific inhibitors of protein kinase C, (PKC), we have shown that PKC activation is likely involved in mediating the signalling of B cell cycle entry induced by Th-B cell contact. At present, we are in vivo labelling B cell internal phosphate stores to locate a membrane substrate that is phosphorylated by PKC as a result of Th contact-dependent B cell activation. Future studies include the monitoring of phosphatidyl inositol turnover and calcium mobilization, the in vitro labelling of phosphate stores as well as the use of PKC inhibitors to inhibit Th-B cell interactions. This system should allow the identification of the B cell second messenger systems that are involved as a consequence of Th-B cell interaction.

M 436 COGNATE INTERACTIONS: INDUCTION OF B CELL CYCLE ENTRY BY PURIFIED PLASMA MEMBRANE VESICLES FROM ACTIVATED T CELLS. Randolph J. Noelle, Ph.D. and William Bartlett. Department of Microbiology, Dartmouth Medical School, Hanover, NH After activation with anti-T3, antigen-specific helper T cell (Th) clones express cell surface molecules that: 1) mediate unrestricted conjugate formation with resting B cells and 2) trigger B cell entry into the G_1 phase of the cell cycle. Conjugate formation between resting B cells and *resting*, antigen-specific Th clones was class II MHC restricted and antigen-specific. After activation of Th clones with anti-T3, the *activated* Th (Th^{T3}) formed physical conjugates with B cells in a class II-unrestricted and antigen-nonspecific manner. Mab blocking studies showed that the binding of CD4 on Th^{T3} to monomorphic domains of class II MHC antigens on B cells was likely involved in the stable conjugate formation between Th^{T3} and B cells.
After activation with anti-T3, Th^{T3}, but not resting Th, fixed with paraformaldehyde induced B cell RNA synthesis when co-cultured with resting B cells. It is proposed that anti-T3 activation of Th resulted in the expression of Th membrane proteins that triggered B cell cycle entry. Kinetic studies revealed that 2-8 hrs of activation with anti-T3 was sufficient for Th^{T3} to express B cell activating function. However, activation of Th with anti-T3 for extended periods of time resulted in reduced effector Th activity. Inhibition of Th RNA synthesis during the activation period with anti-T3 completely ablated the ability of Th^{T3} to induce B cell cycle entry. This indicated that *de novo* synthesis of membrane proteins was required for Th^{T3} to express effector function. The ability of fixed $Th3^{T3}$ to induce entry of B cells into cycle was not due to an increase in expression of CD3, CD4, LFA-1, ICAM-1, class I MHC or Thy-1 on the Th^{T3}. Taken together, these data indicate that CD4 and the *de novo* expression of other Th surface proteins are required for activated Th to induce B cell cycle entry into G_1. Other forms of Th activation (PMA and A23187, Con A) also induced Th effector function. As predicted from the results with fixed Th, purified, purified plasma membrane vesicles (PMV) from activated, cloned Th lines also induced B cell cycle entry. Induction of B cell cycle entry by PMV from activated Th was ablated by anti-CD4, but not anti CD3 MAbs.

M 437 Structural requirements of the membrane IgM molecule for signalling in mature B-cells. V.S.Parikh ,C.Nakai, and P.W.Tucker. Department of Microbiology, UTSMC at Dallas, Dallas,TX.

The carboxyl-terminus of the IgM molecule is essential for transmitting a tolerogenic signal(Webb, et al).This was shown by creating T15-idiotype specific transfectants which expressed either wild-type or hybrid IgM molecules in which the μ-chain C-terminus was replaced with that of other heavy chains (δ,γ2b,α)and an I-A-alpha chain.All hybrids except μ-I-A delivered a negative signal in CH33 cells. When transfected into another cellline,M12.4,this μ-I-A hybrid was also defective in PC-KLH driven Ca++ mobilization although it effectively mediated endocytosis in the same cells.We have created other hybrid constructs where the various segments of the I-A C-terminus (eg,spacer,transmembranal,cytoplasmic residues) are replaced by that of μ-molecule to determine which domains are essential for signalling. We have also created point mutations in the transmembrane and cytoplasmic domains of IgM molecule to pin-point the amino acid residue(s) involved.The effect of these various mutations on changes in intracellular Ca++ levels,growth inhibition and the antigen-induced expression of various genes will be discussed.

Webb et al.(1989)PNAS.86:1977

M 438 DIFFERENTIAL RESPONSIVENESS OF INTESTINAL B CELLS TO MITOGENIC AND ANTIGENIC STIMULI. Greg A. Perry and Marlene K. Shinn, Department of Anatomy, Univ. Nebraska Medical Center, Omaha NE 68105.

B cells of the intestinal tract have been considered by many to be uniform in their antigenic and mitogenic response capacity. However, experiments by Reynolds have demonstrated that the terminal ileal Peyer's patch of sheep is a primary site of B cell differentiation and thus differs from the more proximal Peyer's patches. In the mouse and rat, we have previously described a differential susceptibility of intestinal lymphoid nodules to the lympholytic effects of in vivo hydrocortisone treatment. In this report we examine the differential responsiveness of murine intestinal B cells to both mitogenic and antigenic stimuli. B cells were isolated from murine spleen, Peyer's patches (jejunal and/or terminal ileal), proximal colonic lymphoid tissue (PCLT) and distal colonic lymphoid tissue and tested for their ability to respond to mitogenic (LPS) and antigenic (SRBC, BrMRBC) challenge. A distinct pattern was observed in the ability of B cells from these tissues to proliferate in response to LPS. B cells from the jejunal Peyer's patch responded much like splenic B cells. B cells from the terminal ileal Peyer's patch demonstrated a significantly lower responsiveness compared to jejunal Peyer's patch or spleen, while the PCLT derived B cells showed little or no response. However, B cells from the distal colonic lymphoid nodules again responded much like spleen. This pattern of responsiveness inversely correlates to the previously published patterns of E.coli colonization of the intestine. Results of both SRBC and BrMRBC plaque forming cell (PFC) assays demonstrated that the PCLT contained approximately 30 fold more endogenous PFC than any other tissue examined, and that this PFC frequency was not changed with immunization. These results correlate with the high frequency of $CD5^+$ B cells in PCLT. Taken together, these data strongly suggest that the that the mature, immunologically responsive peripheral B cell pool is not uniform in its regional tissue distributionin the gut. (Supported by AI25222 & AI25820)

M 439 CD19 IS A SECOND COMPONENT OF THE ANTIGEN RECEPTOR COMPLEX ON B CELLS, John M. Pesando, Lisa S. Bouchard, and Brian E. McMaster, Division of Clinical Immunology, The Biomembrane Institute, Seattle Wa 98119. The highly conserved transmembrane sequences of sIgs suggest that these molecules may associate with other integral membrane proteins to execute their dual functions of ligand binding and signal transduction. We now demonstrate that the pan-B and B cell-specific CD19 surface antigen is functionally and physically associated with sIg. Antibodies to sIg induce rapid, specific, concentration dependent, and reversible comodulation of CD19 on all sIg+ B cells. Comodulation is caused by MAb specific for the gamma, mu, kappa, and lambda chains of sIg and by at least one idiotype-specific MAb. Of 18 surface antigens studied, only CD19 comodulates. Comodulation is observed using 15 CD19-specific MAb that detect at least three different CD19 epitopes. Loss of sIg and CD19 occurs concurrently during anti-Ig modulation, suggesting that these are parallel rather than serial events. Studies with directly labelled antibodies indicate that anti-Ig specifically cocaps and internalizes anti-CD19 antibody. Using radiolabelled CD19 antigens or radiolabelled anti-CD19 MAb, we find that both antigen and antibody are rapidly and internalized and degraded during anti-Ig-induced comodulation. CD19 is a phosphoglycoprotein. While anti-Ig comodulates CD19, the reverse is not true, suggesting that ligands induce a change in sIg, probably conformational, to enable it to bind CD19. The long cytoplasmic tail of CD19 (148 AA) relative to that of sIg (4 AA for sIgM) suggests that CD19 may facilitate signal tranduction when antigen is bound to CD19.

M 440 THE PLASMA MEMBRANE ENZYME 5'-ECTO-NUCLEOTIDASE (CD73) REGULATES B CELL ACTIVATION, Antonio Pezzutto*, Linda F. Thompson#, Bernd Dörken*, Kevin Draves+ and Edward A. Clark+, *Dept. of Internal Medicine, Uni. of Heidelberg, FRG, #Oklahoma Med. Res. Foundation, Oklahoma City, OK 73104, and +Dept. of Microbiology/Regional Primate Res. Ctr., Uni. of Washington, Seattle WA 98195.
The CD73 plasma membrane-associated enzyme, 5'-ecto-nucleotidase is expressed on a small subpopulation of human T cells and on a larger fraction of normal human B cells. Using two CD73 specific mAb, 1E9 and 7G2, we evalulated the role of CD73 in B cell activation and proliferation. Both CD73 mAb induce a fraction of tonsillar or blood B cells to leave G0 and enter the G1 phase of the cell cycle. After CD73 antibody binding, a discrete subset of B cells is induced to express CD69 (Ea-1, Leu23, activation-induced molecule), CD71 (transferrin receptor), and CD25 (p55 IL-2 receptor) and to increase expression of Class II MHC. CD73 mAb costimulate with IL-2, with IL-4, with CD40 mAb, and with a low molecular weight B cell growth factor (LMW-BCGF) but not with anti-Ig sera, with CD20 mAb, or with SAC. Thus, signalling via CD73 mediates an activation/competence signal rahter than a progression signal in B cells. CD73 mAb can stimulate cell surface changes and 3H-thymidine uptake in buoyant tonsillar B cells (possible in vivo activated B cells), yet they do not stimulate in vitro activated B cells. CD73 mAb also stimulate increased 3H-thymidine uptake in malignant B cells obtained from patients with chronic lymphoid malignancies. Unlike other competence signals such as anti-Ig, SAC or PMA which stimulate the majority of B cells, CD73 apparently primarily affects only a subset of B cells. Characterization of this subset and the signal transduction pathway induced via CD73 are currently under evaluation. (Supported by Tumor Zentrum Heidelberg Mannheim and NIH grants GM37905, DE08229).

M 441 MOLECULAR COMPONENTS OF THE B-CELL ANTIGEN RECEPTORS OF CLASS IgM AND IgD, Michael Reth, Joachim Hombach, Peter Weiser and Jürgen Wienands, Max-Planck-Institut für Immunbiologie, Stübeweg 51, 7800 Freiburg-Zähringen, West-Germany. Two classes of immunoglobulin, IgM and IgD, are present as antigen receptors on the surface of mature B-lymphocytes. How these membrane-bound Ig chains which lack a cytoplasmic tail generate a cell activation signal is presently unknown. We have found that IgM molecules are non-covalently associated in the B-cell membrane with a disulfide-linked heterodimer consisting of a 34Kd and a 39Kd glycoprotein. The 34Kd protein seems to be the product of mb-1, a B-cell-specific gene encoding a transmembrane protein with an evolutionary conserved cytoplasmic tail. IgM molecules are brought onto the B-cell surface only if complexed with the heterodimer suggesting that the heterodimer is a structural component of the IgM antigen receptor. Indeed both proteins of the heterodimer were labeled by surface iodination of sIgM expressing B-cells. The antigen receptor of class IgD contains also a heterodimer which differs from that of the IgM antigen receptor in one of its components. This structural finding may help to elucidate the function of the two antigen receptors on a mature B-cell.

M 442 TRANSMEMBRANE SIGNALLING VIA THE IL-4 RECEPTOR Kevin Rigley*, M. Finney**, B. Dugas@, R. Callard** and J. Gordon** *Division of Immunology, Institute of Child Health, London W.1, **Dept. of Immunology, Medical School, Birmingham University, Birmingham, B15 2TT, U.K. @ Institute Henri Beaufour, 1 Avenue des Tropiques 91952 Les Ulis, France. Whilst the biological effects of Interleukin-4 have been widely documented the biochemical basis of signal transduction by this important lymphokine remain poorly understood. We now describe a novel signalling pathway that embraces both the activation of the polyphosphoinositide-phosphodiesterase (PPI-PDE) and adenylate cyclase (AD). Uniquely, the activation of the PPI-PDE is essential for the stimulation of AD. These biochemical processes can be mimicked using pharmacological agents which also invoke similar biological effects to those induced by IL-4.

M 443 ESTABLISHMENT OF NORMAL FACTOR DEPENDENT HUMAN B CELL LINES WITH ANTI-CD40 MAB AND CYTOKINES, Françoise Rousset, Eric Garcia, Catherine Peronne, Paolo de Paoli, Alain Vallé and Jacques Banchereau, UNICET, Laboratory for Immunological Research, 27 chemin des peupliers, 69570 Dardilly, France.

Resting human B lymphocytes can be induced to strongly proliferate when cultured in the presence of crosslinked anti-CD40 Mabs. Cytokines such as IL-4, IL-6 and IFN-γ enhance the anti-CD40 induced proliferation. 10^5 purified B cells were cultured with 0.5 µg/ml Mab 89 and 100 U/ml IL-4. After 9 days and one split at day 5, approximately 10 times the input B cells is recovered. Upon regular splitting of the cultures every 5-7 day, a cumulative expansion of 150-400 fold the input B cells can be reached after 35 days (mean doubling time : 33 h). Removal of IL-4 and anti-CD40 antibody halted cell proliferation and resulted in cell death after 7 days. These factor dependent B cell lines could be frozen/thawed and have been maintained for up to ten weeks. They are devoid of EBNA 2 antigen as determined by immunofluorescence staining. Such lines can be generated with resting B cells isolated from peripheral blood, cord blood, tonsils and spleen. B cell lines can be generated from as little as 100 resting B cells. They produce IgG, IgA, IgM and IgE. These normal factor dependent human B cell lines should prove useful 1) to determine the eventual ligand specific for CD40, 2) to study B cell proliferation and differentiation, 3) to study interactions of activated normal T and B cells, 4) to generate human monoclonal antibodies.

M 444 **CHARACTERIZATION OF THE 67 KDa RECEPTOR FOR INTERLEUKIN 1 ON THE HUMAN B CELL LYMPHOMA RAJI** G. Scapigliati, P. Ghiara, P. Bossu', D. Armellini, S. Nuti, D. Boraschi and A. Tagliabue. Laboratory of Immunopharmacology, Sclavo Research Center, Siena, Italy.
Human B cells and murine T cells possess structurally different IL-1 receptors (IL-1R) that can discriminate between IL-1α and IL-1β. We have purified the 67 kDa IL-1R polypeptide from homogenates of dexamethasone stimulated RAJI cells by isopycnic sedimentation of plasma membranes, preparative SDS-PAGE followed by electroelution and affinity chromatography on immobilized IL-1β. The IL-1R detected in RAJI cell homogenates by using a nitrocellulose filter assay and ligand blotting analysis binds almost exclusively ^{125}I-IL-1β and not ^{125}I-IL-1α. This binding is abolished by 2-mercaptoethanol and unaffected by 0.1% SDS. IL-1R enriched fractions from RAJI plasma membranes have been used as antigens in mice to obtain monoclonal antibodies, and screening of obtained hybridomas is currently in progress. RAJI cells have been cloned by limiting dilution and clones with high binding capacity for ^{125}I-IL-1β were obtained. Two clones (3D4 and 4A3), showing the highest binding, also show an high level of the lymphocyte membrane molecule CD69, that has been reported to be involved in the IL-1-dependent activation of lymphocytes. Work is in progress to better elucidate the molecular structure of the 67 kDa IL-1R, and to relate the presence of the receptor to possible biological effects exerted by IL-1 on B cells.

M 445 A $CD5^+$ B CELL HYBRIDOMA DERIVED FACTOR(S) WHICH INDUCES MATURATION OF $CD5^+$, AUTOIMMUNE B CELL POPULATIONS, Sherr D.H. and J. Hardin, Department of Pathology, Harvard Medical School, Boston, MA 02115
A number of investigators have demonstrated the association of $CD5^+$ (Ly-1/Leu-1) B cells with autoimmunity, excessive B cell proliferation, and transformation. Therefore, it was of particular interest to evaluate the factors which contribute to $CD5^+$ B cell maturation. The possibility that $CD5^+$ B cells themselves produce a factor(s) capable of influencing their own development was the focus of the present investigation. Autoimmune $CD5^+$ B cells were shown to be induced from $CD5^-$ B cell populations following culture with a 19-22kd factor(s) derived from a $CD5^+$ B hybridoma. Functional and molecular analyses suggest that this $CD5^+$ B cell inducing activity is not mediated by IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IFN-gamma, GM-CSF, and/or TNF. The role that such a B cell derived, B cell directed factor may play in immunity and disease is discussed.

M 446 **RECOMBINANT LMW-BCGF DOES NOT POSSESS CD23 CLEAVING ACTIVITY** J.G. Shields, S. Henchoz, A. Proudfoot, S. Sharma(*) and J.Y. Bonnefoy, Glaxo IMB, 46 Rte des Acacias, 1211 Geneva 24, Switzerland and (*)Roger Williams General Hosp-Brown University, Providence, RI 02908. Low molecular weight B cell growth factor (LMW-BCGF) is one of a number of cytokines which shows proliferative activity on activated human B lymphocytes and certain B cell lines. A partially purified preparation of LMW-BCGF also contains a second activity - cleavage of membrane CD23 expressed on B cells (Gordon & Guy, (1987) PNAS 84: 6239). The soluble CD23 thus produced is also capable of acting as a B cell growth factor. We sought to determine whether the BCGF and CD23-cleaving activities were mediated by the same molecule. E. Coli derived recombinant LMW-BCGF (Sharma et al, (1987) Science 235: 1489) had the ability to stimulate proliferation of B cell lines (HFB1 and P3HR1) and PMA-activated normal B cells. The same material was unable to reduce the expression of CD23 on IL-4 stimulated B cells. Thus recombinant LMW-BCGF appears not to have both activities ascribed to the partially purified natural LMW-BCGF. Work is currently in progress to separate the two activities from the natural source.

M 447 **INTERLEUKIN 4 INDUCES SELECTIVE PRODUCTION OF INTERLEUKIN 6 FROM NORMAL HUMAN B LYMPHOCYTES**, Erlend B. Smeland[1], Heidi Kiil Blomhoff[1], Steinar Funderud[1] and Terje Espevik[2], [1]Immunology lab., Inst. for Cancer Research, The Norwegian Radium Hospital, Oslo, Norway; [2]Institute for Cancer Research, University of Trondheim, Trondheim, Norway.

The growth and differentiation of B lymphocytes are regulated by complex mechanisms. A number of cytokines have been shown to potently influence functional B cell responses. In this study we have examined the production of TNF-α/β and IL-6 by extensively purified human B lymphocytes activated by different growth stimuli. The combination of phorbol esters and the calcium ionophore ionomycin was particularly potent in inducing the production of these cytokines. Notably, IL-4 induced significant production of IL-6 in a dose-dependent fashion when added to resting B cells. However, in contrast to several other stimuli, IL-4 did not induce TNF-α or TNF-β production, implying a selective action of IL-4 on IL-6 induction.

Anti-μ alone induced only a modest increase in TNF and IL-6 secretion. However, anti-μ synergized markedly with phorbol esters and IL-4 for cytokine production. None of several other cytokines tested were able to induce IL-6 production, when given to resting B cells. Available data therefore suggest that B cells may produce and respond to several important cytokines, adding to the complexity of the regulation of cell responses.

M 448 The Delivery of the Major Growth Stimulus to Resting B cells: the Induction of Ornithine Decarboxylase following Direct Contact between B and T Cells, E. Charles Snow and Randolph J. Noelle, Department of Microbiology and Immunology, University of Kentucky Medical Center, Lexington, KY. 40536; and Department of Microbiology, Dartmouth Medical School, Hanover, NH 03755

Previous results from our laboratory implicated that the physical interaction between B and T cells delivers the major growth stimulus to resting B cells. For this purpose, paraformaldehyde treated helper T cells were employed to form conjugates with resting B cells. Following such conjugate formation, the B cells entered a stage of high-rate RNA synthesis and became responsive to the growth promoting actions of soluble mediators. The earliest biochemical change within the B cells was a 4-6 fold transient increase in cAMP. The present study provides evidence for this conjugate formation inducing the expression of ornithine decarboxylase (ODC) activity within the B cells, detectable at 4 hours, peaking at 18 hours, and depleted by 30 hours. Neither the appearance, magnitude or longevity of this ODC expression was modulated by soluble mediators. The expression of enhanced ODC activity appears to be regulated at the level of gene transcription. The production of polyamines by ODC apparently participates early during the process of thymus-dependent B-cell activation.

M 449 CHARACTERIZATION OF THE MURINE HOMOLOG FOR THE CD22 B CELL-ASSOCIATED SURFACE ANTIGEN, Raul M. Torres, Tom St. John*, Ivan Stamenkovic+, and Edward A. Clark, Univ. of Washington, *Fred Hutchinson Cancer Research Center, Seattle, WA, and +Mass. Gen. Hosp., Boston, MA

The human B cell surface glycoprotein CD22 has been implicated with early events in B cell signaling through surface IgM (sIgM). Cross-linking of sIgM results in an increase of intracellular free calcium ([Ca^{++}]i) only in CD22+, and not CD22-, B lymphocytes. Furthermore, the binding of a monoclonal antibody to CD22, while not altering [Ca^{++}]i alone, will augment both the calcium and proliferative signal(s) delivered by anti-immunoglobulin. A cDNA encoding human CD22 has been isolated and sequenced revealing homology with the rat nervous system adhesion molecule, myelin-associated glycoprotein (MAG), suggesting a possible functional role for CD22 as a B cell-specific cell adhesion molecule. Northern analyses using human CD22 cDNA as a probe shows a 2.1 and 2.4 kb message with Daudi and 70Z/3 poly A+ RNA, respectively. However, initial attempts using the human cDNA to screen four murine B cell cDNA libraries failed to identify any putative murine homologs. As an alternative approach, oligonucleotides were designed, based on regions of high similarity between human CD22 and rat MAG sequences, to be used as primers in the polymerase chain reaction (PCR) with murine B cell cDNA as template. An approximate 272 bp sequence just 5' of the transmembrane domain has been amplified, using as templates both cDNA and genomic DNA from the pre-B cell line 70Z/3, and cDNA from the mature B cell line 38C13. Rescreening the murine B cell cDNA libraries with this murine CD22 fragment has led to the isolation of a putative CD22 mouse counterpart. After the isolation, sequencing and verification of a murine CD22 cDNA, we have analyzed CD22 tissue distribution and ontogenetic expression in both normal and diseased mice in attempt to gain insight into a possible functional role for this surface phosphoglycoprotein (Supported by NIH grants GM37905, GM42508, and AG00057).

M 450 AFFINITY THRESHOLDS FOR B CELL ACTIVATION VERSUS INACTIVATION-STUDIES WITH MONOCLONAL ANTI-MOUSE IGM ANTIBODIES (mab). V. Udhayakumar and B. Subbarao Dept. of Micro & Immunology & Center on Aging, University of Kentucky, Lexington, KY 40536. To evaluate the differences in the affinity threshold required for B cell activation versus tolerance we measured the avidities of a set of well characterized anti-IgM mab. The results indicated that there was no direct relationship between the avidity of an antibody and its ability to activate B cell. At least three nonstimulatory mab have avidities very similar to b7-6 which is the only stimulatory mab in soluble form. This failure could not be related to their fine specificity because all the mab became highly stimulatory when immobilized on to Sepharose beads. On the contrary, all the mab irrespective of their avidity and fine specificity were capable of inhibiting the growth of an immature B lymphoma, BKS-2, which represents a clonal model for B cell tolerance. Further, a direct correlation was found to exist between the avidity and the dose required to cause 50% inhibition of BKS-2 growth. Although we failed to see a significant difference in the tolerogenic potential of soluble versus immobolized anti-IgM mab in directly inactivating BKS-2 cells, a remarkable difference became apparent when we added rIL-5 to these cultures. It was found that rIL-5 was effective in overcoming the tolerogenic effect of soluble but not immobilized anti-IgM mab. The implications of these findings with reference to the affinity threshold required for B cell activation versus inactivation will be discussed. Supported in part by the NIH grants AI21490, AG05731, AG00422.

M 451 IDENTIFICATION OF SURFACE IgM-ASSOCIATED MOLECULES ON HUMAN B CELLS, Carel van Noesel,* Jannie Borst,** Evert de Vries** and René van Lier*, *Central Laboratory of the Netherlands Red Cross Blood Transfusion Service & Lab. Exp. Clin. Immunology of the University of Amsterdam, and **The Netherlands Cancer Institute, Amsterdam, The Netherlands. Both T and B lymphocytes express a clonotypic receptor, responsible for recognition of antigen. Signals elicited via the T-cell receptor (TCR) are thought to be transduced into the cytoplasm by the non-polymorphic CD3 molecules. We have addressed the question whether membrane-bound immunoglobulin (Ig) on human B cells is associated with an analogous complex of proteins. From radioiodinated B-cell lines and tonsil B cells a disulphide-linked dimer of 85 kDa molecular mass was isolated, found in non-covalent association with IgM. Under reducing conditions, the dimer resolved into subunits of 47 and 36 kDa, both of which carry N-linked carbohydrate. Preliminary HPLC mapping of tryptic peptides derived from the iodinated components, suggests that they may represent independent gene products. Upon B-cell stimulation with the PKC activator phorbol myristate acetate (PMA), both subunits were phosphorylated. We are investigating further characteristics of these two proteins and examining the possible existence of additional members of this IgM-associated complex.

M 452 BONE MARROW STROMAL CELLS AND INTERLEUKIN-7 INDUCE COORDINATE EXPRESSION OF THE BP-1/6C3 ANTIGEN AND PRE-B CELL GROWTH, Pamela A. Welch, Peter D. Burrows, Steven Gillis, and Max D. Cooper, University of Alabama at Birmingham and the Howard Hughes Medical Institute, Birmingham, AL 35294, Immunex Corporation, Seattle, WA 98101.

The BP-1/6C3 molecule expressed by early B lineage cells and some stromal cells is a type II integral membrane glycoprotein that belongs to the zinc-dependent family of metallopeptidases. In order to explore the potential role of the BP-1/6C3 molecule in precursor B cell proliferation, we established a stromal cell line (BHM) from long-term bone marrow cultures of the Whitlock-Witte type and developed a rapid bioassay for the detection of responsive target cells. When non-adherent bone marrow cells were cultured with BHM stroma, a selective up-regulation of BP-1 expression on B cell precursors within 72 hours coincided with the induction of proliferation. Because the BHM inductive effects on precursor cells could be reproduced with supernatant, soluble growth factors were evaluated in the assay. Interleukin-7 was unique in its ability to induce the BP-1/6C3 expression and concomitant cell growth; interleukins 1-6 had no effect on BP-1/6C3 expression. Northern blot analysis revealed that the BHM stromal line constitutively expressed high levels of variable length transcripts for IL-7. Finally, the IL-7-induced proliferative response of early B lineage cells to IL-7 was partially inhibited by the BP-1 antibody. The data suggest the participation of the BP-1/6C3 molecule in the IL-7 induced proliferation of B cell precursors. (NIH grants CA16673 and CA 13148; MDC is a HHMI investigator.)

M 453 CHARACTERIZATION OF THE MURINE IL-5 RECEPTOR, Naoto Yamaguchi, Yasumichi Hitoshi, Yoshiyuki Murata, Seiji Mita, Akira Tominaga, and Kiyoshi Takatsu, Department of Biology, Institute for Medical Immunology, Kumamoto University Medical School, Kumamoto 860, JAPAN

Recently, we have generated rat monoclonal antibodies (mAbs) directed against the murine IL-5 receptor. One of these mAbs, designated H7, completely inhibited the high- and low-affinity binding of IL-5 to the cells. IL-5 was also capable of blocking the binding of H7 mAb to the cells reciprocally. H7 mAb was reactive exclusively with IL-5-responsive cells, such as T88-M cells (IL-5-dependent line), BCL_1-B20 cells (B-CLL), and MOPC 104E cells (myeloma). H7 mAb immunoprecipitated a major band with approximate M_r of 60 kDa from the extracts of cell surface-radioiodinated T88-M, BCL_1-B20, and MOPC 104E cells. The precipitation of the 60-kDa molecule was inhibited when the excess amounts of unlabeled IL-5 was present. Analysis with immobilized IL-5 also revealed that a 60-kDa molecule bound specifically to IL-5-coupled beads as compared to control beads when the extract of surface-radioiodinated T88-M was applied. Thus, these features reflect that the IL-5 receptor is the 60-kDa molecule defined by H7 mAb. Furthermore, the IL-5 receptor does not appear to be covalently linked to other molecules with intermolecular disulfide-bridges because of no apparent major bands of higher M_r under nonreducing conditions. Also, treatment with N-Glycanase indicated that the IL-5 receptor is a glycoprotein carrying N-linked carbohydrates. We are now trying to analyze protein kinase reaction involved in IL-5 receptor-mediated signalling.

M 454 IFN-τ INDUCES IgM^+ LYMPHOMA CELL LINE 29M10 TO DIFFERENTIATE TO IgG1 BEARING CELLS, AND TO SECRETE IgG1 ISOTYPE, Israel Zan-Bar, Irit Altboum and Yael Porat, Department of Human Microbiology, Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel.

Murine B cell lymphoma I.29 cell lines can be stimulated by LPS, IL-4, IL-5 and other reagents to secrete Ig and to differentiate from IgM^+ to $IgG2a^+$, $IgG1^+$, IgE^+, or IgA^+ cells. In the present work the effect of IFN-τ on the Ig secretion, proliferation, and differentiation of I.29 cell lines were examined. No direct or indirect effect of IFN-τ on the cells growth were detected. IFN-τ induces in LPS stimulated I.29 cells isotype switch and secretion of IgG1. In addition IFN-τ induces augmentation in the secretion of IgM, and IgA, and has no effect on the secretion of IgG2a, IgG2b or IgG3. Simultaneous stimulation of the lymphoma cells with IL-4, IFN-τ, and LPS reveals that IFN-τ antagonized the effects of IL-4 in elevation of IgM and total Ig secretion and abrogates the secretion of IgE. IFN-τ synergizes IL-4 in IgG1 secretion. Preincubation of the cells with IFN-τ for 48 hrs prepares them to secrete IgG1 on subsequent stimulation of the cells with LPS. Similar observation of preeducation of the lymphoma cell lines with IL-4 or IL-6 were obtained.

M 455 INTRATHYMIC PRESENTATION OF NOMINAL ANTIGEN BY B CELLS. Margot Zöller, Institut of Radiology and Pathophysiology, German Cancer Research Center, Heidelberg, Fed.Rep.Germany.

Prenatal tolerization with trinitrobenzenesulfonic acid (TNBS) leads during the first postnatal week to a transient increase in intrathymic TNP-specific B cells. To evaluate, whether this may influence selection of the T cell repertoire and establishment of tolerance towards the neoself determinant TNP, the capacity of intrathymic B cells to present TNP as well as an anti-TNP antibbody (Sp6), which carries a recurrent idiotype, to thymocytes of prenatally untreated and TNBS-treated litters was explored.

Deletion and reconstitution experiments revealed that in the thymus of 10 days old litters Sp6 as well as TNP was presented exclusively by B cells. After stimulation with TNP-HRBC, B cells could still present the antigen, but depletion of B cells did no longer abolish proliferation, i.e. after stimulation intrathymic B cells were no longer the only source for presentation of external antigen. B cells of prenatally tolerized mice differed inasmuch as they initiated only proliferation in response to Sp6, but not in respons to the neoself determinant TNP. Furthermore, after challenge with TNP in an immunogenic form, thymic as well as splenic B cells had lost the capacity to provide positive signals to T cells specific for the neoself determinant as well as to T cells specific for the cognate antibody.

It is concluded that B cells are of importance in the intrathymic selection of the T cell repertoire and that they are invovled in the process of tolerization towards nominal antigen.

M 456 EFFECTS OF CYTOKINES AND CELL-CONTACT SIGNALS ON HUMAN B CELL RESPONSE AND Ig ISOTYPE-SWITCH. Rudolf H. Zubler, Xiaohong Zhang, Heidi James, Christiane Werner-Favre. Division of Hematology, University Hospital, 1211 Geneva, Switzerland.

A human B response occurs in the presence of mutant EL-4 thymoma cells (clone 6.1.5.5) in two different manners : 1) El-4 cells can stimulate B cells via a cell-contact signal which is cyclosporine A (CsA)-resistant and acts in conjunction with human T cell supernatant (T-SUP). 2) In addition, EL-4 cells that have been activated with phorbol ester (PMA) or IL-1, provide a helper activity which can replace T-SUP, is CsA-sensitive, and also occurs via cell-contact. In the response type 1, T-SUP could not be replaced by combinations of recombinant IL-1, 2, 4, 5, 6, IFNg, TGFß. On the other hand, in the response type 2 no soluble (murine or human) T cell factors seem to participate at all. IgE secretion was over twenty times increased with added IL-4 in both, reponse types 1 and 2. Analysis of the clonal responses of single B cells added by FACS (whereby 60% B cells responded and one in 6 ot these cells generated an IgE response) indicated, that switch from either IgM-, G- or A- to IgE-secretion occurs with IL-4. Switch to IgA secretion also occurred in responses 1 and 2, but this was not influenced by any of the tested cytokines. Thus, our data indicate that human B cells switch to IgA secretion at some preprogrammed rate, uninfluenced by different culture conditions. Differential distribution of IgA cells into different organs could then be explained by selective homing.

M 457 SURFACE IMMUNOGLOBULIN CROSSLINKING ACTIVATES A TYROSINE KINASE PATHWAY IN B CELLS, Mark Brunswick, Lawrence E. Samelson and James J. Mond, Uniformed Services University, Bethesda, MD 20814 and National Institutes of Health, Bethesda, MD 20892

Many investigators have suggested that the principal biochemical pathway that is activated in B cells stimulated by antigen or anti-Ig mediated crosslinking of surface immunoglobulin is that which results in hydrolysis of phosphatidyl inositol bisphosphate with generation of DAG and IP_3. Recent evidence from our laboratory suggests, however, that sIg mediated B cell activation can proceed in the absence of detectable increases in the concentration of diacylglycerol or of $[Ca^{2+}]_i$ and thus implicates the involvement of other non-PKC/Ca dependent pathways of activation. We therefore sought evidence for the activation of a signalling pathway which is associated with growth in other cell types including T lymphocytes, i.e. the protein tyrosine kinases. The data in this manuscript demonstrates that crosslinking of sIg by mitogenic and non-mitogenic monoclonal anti-Ig antibodies leads to the rapid tyrosine phosphorylation of cellular substrates, consistent with their activation of a tyrosine kinase activity. This tyrosine kinase dependent activation is not stimulated by other B cell mitogens including phorbol esters and ionophores and does not require the presence of detectable PKC. Furthermore, inhibition of anti-Ig stimulated PIP_2 hydrolysis does not inhibit activation of this tyrosine kinase dependent pathway. These findings suggest that occupancy of the sIg receptor may induce multiple pathways of activation.

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M 500 PREFERENTIAL LINKAGE OF BCL-2 TO IMMUNOGLOBULIN LIGHT CHAIN GENES IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL), Masaaki Adachi, Ayalew Tefferi+, Thomas J. Kipps* and Yoshihide Tsujimoto, The Wistar Institute, Philadelphia, PA 19104. +Dept. of Internal Medicine, Mayo Clinic, Rochester, MN 55905 and *Research Institute of Scripps Clinic, CA 92037.

Most human follicular lymphoma possess the t(14;18) chromosome translocation that juxtaposes the IgH gene to the 3' regions of bcl-2 gene. Here we show that the rearrangements of the bcl-2 gene occurred in a significant fraction (approximately 10%) of B cell CLL although no karyotypic abnormality involving bcl-2 gene has been described. In all 3 CLL cases analysed, the breakpoints on the chromosome 18 clustered at the 5' flanking region of the bcl-2 gene and the rearranged bcl-2 genes were juxtaposed with the Igλ or Igκ genes. These results indicate that the bcl-2 gene is preferentially linked to the Ig light chain gene in CLL. The breakpoint clustering region within the 5' flanking region of bcl-2 gene includes three streches of stable Z-DNA elements, suggesting the involvement of Z-form structure in the bcl-2 rearrangements in CLL. These results are in sharp contrast to the t(14;18) chromosome translocation of follicular lymphoma cases. This striking difference could reflect the processes of CLL or follicular lymphoma development.

M 501 Expression of Immunoglobulin Heavy Chain Variable Genes in Follicular Small Cleaved Cell Lymphoma : Predominate Use of the VH3 Family. *David W. Bahler, Michael J. Campbell, Thomas Chen, Andrew D. Zelenetz, Shoshana Levy, and Ronald Levy. Division of Medical Oncology, Room M207, Stanford University Medical Center, Stanford CA, 94305*

We have sequenced the immunoglobulin heavy chain variable region genes (VH) expressed by follicular small cleaved cell (FSC) lymphoma cells from 12 different patients. Most of these sequences were obtained without cloning using an asymmetric amplification technique. All of the genes we have sequenced are members of just one of the six known VH families, VH3. Although VH3 appears to be the largest VH family containing approximately 50 percent of the VH genes, finding only VH3 genes expressed in these 12 tumors suggests that the usage of VH genes by FSC lymphoma is strongly biased. Biased usage of VH genes has also been reported in chronic lymphocytic leukemia (CLL) . However, in contrast to FSC lymphoma, the predominate variable genes used in CLL belong to the VH4, VH5 and VH6 families. The significance of biased VH gene usage to the development of FSC lymphoma will be discussed.

M 502 INTERLEUKIN-1 AND INTERLEUKIN-6 EXPRESSION IN HUMAN MULTIPLE MYELOMA, Marleen H.C. Bakkus[1], Karin M.J. Brakel-van Peer[2], Henk J. Adriaansen[2] and Robbert Benner[2], [1]Department of Hematology-Immunology, Free University Brussels, Belgium, [2]Department of Immunology, Erasmus University Rotterdam, The Netherlands.

Multiple Myeloma (MM) is a malignancy characterized by the clonal development of plasma cells in the bone marrow. Myeloma cells in culture have been reported to produce several cytokines which may be involved in the regulation of tumor growth and differentiation and/or activation of osteoclasts causing bone resorption. IL-6 is suggested to play a role in autocrine growth stimulation of myeloma cells (1) and IL-1ß, among other cytokines, is likely responsible for bone resorption (2). To investigate which cell types in the bone marrow of MM patients express mRNA for these cytokines, we used a fluorescent *in situ* hybridization (ISH) technique, using biotinylated ssRNA-probes in combination with cell surface marker staining. The ISH studies clearly revealed expression of IL-1ß by plasma cells ($CD38^+$), T-cells ($CD3^+$) and macrophages ($RFD9^+$). IL-6 mRNA expression was detected in T-cells and monocytes ($CD14^+$), but not in plasma cells. We therefore conclude that IL-6 is more likely to be active in a paracrine rather than in an autocrine pathway and that myeloma cells express IL-1ß, also in the *in vivo* situation, suggesting a role in bone resorption.

This study was supported by the Netherlands Cancer Foundation.

1) Kawano, M. et al. Nature 1988; 332 : 83.
2) Dewhirst, F.E. et al. J. Immunol. 1985; 135 : 2562.

M 503 **Mutations in the IgH enhancer alter tumor spectrum in *c-myc* transgenic mice.** Steven R. Bauer and Richard H. Scheuermann, Basel Institute for Immunology, Basel, Switzerland.

Our recent analysis of the IgH enhancer structure has localized negative regulatory sites flanking the enhancer core. These cis-acting elements serve as binding sites for a cell-type specific nuclear protein, NF-μNR, which functions as an enhancer suppressor, keeping the IgH gene silent in non-B cells.

We have used a *c-myc* transgenic mouse model system to study the effects of NF-μNR binding site deletions on enhancer activity. In agreement with published data we find that the *c-myc* oncogene driven by a wild-type enhancer causes a polyclonal expansion of the preB compartment usually resulting in tumors of the B or preB type. In addition, we find that NF-μNR binding site deletion mutations broaden the spectrum of *c-myc* induced tumors throughout the lymphoid lineages to include not only preB but also preT and mature T cells. Some tumors express both myeloid and lymphoid markers supporting the idea that we are transforming early precursor cell types due to mutant enhancer driven expression of *c-myc* early in hemopoietic differentiation. As these transgenic lines progress they may yield interesting insights into early myeloid/lymphoid lineage relationships and allow us to establish transformed precursor cell lines. Implications with respect to the mechanism of enhancer regulation and tumorigenesis will be discussed.

M 504 CONSTITUTIVE AND CELL CYCLE-REGULATED EXPRESSION OF C-*MYB* mRNA IS RELATED TO THE STATE OF DIFFERENTIATION IN MURINE B-LYMPHOID TUMORS AND IS REFLECTED IN PROTEIN/DNA INTERACTIONS. Timothy P. Bender[1], Peter Isakson[2], Katrina M. Catron[1] and Charles R. Toth[1]. Departments of Microbiology[1] and Pharmacology[2], University of Virginia, Charlottesville, VA. 22908. We have previously shown that the steady-state level of *c-myb* mRNA is differentially regulated in murine B-lymphoid tumors. Pre-B cell lymphomas contain 10 to greater than 100 fold more *c-myb* mRNA than B cell lymphomas and plasmacytomas. This difference appears to be maintained primarily by a block to transcription elongation (attenuation) which occurs in the first intron of the gene. We have recently investigated the low level of *c-myb* mRNA expression in the BCL_1 B cell lymphoma using amino acid starvation to block BCL_1 cells in a G_0/G_1 state or in combination with aphidicolin to prevent progression into S-phase. Starved cells express little detectable *c-myb* mRNA. However, upon release into the cell cycle the level of *c-myb* mRNA increases 7-10 fold during late G_1/S-phase as measured by DNA synthesis. Levels of *c-myb* mRNA then decrease during progression through the cell cycle. Aphidicolon does not inhibit the increase in *c-myb* mRNA expression indicating that this increase is independent of DNA synthesis. We have used elutriation to confirm that *c-myb* mRNA levels are cell cycle regulated in cycling BCL_1 cells and extended this result to the A20 B cell lymphoma. Interestingly, the level of *c-myb* mRNA in pre-B cell lymphomas is not cell cycle regulated indicating that a switch from constitutive to a cell cycle related mode of expression occurs during B cell development. We find that changes in *c-myb* mRNA levels during the cell cycle are regulated, at least in part, by attenuation. Finally, we have used gel shift analysis and a T7 gene 6 exonuclease assay to scan the *c-myb* attenuation region for differentially expressed protein/DNA interactions. We detect two such interactions in pre-B cell lymphomas but not B cell lymphomas and a third interaction in B cell lymphomas but not plasmacytomas. Thus, changes in the mode of *c-myb* mRNA expression are correlated with differences in protein/DNA interactions in the murine *c-myb* attenuation region.

M 505 **IN VITRO EFFECTS OF CLONED HUMAN INTERLEUKIN-7 ON CELLS FROM ACUTE LYMPHATIC LEUKEMIAS,** Heidi Kiil Blomhoff, Erlend B. Smeland, Bjørn Erikstein, Steinar Funderud and Cecilia Skjønsberg, Immunology laboratory, Institute for Cancer Research, The Norwegian Radium Hospital, Oslo, Norway.

The ability to respond to lymphokines may provide a key to understanding the regulation of normal and abnormal lymphoid proliferation and differentiation. The recently cloned human interleukin-7 (IL-7) has been shown to induce proliferation of B-cell precursors from normal bone marrow, without significantly affecting the differentiation process. We have examined the effects of recombinant IL-7 on purified neoplastic B-cell precursors from blood or bone marrow samples of 10 patients with acute lymphatic leukemia, the major form of childhood cancer. The most important finding was that IL-7 markedly reduced the DNA-synthesis in 3 cases (group 1), accompanied by a pronounced maturation of the cells. In another 3 cases (group 2) IL-7 had no effect either on DNA synthesis or maturation, whereas we observed that IL-7 stimulated the DNA synthesis of the last 4 cases (group 3) without affecting differentiation. When comparing the effects of IL-7 with BCGF, we found that partially purified BCGF synergistically enhanced the IL-7 stimulated DNA synthesis in group 3 cells and potentiated differentiation of group 1 cells. Finally, our results indicated that IL-7 inhibited the group 1 cells late in G_1.

M 506 EXPANDED BONE MARROW CD10+ LYMPHOID PROGENITOR POPULATIONS MIMICKING COMMON ALL IN PATIENTS WITH NEUROBLASTOMA. F. Brok-Simoni, I. Hakim, M. Mandel, M. Biniaminov, N. Amariglio, G. Rechavi & B. Ramot. Institute of Hematology, The Chaim Sheba Medical Center and Sackler School of Medicine, Tel Aviv University, Israel.
The analysis of BM mononuclear cells of two infants with stage IV-S neuroblastoma revealed the presence of many lymphoblasts and immature lymphocytes. Immunophenotypin showed that a high percentage of the BM cells are positive for the HLA-DR, CD19, CD20 and CD10 phenotype, similar to common ALL cells. Within one year of follow-up without any treatment, a complete regression of the tumors was observed. Immature lymphocytes carrying the same surface markers can be detected as a minute population (1-3%) of th normal hematopoietic results suggest that such populations are a favorable prognostic factor.
The CD10 antigen was recently cloned and found to be an enkephalinase. Enkephalins ar synthesized in the adrenal medulla and NB cells. It is tempting to suggest that secretion of enkephalins by NB cells is a stimulus for the expansion of CD10 (enkephalinase)-bearing lymphoid cells.

M 507 COEXPRESSION OF NATIVE AND TRUNCATED ISOFORMS OF IG μ CHAINS IN HUMAN B CELL TUMORS RESULTS FROM ALTERNATIVE RNA SPLICING, James Caldwell, Patrick McElhone, Jane Brokaw, Roberto Anker and Brian Pollok, Wake Forest University Medical Center, Winston-Salem, NC 27103

Several human B cell lines derived from mature B cell tumors express truncated forms of μ heavy chains (μ'_m and μ'_s) which immunoprecipitate from biosynthetically labelled cell lysates as 55-57 kD proteins, along with native membrane and secretory forms (μ_m and μ_s). Anti-κ antibodies coprecipitate native size μ chains but not μ', indicating that the truncated isoforms do not pair with light chain. Cell surface labeling with ^{125}I followed by immunoprecipitation does not detect μ' on the surface of the cell. Endo H glycosidase sensitivity of the N-linked sugars on μ' chains also demonstrates an absence of intracellular transport for μ' chains in mature B cell lines. *In vitro* translation of μ' chains using cytoplasmic polyA$^+$ selected RNA from these cell lines confirms that the truncated proteins are not degradation products of native μ chains. Northern blot analysis reveals the presence of μ' transcripts of 2.3 and 2.0 kb, as well as native size μ mRNA of 2.7 and 2.4 kb. Further Northern analysis and nuclease protection studies using leader region specific oligonucleotide probes indicates that these μ' transcripts arise from a direct leader-to-Cμ1 exon splicing event, removing the entire V_H coding region. Sequence analysis of the μ' transcript in a cell line that produces almost exclusively μ' confirms this unusual processing pattern. PCR amplification of heavy chain mRNA in other μ'-producing cell lines, using primers derived from the sequences of native size transcripts, generates products of 136 nt, the size predicted by a leader to Cμ1 splice, and 491 nt, as predicted by conventional splicing. Current experiments are directed toward analyzing the presence of μ' in plasma cell lines and in untransformed B cells.

M 508 THE INTERACTIONS BETWEEN CIRCULATING MALIGNANT PLASMA CELL PRECURSORS AND BONE MARROW STROMAL CELLS IN MULTIPLE MYELOMA,
Federico Caligaris-Cappio, Luciana Bergui, GianLuca Gaidano, Marina Schena, Margherita Riva, Luisa Tesio, Clinica Medica A, Dip. Sc. Biomediche e Oncologia Umana, Università di Torino, Torino, ITALY.
A culture system was devised to investigate the relationships between circulating malignant precursors and bone marrow (BM) microenvironment in multiple myeloma (MM). Adherent stromal cell cultures were obtained from the bone marrow (BM) of 5 patients with MM, by culturing 1 - 2 x 10^6 BM cells / ml of RPMI medium. Stromal cells reached the confluence after 6 - 8 weeks of culture, when no residual lymphoid populations could be detected by morphology and immunophenotyping. Peripheral blood mononuclear cells (PBMC, 1 - 2 x 10^6/ml) from each individual patient were then layered onto their own BM confluent stromal cells in presence of RPMI medium and 10% autologous serum. After three further weeks of culture at 37°C in presence of 5% CO_2 a monoclonal B cell population became apparent and included variable proportions of monoclonal lymphocytes and plasmacells. The monoclonal B cell population (including plasmacells) was tightly adherent to the meshwork of stromal cells and was scattered amidst macrophages and activated T lymphocytes which were, instead, both adherent and floating in the medium. This culture system may prove valuable to analyze the cytokines and the adhesion molecules relevant in the growth and dissemination of MM.

M 510 DEVELOPMENT OF AUTOIMMUNITY IN A "NORMAL" MOUSE STRAIN WITH THE NEONATAL INJECTION OF AN ANTI-IDIOTYPIC ANTIBODY DIRECTED AGAINST A SELF-ERYTHROCYTE-SPECIFIC AUTOANTIBODY. Michael J. Caulfield and Deborah Stanko, Section of Immunology, Research Institute of the Cleveland Clinic Foundation, Cleveland, OH 44195

Recently, we characterized a pathogenic anti-self-erythrocyte monoclonal antibody (G8) derived from an unimmunized Coombs-positive NZB mouse. This autoantibody recognizes native erythrocytes from mice but not from other species and appears to have the same specificity as naturally occurring autoantibodies from NZB mice. Sequence analysis of the V region genes encoding G8 indicating that it is closely related to germline V_H and V_L genes. Furthermore, an anti-idiotypic mAb (E8) prepared against the G8 autoantibody inhibits ~ 60% of anti-erythrocyte AFC from aged Coombs-positive NZB mice indicating that the G8 mAb expresses a recurrent idiotype characteristic of spontaneously arising Coombs autoantibodies. In an attempt to abrogate the development of autoimmunity in NZB mice, we injected neonatal mice with a single dose of 10 μg of E8 and followed cohorts of mice for the development of Coombs-positivity. Control groups consisted of NZB mice injected with saline alone, and neonatal BALB/c mice injected with either 10 μg of E8 or saline. We found that the administration of E8 to NZB mice did not alter the course of the development of Coombs-positivity in that strain. Unexpectedly, however, a small number (2/18) of BALB/c mice injected with E8 became Coombs-positive. None of the BALB/c mice injected with saline became positive as BALB/c is a "nonautoimmune" strain of mice not known to develop Coombs-positive hemolytic anemia. Even more striking results were observed on mortality of the neonatally E8-injected BALB/c mice. These mice failed to live as long as their saline-injected counterparts. Indeed, their life-expectancy was similar to that of NZB control mice which had a mean survival of ~ 15 months whereas normal BALB/c mice have a life-expectancy of > 24 months. Thus, perturbation of the development of the neonatal immune repertoire resulted in eventual development of autoimmunity and a shortened life-span.

M 511 CHARACTERIZATION OF THE EXPRESSION OF EBV-RELATED ANTIGENS (LMP and EBNA) IN EBV-NEGATIVE, EBV-POSITIVE, EBNA2 AND LMP TRANSFECTED CELL LINES: COMPARISON OF REACTIVITIES OF DIFFERENT ANTIBODIES. Christensson B., Srinivasan G., Li-Fu.H., Trivedi.P., Klein G. Departments of Pathology and Tumor biology, Karolinska Hospital and Institute, S-10401, Stockholm, SWEDEN.

Immunofluorescent (IFL) and immunoenzyme (IE) methods were used to investigate the expression of EBV related antigens (LMP and EBNA2) in cell lines either uninfected with EBV (Ramos, DG75), EBV-infected (Raji, IB4) or transfectants expressing the LMP (DG75-HSP39) or EBNA2 (DG75-FA) genes. EBNA and LMP antigen expression was seen with both IFL and IE techniques in cytological smears in EBV positive cell lines and EBNA2 and LMP transfectants, respectively and was verified using western blots. Using sensitive immunoalkaline phosphatase methods and monoclonal antibodies (S12 and CS 1-4) cytoplasmic staining was also seen in the EBV-negative cell lines Ramos and DG75. In immunohistochemical stainings of normal tonsil sections these antibodies seemed to detect an antigen related to vascular endothelial cells. Although the staining of EBV-neagtive cells was weaker than that of LMP-expressing EBV-positive cell lines and LMP-positive transfectants these data suggest that the use of these antibodies for in vivo detection of EBV positive cells should be made with caution. The possibility of the presence of a cellular homologue of the LMP gene may warrant further study.

M 512 LEUKEMIC B CELLS DISPLAY DIFFERENT PATTERNS OF REPONSE TO IL-2 AND IL-4 DEPENDING ON THE MODE OF *IN VITRO* ACTIVATION, Thierry Defrance, Anne-Catherine Fluckiger, Béatrice Vanbervliet and Jacques Banchereau, UNICET, Laboratory for Immunological Research, 27 chemin des peupliers, 69570 Dardilly, France.

The proliferative response of B-CLL and non Hodgkin B lymphomas to IL-2 and IL-4 was studied using different types of B cell activation signals. When leukemic B cells were stimulated with anti-Ig reagents (insolubilized anti-IgM or SAC) they failed to mount a proliferative response to IL-4, whereas IL-2 stimulated DNA synthesis in the same culture system. Moreover, addition of IL-4 together with IL-2 profoundly suppressed IL-2 driven proliferation of anti-Ig activated B cells. In contrast, when leukemic B cells were activated with PDBU and ionomycin or immobilized anti-CD40 mab, opposite results were obtained. Under these conditions of activation, IL-4 was able to stimulate growth of the leukemic B cell clones and combinations of IL-4 and IL-2 were found to have additive effects on B cell proliferation. Taken together, our data suggest that the nature of the activation signal determines the outcome of cytokine stimulation on leukemic B cell growth.

M 513 DIFFERENTIATION OF B-CLL CELLS INDUCED BY BRYOSTATIN 1, Hans G. Drexler, Suzanne M. Gignac, Michael Buschle, George R. Pettit and A. Victor Hoffbrand, Department of Haematology, Royal Free Hospital School of Medicine, London NW3 2QG, U.K.
B-chronic lymphocytic leukemia (B-CLL) cells are arrested in their differentiation and do not respond adequately to most physiological agents, but can be induced to differentiate by direct activation of protein kinase C (PKC) which is a key enzyme in the phosphoinositol lipid signal transduction pathway, e.g. by the phorbol ester TPA. Bryostatins, naturally occurring macrocyclic lactones isolated from the marine animal Bugula neritina, are structurally unrelated to phorbol esters but stimulate PKC to a comparable degree as TPA. Peripheral blood B-CLL cells were incubated in-vitro with Bryostatin 1 (Bryo) at nanomolar concentrations under standard culture conditions. While the cells remained proliferatively inert, Bryo triggered activation and differentiation of B-CLL cells in all cases as documented by the induction of morphological changes, RNA synthesis, monotypic immunoglobulin production, expression of tartrate-resistant acid phosphatase (TRAP) and transcription of the (proto-)oncogenes fos, jun and myc. Addition of the calcium ionophore A23187 increased RNA synthesis, Ig production and fos, myc and jun mRNA expression; morphologically, the cells acquired plasmacytoid features and were TRAP-negative. Bryo mimicked the inducing effects of TPA with or without A23187, respectively. Bryo was less active at equivalent concentrations than TPA and partially antagonized TPA-mediated responses. The data show that Bryo has effective differentiation-inducing properties on B-CLL cells that can be increased by double-stimulation with a calcium ionophore.

M 514 A MURINE MODEL SYSTEM FOR B CELL LYMPHOMAGENESIS IN IMMUNOCOMPROMISED HOSTS, Dean W. Felsher and Jonathan Braun, Department of Pathology, UCLA School of Medicine, LA, CA 90024.
We have isolated four new murine B cell lines arising spontaneously from long-term culture of lymphoid tissue from normal mice. Phenotypically, they have uniformly activated a *c-myc* locus, exhibit an unusual preferential λ light chain rearrangement and express the CD5 lineage marker. Functionally, although derived from normal animals, all four cell lines are rapidly tumorigenic when reintroduced into the immunocompromised C.B-17 *scid* mouse, whereas they are significantly less or nontumorigenic in untreated syngeneic animals. Conventional T cell dependent reponses are not critical to host resistance. However, direct *in vitro* studies demonstrated that these cell lines had marked NK cell sensitivity that declined following host passage. Fully malignant variants derived from these cell lines are NK resistant. Moreover, the *in vivo* depletion of NK cells increases the frequency and decreases the latency of tumor induction. These findings raise the unexpected possibility that non-immune (versus immune CTL) host mechanisms may be the key deficit promoting B cell neoplasia in the setting of immunodeficiency disease. Presently, we are studying the molecular basis for the acquired NK resistance as a putative secondary event in the malignant progression of these cell lines.

M 515 BURKITT LYMPHOMA LINES CONTAIN NUCLEAR PROTEINS THAT INTERACT WITH THE IL6-RESPONSE ELEMENT OF LIVER ACUTE PHASE GENES, Georg H. Fey, Masahira Hattori, Thomas Brechner, Charles Abney and Gertrud Hocke, Department of Immunology, Research Institute of Scripps Clinic, La Jolla, CA 92037.
Acute phase genes code for plasma proteins that are synthesized in the liver and dramatically altered in their plasma concentrations during acute and chronic inflammations. These proteins provide important protective functions to the body against tissue injury and infections. Interleukins 1 and 6 are the major regulators of the transcriptional induction of acute phase genes. We have mapped the IL6-responsive cis-acting transcriptional control element (IL6-RE) in the promoter region of the rat α_2-macroglobulin (α_2M) gene, a prototype acute phase gene. This element, CTGGGA, is conserved in other major rodent acute phase genes and is sufficient to confer IL6-responsiveness to other genes. We have identified nuclear proteins from hepatic cells that form complexes with this element, and are altered in a characteristic fashion by treatment with IL6. Proteins forming indistinguishable complexes with the IL6-RE of the rat α_2M gene were also detected in the Burkitt cell lines Raji, Namalwa and Cess. However, only Cess cells have the IL6-receptor, and only in Cess cells these complexes were characteristically altered by treatment with IL6. Thus, common elements probably exist in the IL6 signal transduction machinery between hepatic cells and B-lymphocyte derived cells.

M 516 ROLE OF AUTOCRINE B CELL GROWTH FACTORS IN THE CONTROL OF PROLIFERATION AND DIFFERENTIATION IN NON-HODGKIN'S LYMPHOMAS. Ford, Richard J., Daijun Li, Archito Tomayo, Linda Yoshimura. M.D. Anderson Cancer Center, Houston, TX 77030 The non-Hodgkin's lymphomas (NHL-B) are a diverse group of common human lymphoid neoplasms, primarily derived from the B cell lineage. We have developed NHL-B cell lines using natural product, 12kD BCGF. The cell lines accurately reflect the immunophenotypic and genotypic characteristics of the original biopsies from which they were derived. The NHL-B cells were assayed for in vitro proliferative activity using BCGF, and a variety of other putative B cell growth stimulating cytokines, including IL-2, IL-4, IL-6, and Lymphotoxin. BCGF stimulation resulted in at least a seven-fold increase in proliferative response of the NHL-B cells, while the other cytokine growth factors showed no significant stimulation, or were inhibitory. The NHL-B cell lines also secreted growth factors into culture supernatants which stimulated cell growth in a dose dependent fashion in both autochtanous lymphoma cells as well as BCGF-dependent normal B cell lines. These studies suggest that a BCGF-like autocrine growth factor is responsible for the proliferative capacity of the tumor cells, which molecular studies have corroborated. The NHL-B cell lines did not secrete Ig spontaneously in vitro, or when stimulated with differentiation-inducing lectins such as Pokeweed mitogen or Staphylococcal protein A (SAC), with or without the addition of IL-6. This suggests that the lymphoma cells may either lack the receptor for IL-6 or exhibit a defect in signal transcution for this cytokine. Molecular studies have indicated that both mechanisms can be observed in NHL-B cells.

M 517 ALTERATION OF BONE MARROW FUNCTION BY A NUTRITIONAL DEFICIENCY. Pam Fraker and Louis King, Dept. of Biochemistry, Michigan State Univ., E. Lansing, MI 48824 Malnutrition accompanies many Western diseases (e.g., A.I.D.S., cancer, gatrointestinal disorders, alcoholism, renal disease) and can further compromise host defense in such patients. Little is known about how such nutritional deficiencies effect bone marrow function and whether production of new leukocytes is compromised. Young adult A/J mice were given diet containing suboptimal levels of zinc for 30 days to ascertain the effects of a deficiency in this essential trace element on lymphopoietic processes. The number of nucleated cells in the marrow was reduced 30% in mice which were moderately zinc deficient and 50% in those that exhibited a more severe form of the deficiency. Flow cytometric scatter profiles of nucleated cells of the marrow indicated a significant reduction in the proportion of small cells that contain precursor cells. Focusing initially on the phenotypic distribution of B-cells of BM, it was found that the proportion of immature B-cells was reduced about 40% in moderately deficient mice and about 50% in those more severely deficient. The functional capacity of immature B-cells to respond to stimulation with trinitrophenylated lipopolysaccharide in short term culture was reduced, though it correlated with the overall decline in the proportion of sIgM bearing B-cells. Thus it would appear that nutritional deficiencies can substantially alter bone marrow function. (Supported by N.I.H. HD10586)

M 518 DEVELOPMENT OF PRE-B CELLS IN TRANSGENIC SCID MICE CARRYING A FUNCTIONAL μ-HEAVY CHAIN GENE, Michal Fried, Richard R. Hardy and Melvin J. Bosma, Institute for Cancer Research, Philadelphia, PA 19111. The absence of detectable pre-B, B and T cells in the mouse mutant scid has been postulated to result from a defective V(D)J recombinase activity. This postulate is based on the detection of aberrant Ig and TCR gene rearrangements in transformed B and T lineage cells of scid mice. To test this hypothesis, we introduced a functionally rearranged μ-heavy chain gene into the scid mouse genome to see whether pre-B cells would now appear in such mice, (μ-transgenic scid mice) as predicted by the hypothesis. Examination of bone marrow cells from μ-transgenic scid mice revealed the presence of pre-B cells at levels comparable to those found in μ-transgenic control littermates. However, cells bearing surface IgM were not detectable in the lymphoid tissues of μ-transgenic scid mice. It was of interest therefore, to determine whether the pre-B cells arising in μ-transgenic scid mice attempt light chain gene rearrangements, and if so, whether these rearrangements are aberrant. For this purpose, we employed the polymerase chain reaction (PCR) technique to amplify fragments containing $V_\kappa J_\kappa$ rearrangements. Preliminary results show the existence of cells with $V_\kappa J_\kappa$ rearrangements in μ-transgenic scid bone marrow, however, the frequency of such cells in ~3 orders of magnitude lower than that observed in bone marrow of μ-transgenic control mice. No $V_\kappa J_\kappa$ rearrangements were detected in bone marrow of non-transgenic scid mice. Cloning and sequencing of several amplified $V_\kappa J_\kappa$ fragments from μ-transgenic scid bone marrow should be informative regarding the nature and diversity of these rearrangements.

M 519 V_H GENE ALTERATIONS DURING THE EVOLUTION OF A HUMAN B CELL LYMPHOMA WITH A CONSERVED AUTOIMMUNE SPECIFICITY, David F. Friedman, June Goldman, and Leslie E. Silberstein, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.
We have previously described EBV transformed human B cell lymphoma lines from a patient with cold agglutinin disease; the cell lines have the same abnormal karyotype as the tumor, and the line's secreted immunoglobulin has the same specificity (auto-anti-Pr_2) and IEF pattern as the pathogenic autoantibody. The expressed V_H and V_L genes of seven subclones were nearly identical, demonstrating that the mutation rate in the tumor at diagnosis was low. We now report several changes in the expressed V_H gene in tumor recurrences 3 and 5 years later. Southern Blot analysis using HindIII and XBA1 reveals 2 identical J_H hybridizing bands at all time points. In the ECOR1 digest, 1 of the 2 J_H fragments is replaced by a smaller fragment in the 3 year specimen, and the other J_H fragment is replaced by a different, smaller band in the 5 year specimen. From the 3 year tissue, we prepared cDNA with a C_u specific primer, and amplified the product with PCR, using primers taken from the leader and the DJ regions. The amplified material was sequenced directly (consensus sequence) using the same 2 primers, and was also ligated into PBS, subcloned, and 9 subclones sequenced from the M13 universal primer. The consensus sequence had 12 basepair changes from the original sequence, 4 replacement and 8 silent mutations. Seven of the 9 subcloned sequences were identical to the consensus, 2 had single basepair changes from the consensus. These preliminary findings suggest that V gene replacement with a closely related V gene may account for the different ECOR1 restriction pattern and the 12 nucleotide changes. The near identity of the subcloned sequences to the consensus means that at relapse, as at diagnosis, there is little microheterogeneity of Ig gene sequence within the tumor, suggesting that selection, possibly by the autoantigen, may play a role in V gene alterations during tumor progression.

M 520 CD23 AND SERUM THYMIDINE KINASE AS MARKERS OF DISEASE ACTIVITY IN B CLL. J Gibson, S Neville, R Brown, D Joshua and H Kronenberg. Haematology Department Royal Prince Alfred Hospital Sydney, Australia. Although the Rai and Binet staging systems are of value in estimating survival in B CLL it is recognised that they are often unable to accurately predict disease progression. The existence of patients with advanced stage disease but with a clinically stable course as well as patients with apparently early stage disease but with a progressive course indicates the need for additional prognostic markers. The studies described in this abstract evaluated five variables as potential markers of disease activity in B CLL: the distribution of four cell surface antigens CD5, CD23 the IL2-receptor and the antigen detected by FMC7 antibody on peripheral blood mononuclear cells and serum levels of the enzyme thymidine kinase (STK). In 55 patients with B CLL these variables were correlated with Rai stage (0-II vs III-IV) and whether the patient was considered to have indolent (n=24) or active (n=31) (chemotherapy-requiring) disease. The most interesting correlation was found with CD23, a B cell activation antigen. A highly significant correlation existed between the mean percent (±SEM) (and absolute number) of CD23 positive cells and the presence of active or indolent disease (53.5±3 vs 30.2±5.3, $2p<0.001$). In contrast no correlation was found between the levels of CD5, IL2-R or FMC7 positivity and disease activity. STK levels were also found to correlate with disease activity with significantly higher levels found in patients with active disease than in patients with indolent disease (12.9±2.5 vs 3.0±1.8, $2p<0.005$). STK was independent of CD23. In summary, the activated B cell phenotype (CD23+) and high levels of STK are independent markers of disease activity in B CLL. Longitudinal studies in individual patients are now in progress.

M 521 WILD-TYPE V(D)J RECOMBINATION IN *SCID* PRE-B CELLS, Eric A. Hendrickson[1], Mark S. Schlissel[2] and David T. Weaver[1], [1]Division of Tumor Immunology, Dana Farber Cancer Institute and the Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts 02115 and [2]The Whitehead Institute and the Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139.
Homozygous mutation at the *scid* locus in the mouse results in the aberrant rearrangement of immunoglobulin (Ig) and T-cell receptor gene segments. We introduced a retroviral vector containing an Ig rearrangement cassette into *scid* pre-B cells. Following inversional rearrangement, we identified two clones containing perfect reciprocal fragments and wild-type coding joints, documenting for the first time the ability of *scid* pre-B cells to generate functional protein-coding domains. Subsequent induced rearrangement of the endogenous kappa (k) loci in these two cell clones resulted in a mixture of *scid*-and wild-type V-Jk joints as assayed by a polymerase chain reaction and DNA sequencing. Additionally, 3 Ig m^- *scid* pre-B cell lines showed both *scid* and wild-type V-Jk joins. These experiments strongly suggest that the V(D)J recombinase activity in *scid* lymphoid cells is diminished, but not absent, thus providing the molecular foundation for the known "leakiness" of the *scid* mutation.

M 522 GENERATION OF HUMAN MYELOMA CELL LINES FROM PRIMARY MYELOMA COLONIES
J.Hitzler, T.Takahashi, G.Wandl, N.Jamal, H.A.Messner, Ontario Cancer Institute, 500 Sherbourne Street, Toronto, Ontario M4X 1K9, Canada
Patients with advanced multiple myeloma have a high probability of forming large myeloma colonies when bone marrow or peripheral blood samples are cultured in methylcellulose supplemented with Iscove's medium, human plasma, and PHA-LCM as a source of growth factors. Primary myeloma colonies may reach a size of 20-50000 cells and can either be recloned in semisolid medium or propagated in liquid suspension culture. Using this system, 7 human multiple myeloma cell lines (OCI-Myl to 7) were established. 6 of these lines proliferate without addition of exogenous growth factors whereas growth in one line is dependent on the addition of IL-6. All lines retain the secretion of their respective M-protein. Characterization using a panel of B-cell related monoclonal antibodies demonstrated for the majority of these lines a mature plasma cell-like phenotype: 6/7 lines reacted with PCA-1, 3/7 in addition with CD38; one line was negative for both. Earlier antigens like CD19, CD10 were either completely absent or weakly expressed in only one line. None of these lines reacted with CD34, two however were positive for CD33. The subpopulation of clonogenic cells was shown to have a size distribution similar to that of the unseparated population while cells with high self-renewal capability were predominantly found in fractions of small-sized cells. A discriminating cell surface marker for clonogenic cells could not be identified, although B1 enriched clonogenic cells in one population. Further studies to identify the clonogenic cell in multiple myeloma and to investigate possibly autocrine growth factor production in the factor-independent lines are in progress.

M 523 Possible Role of Fc Receptor Phosphorylation in Modulating B-Cell Activation by Membrane Immunoglobulins. Walter Hunziker, Terry Koch, Andrew Whitney and Ira Mellman. Department of Cell Biology, Yale University School of Medicine, New Haven, CT 06510.
Fc receptors for IgG (FcRII) mediate a wide range of functions including endocytosis of antigen-antibody complexes, triggering the release of inflammatory and cytotoxic agents and modulating the activation of B lymphocytes. cDNAs encoding three distinct murine FcRII isoforms (FcRII-A, -B1 and -B2) have been cloned, each of which may be associated with specific functions. The FcRII-B2 isoform, expressed predominantly by macrophages, is responsible for the coated pit-coated vesicle mediated internalization of antibody-antigen complexes. In contrast, the closely related FcRII-B1 isoform, expressed by B-lymphocytes, is comparatively unable to mediate endocytosis. Due apparently to cell type-specific alternative mRNA splicing, FcRII-B1 contains an in-frame insertion of 47 amino acids in its cytoplasmic tail that prevents coated pit localization. While the functions of FcRII-B1 on B lymphocytes remain poorly defined, this isoform is known to play a role in regulating B-cell activation by membrane immunoglobulins (mIg). Crosslinking mIg can result in B-cell differentiation and proliferation via stimulation of phospholipase C, mobilization of cellular calcium and activation of protein kinase C. However, when FcR are cross-linked together with mIg the receptors confer a dominant inhibitory signal on B-cell activation. The mechanism by which FcR prevent B-cell activation is unknown. Here we show that the FcRII-B1 isoform is serine-phosphorylated in response to stimuli that result in B-cell activation via mIg or phorbol esters; phosphorylation does not occur when mIg is directly cross-linked to FcR. Since the FcRII-B2 is not phosphorylated under any conditions, it is clear that the cytoplasmic tail insertion in FcRII-B1 introduces specific phosphorylation sites that may play a role in regulating B-cell activation, in addition to disrupting a domain needed for coated pit localization.

M 524 V GENES ENCODING AUTOANTIBODIES IN MOTHEATEN MICE, Kuppuswamy N. Kasturi and Constantin A. Bona, Department of Microbiology, Mount Sinai Medical Center, New York, NY 10029. We have investigated the genetic and structural basis of the specificity of autoantibodies produced by mev mice. More particularly we have examined whether somatic mutations contribute to the specificity of these autoantibodies since Ly 1 subset of B cells predominates in this strain. The nucleotide sequence of V genes coding for 3 autoantibodies(mAbs) specific for thymocytes and erythrocytes, random selected from a panel of hybridomas derived from mev mice, were determined. All three antibodies were found to use Vk genes from Vk 9 gene family and Vh genes from J606 and S107 gene families. All Vk genes and the Vh S107 do not show any apparent somatic mutations. The nucleotide sequence of the Vh(J606) genes used by 2 autoantibodies are identical although they bear different isotypes. The sequence data indicate that this Vh gene is distinct from any known germline genes. Whether somatic mutations have occurred in the Vh genes coding these antibodies (conferring the particular autoantibody specificity) was investigated by Southern analysis of genomic DNA using CDR specific oligonucleotide probes corresponding to CDR1 and CDR2 of the mRNA. The results show that there were no somatic mutations in CDR2 while mutations might have occurred in CDR1. To determine the degree of somatic mutation we are currently cloning the germline gene corresponding to the Vh genes used by these autoantibodies.

M 525 FUNCTIONAL PRE-B CELL CLONES FROM LEAKY SCID MICE. Debra B. Kotloff, Norman R. Ruetsch and Melvin J. Bosma. Fox Chase Cancer Center, Institute for Cancer Research, Philadelphia, PA 19111

Severe combined immunodeficient mice (scid mice) bear an autosomal recessive mutation which manifests as a severe deficiency in functional B and T lymphocytes. This deficiency appears to result from a defect in the recombinase enzyme system which mediates the assembly of V, D and J gene segments into coding sequences for immunoglobulin and T cell receptor variable regions. Successful assembly of V, D and J elements does occur in some scid lymphocytes, however, as reflected by the emergence of a limited number of functional B and T cell clones in some scid mice. In order to understand how such functional lymphocytes may arise in the presence of a defective recombinase enzyme system, we attempted to obtain potential precursors of functional scid B cells by Abelson murine leukemia virus (A-MuLV)-transformation of pre-B cells from leaky scid mice. Four cytoplasmic μ^+ clones have been isolated from these mice. These clones represent the first reported examples of A-MuLV-transformed scid cells which express productive Igh gene rearrangements. Southern blot analyses of genomic DNA revealed conventional gene rearrangements in three of the four clones, while one clone deleted the entire Jh region of its nonproductive allele. Thus far, VDJ recombination activity has been evaluated in one clone by a transient transfection assay employing extrachromosomal recombination substrates. The recombination activity was found to be normal in this clone, suggesting the occurrence of a somatic event that resulted in an apparently normal recombinase phenotype.

Supported by NIH grants and a Cancer Research Institute Fellowship to D.B.K.

M 526 INHIBITED INDUCTION OF NEUROMUSCULAR DYSFUNCTION CAUSED BY ANTI-ACETYLCHOLINE RECEPTOR ANTIBODY IN LEWIS RATS: CHARACTERIZATION OF "PROTECTIVE" ANTIBODIES REACTIVE WITH DENATURED EPITOPES. Keith A. Krolick and Trai-Ming Yeh. Department of Microbiology, University of Texas Health Science Center at San Antonio, San Antonio, Texas 78284

Antibodies produced in rats against an irreversibly denatured form of the acetylcholine receptor (AChR) were capable of reacting with AChR of intact conformation. However, antibodies produced against denatured AChR were not capable of producing the AChR-dependent neuromuscular impairment seen following immunization with AChR of intact conformation; this immunopathological difference was observed despite the fact that both immunizations resulted in the production of clonotypically heterogeneous antibodies with similar titers and relative binding avidities for conformationally intact AChR, and a dominance by the IgG2a isotype. Although they had no apparent disease-causing potential of their own, antibodies produced against denatured AChR could mediate AChR-dependent neuromuscular impairment if conditions were provided leading to enhanced AChR crosslinking. Finally, when serum antibodies produced against denatured AChR were present (either by active immunization or by passive transfer), rats were observed to be refractory to the usual pathological effects of antibodies produced against intact AChR. These observations suggest a that disease severity in this system may be influenced by the relationships between at least two subsets of the total antibody repertoire for AChR, one that is conformation-dependent and disease-causing, and a second that can be potentially protective.

M 527 RESCUE OF THE B-CELL LINEAGE IN SCID MICE WITH Ig TRANSGENES, Marinus C. Lamers, Horst Mossmann, Georges J.F. Köhler, Max-Planck-Institut für Immunbiologie, 7800 Freiburg, FRG.

We have crossed S(evere) C(ombined) I(mmuno) D(eficiency) mice with mice carrying Ig transgenes, and we analysed the B-cell lineage in SCID transgenic offspring. In SCID mice with μ and κ transgenes a complete rescue of the $B220^+$, $s\mu^-$ pre-B-cell pool in the bone marrow was found. However, $s\mu^+$ cells in the bone marrow were rare, and absent in the spleen. Some $s\mu^+$ cells were found in the peritoneal cavity. These data show that a μ chain is essential for normal pre-B-cell development. We are in the process of analysing mice with μ and δ transgenes, and deletional variants of the μ transgene to establish minimal requirements of the μ chain for B-cell development.

M 528 ISOLATION OF A 26KD MEMBER OF A NEW TRANSMEMBRANE PROTEIN FAMILY, Shoshana Levy, Rachel Oren, Shuji Takahashi, Carol Doss and Ronald Levy Stanford University School of Medicine, Stanford, CA 94305

A murine monoclonal antibody which inhibited the proliferation of a human lymphoma cell line was isolated. The antibody reacted with a 26 kd cell surface protein and induced a reversible anti-proliferative effect on the cells. Most human cell lines, including hematolymphoid, neuroectodermal and mesynchymal, expressed the p26 antigen. However only a subset of these cell lines, including several large cell lymphomas, were susceptible to the anti-proliferative effects of the antibody. A cDNA clone coding for the protein was isolated by using the monoclonal antibody to screen an expression library in COS cells. Analysis of the deduced amino acid sequence indicates that the protein is highly hydrophobic and that it contains multiple transmembrane domains. The p26 showed strong homology with the CD37 antigen and with a previously described protein expressed in the early stages of tumorogenesis of melanoma.

M 529 **Expression of MMTV antigen(s) on bone marrow pre-B cells of BALB/c mice.**

Mayra M. Lopez-Cepero, Yang Wang, Richard Riley and Diana M. Lopez
Department of Microbiology and Microbiology,
University of Miami, School of Medicine, Miami Fl.

Mouse mammary tumor virus (MMTV) is a type B retrovirus capable of inducing mammary carcinomas in mice. Restriction endonuclease mapping and molecular hybridization studies have revealed the presence of MMTV genomes integrated in the DNA of germinal and somatic normal cells of all inbred mouse strains. BALB/c mice, which lack the exogenous milk transmitted MMTV, have two genome size proviruses designated as Mtv-8, Mtv-9 and a subgenomic provirus designated as Mtv-6. Previous studies in our laboratory have demostrated preferential RNA expression of genes from the endogenous retrovirus in B cells from the BALB/c mice. Western blot analyses show a band of 70-72,000 m.w. in purified splenic B cell preparations. This molecular weight corresponds with a precursor of MMTV env protein. In contrast, Western blot analyses of a purified subset of bone marrow cells, pre-B ($B\ 220^+$, sIg^-), shows a band of 75-77,000 m.w. that specifically reacts with a polyclonal anti MMTV antibody. Precursor proteins of gag and env MMTV genes show similar m.w. DNA probes that individually code for the env, gag, and LTR regions of MMTV are being used to identify the mRNA that codes for the protein expressed on bone marrow pre-B cells. Proliferative responses to the products of the endogenous MMTV have been described previously. Positive responses correlate with a certain degree of protection against the development of exogenous MMTV expressing mammary tumors. Thus, the characterization of endogenous MMTV antigen(s) present on B cells and B cell precursors is of great importance to document the origen of the immune responses so effective in the host defense against tumorigenesis.

M 530 IDIOTYPIC INTERACTIONS IN AUTOIMMUNITY: ANTI-IDIOTYPES AGAINST AUTOANTIBODIES IN NORMAL HUMAN IMMUNOGLOBULINS. Inger Lundkvist and Anneke Brand, Department of Immunohaematology and Blood Bank, Academic Hospital Leiden, The Netherlands.

Some autoimmune diseases are today successfully treated with intravenous administration of high doses of pooled, polyspecific human immunoglobulin G (IVIg). We have investigated the role of idiotype-anti-idiotype interactions in spontaneous and IVIg-induced recovery from an autoimmune disease of the peripheral nervous system: the Guillain-Barré syndrome (GBS) and its chronic/relapsing form CIDP. Anti-peripheral nerve autoantibody activity was measured by indirect immunofluorescence using a selected neuroblastoma cell line (NBL 108cc15) cross-reactive with human peripheral nerve tissue. In vitro analyses show that IVIg, as well as serum from spontaneously recovered GBS patients, contain antibodies from which the $F(ab')_2$ fragments could inhibit autologous and allogeneic anti-NBL autoantibody activity in the pre-recovery phase. Interestingly, these anti-idiotypes are also found in all normal, healthy individuals so far tested, albeit to various extents. Comparison of the immunoglobulin molar ratios giving 50% inhibition of anti-NBL autoantibody activity in individual patients showed that the degree of inhibition appeared to be independent of the source of the anti-idiotypes (IVIg, serum from recovered GBS patients or individual donors). We have observed similar effects after treatment in vivo, sustaining the notion that the outcome is patient-related. Taken together, these results suggest that prevention of an autoimmune disease and spontaneous as well as therapeutically-induced recovery is due to successful suppression of autoantibodies by anti-idiotypic antibodies.

M 531 CONSTRUCTION OF RAT IgE CONSTANT REGION DOMAIN DELETION MUTANTS TERMINATING IN EXONS OTHER THAN CH4, D. Randy McMillan and Charles Faust, Department of Biochemistry and Molecular Biology, Texas Tech University Health Sciences Center, Lubbock, TX 79430. IgE is a central figure in allergic reactions, as a consequence of its very specific interaction with a high affinity receptor on the surface of mast cells and basophils, and the subsequent cross-linking of this receptor-bound IgE rat with allergen. Here we report the construction and characterization of a series of mutant IgEs, all lacking the epsilon heavy chain CH4 domain. The authentic rat epsilon rearranged (Molec. Cell. Biol. 7:2614-2619, 1987) and germline epsilon heavy chain (J. Biol. Chem. 264:1846-1853, 1989) clones were described previously. Single exon modules were constructed from these, characterized and reassembled into various combinations of assemblies encoding mutants of the epsilon heavy chain gene without the CH4 exon. The missing CH4 exon was replaced with a synthetic exon module facilitating transcriptional termination and polyadenylation, as well as translational termination. Each assembly was verified and then electroporated, together with the xgprt gene, into the rat kappa light chain secreting myeloma, Y3. Co-transfectants were selected for resistance to mycophenolic acid. Northern blot analysis was performed on each transfection, confirming the expected mRNA product. Each transfectant was labeled with [35-S] methionine and cysteine, and the secreted product was characterized by immunoprecipitation and SDS gel analysis. In each case the expected protein product was produced. These IgE products will be useful in examining the molecular interaction of IgE with the high affinity Fc receptor on the mast cell. This work was supported in part by NIH grant, AI-23456.

M 532 DIFFERENTIAL METHYLATION OF THE EPSTEIN BARR-VIRUS GENOME IN BURKITT'S LYMPHOMAS AND LYMPHOBLASTOID CELL LINES, Janos Minarovits, Ingemar Ernberg, Kerstin Falk and George Klein, Department of Tumor Biology, Karolinska Institute, S-104 01 Stockholm, Sweden
We studied the methylation pattern of Epstein-Barr virus (EBV) genome in DNA samples isolated from Burkitt's lymphoma (BL) biopsies, in vitro growing BL cell lines and lymphoblastoid cell lines (LCLs) transformed by EBV using the isoschizomers HpaII (methylation sensitive) and MspI (methylation insensitive). We found that the EBV DNA is methylated in BL biopsies and in the BL line RAEL (a group 1 cell line expressing only EBNA 1) while unmethylated in LCLs (including CB-MI-RAL-STO transformed by the virus rescued from RAEL) in the regions investigated (BamHI W, H, E, M, K, and the coding region of the latent membrane protein, LMP). The group 2 and 3 BL cell lines studied (expressing EBNA 1, 2, 3, 4, 6 and LMP, like the LCLs) showed a mixture of methylated and unmethylated sequences in the regions investigated. This is probably due to lytic replication of EBV in a minority of the cells since treatment of the cells with phosphonoformic acid, an inhibitor of the lytic cycle, resulted in the disappearance of unmethylated HpaII fragments in the BamHI W and BamHI E regions investigated. We compared the methylation pattern of cellular DNA in RAEL and CB-MI-RAL-STO using a series of methylation sensitive enzymes and sheared human placental DNA as probe. We found that the cellular DNA is more methylated in RAEL than in the corresponding LCL. We conclude that the pattern of methylation of the EBV genome is cell type specific. This pattern reflects the general methylation pattern of the cellular genome in case of RAEL and CB-MI-RAL-STO.

M 533 ANTI-HISTONE H1 AUTOANTIBODIES IN AUTOIMMUNE MICE, Marc Monestier and Thomas M. Fasy, Center for Molecular Medicine and Immunology, Newark, NJ 07103 and Department of Pathology, Mount Sinai Medical Center, New York, NY 10029.
Anti-histone H1 autoantibodies are detected in the sera of human patients or mice with systemic autoimmune diseases. Even though these autoantibodies are useful in the classification of autoimmune syndromes, their possible pathogenic role remains unknown. Histone H1 is a 23 kD protein composed of 3 domains: N-H1 (NH_2-terminal), G-H1 (globular) and C-H1 (COOH-terminal). IgM anti-H1 autoantibodies found in the sera of autoimmune mice bind mostly to determinants located in the C-H1 domain whereas IgG antibodies also recognize conformational epitopes requiring the integrity of both the G-H1 and C-H1 domains. These IgG anti-GC-H1 antibodies rise steadily with age in MRL lpr/lpr and NZB mice while IgM and IgG antibodies directed against the C-H1 domain stay at constant levels during the evolution of the disease. Using H1 molecules from various species, IgM antibodies were found to bind equally to all H1 variants tested, whereas IgG antibodies show decreased binding to phylogenetically distant H1 molecules. The increase in the specificity of anti-H1 antibodies following the IgM-IgG switch suggests that the activation of histone-specific autoreactive clones is triggered by the autoantigen itself. The biodistribution of radiolabeled histones and anti-histone antibodies injected into normal BALB/c mice was also studied. The results suggest that anti-histone antibodies can mediate kidney injury through binding to histones deposited on the glomerular basement membrane. Supported by NIH grant AI26665.

M 534 EXPERIMENTAL MODEL FOR THE ANALYSIS OF AUTOIMMUNITY IN PROGRESSIVE SYSTEMIC SCLEROSIS, Tai Muryoi, Kuppuswamy N. Kasturi and Constantin A. Bona, Department of Microbiology, Mount Sinai Medical Center, New York, NY 10029. Tight skin mice develop cutaneous hyperplasia and histopathological alterations of skin similar to those described in diffuse type of scleroderma. These mice also produce anti-topoisomerase I specific autoantibodies, characteristically present in the sera of a subpopulation of the scleroderma patients. However, the etiology of the human disease is unknown. From unimmunized tight skin mice spleen cells we have isolated a number of hybridomas secreting monoclonal antibodies specific for topoisomerase I. We have examined the immunochemical and functional properties of these antibodies and compared with that of scleroderma patients. Results of Western blot analysis and competitive inhibition in RIA indicate that mouse anti topoisomerase I antibodies recognize the same or similar epitopes recognized by autoantibodies found in scleroderma patients. Further more, one monoclonal antibody (34A1-26) also neutralises the enzymatic activity of topoisomerase I similar to autoantibodies present in scleroderma. These results suggest that the autoantibodies present in scleroderma patients and tight skin mice recognize some conserved epitopes present on the topo I polypeptide which confer the catalytic property for the enzyme.

M 535 ANALYSYS OF THE PHENOTYPE INDUCED BY THE ras ONCOGENE IN HUMAN B LYMPHOCYTES, Sergio Nasi, Isabella Sirinian,* Gianna Panetta, Alessandra Marchetti and Richard Jucker, Centro Acidi Nucleici C.N.R., Università "La Sapienza", 00185 Roma, Italia, tel. (6) 49912227

Human lymphocytes immortalized by the Epstein-Barr virus (EBV) are an attractive model system for studying B cells transformation mechanisms. By electroporation with plasmids DNA carrying the entire T24-ras oncogene, we have isolated stably transformed cell lines which express different levels of the oncoprotein and studied their phenotype. The presence of ras causes tumorigenicity in nude mice according to its expression level and the transformed lymphocytes are immature and morphologically similar to cells of large cell lymphoma. Parameters of growth in culture, e.g. cloning efficiency in agarose, vary among different ras transformed lines and are intermediate between parental lymphoblastoid cells and reference neoplastic lymphocytes such as Burkitt lymphoma cells Raji.

It was reported that an overexpressed myc oncogene induces the tumorigenic conversion of EBV immortalized lymphocytes and therefore we suggest that the two oncogenes myc and Ha-ras may act on the same regulatory switch. In this regard, preliminary results showed an increased myc mRNA level in our ras transformed lymphocytes.

We are investigating the expression of lymphocytes maturation markers and regulatory molecules, because we think that such a study can shed light on the regulation of B cells transformation and/or differentiation by the Ha-ras gene.

*recipient of an AIRC fellowship

M 536 IGA RECEPTORS OF T560, A B-LINEAGE LYMPHOMA FROM GALT, Julia M. Phillips-Quagliata, T. Dharma Rao, Andres Gonzalez and Azzam Al Maghazachi, Dept. of Pathology, NYU Medical Center, New York, NY 10016. Lymphoma T560 originated in the GALT of a (B10.A X B10.H-2^aH-4^b)F1 hybrid mouse recipient of B10.H-2^aH-4^b cells in the laboratory of Dr. G. Haughton, U. N.C. It lacks Thy 1, Lyt 1, Lyt 2, Mac1 and Mac 2 and bears B220 and Ia . It is, however, Ig-negative and secretes not only IL-1 and IL-6 but also IL-4 so is not a typical B cell. Both of 2 cultured lines of T560 are virtually 100% Ia-positive, but T560.2 secretes more lymphokine than T560.1 and is much more efficient at stimulating in an MLR and presenting antigen to an antigen-reactive T cell line. Receptors for IgA, IgG2a and IgG2b on the lines have been examined by rosette assays. Line T560.1 bears IgA receptors (IgAR)on only a low percentage of cells but has a moderately high percentage of IgG2b receptor (FcRII)-bearing cells; T560.2 has a high percentage of IgAR-bearing cells and a low percentage of FcRII-bearing cells. The IgAR differ from those previously described on T cells in being readily inhibitable not only by soluble IgA but also by IgM and by high concentrations of IgG2a and IgG2b. In their cross-reactivity with IgM, they resemble the poly-Ig receptor of glandular epithelial cells but the poly-Ig receptor is not thought to be present on lymphoid cells. T560 IgAR are not inhibitable by any of a variety of sugars so probably do not bind IgA and IgM through carbohydrate sidechains on the Igs. They are 100% trypsin-sensitive and neuraminidase-resistant and about 65% sensitive to phosphatidylinositol (PI)-specific phospholipase C (PIPLC). IgAR activity is abrogated by exposing T560.2 cells to phorbol myristate acetate (PMA) and the PMA effect is completely reversed by staurosporine, a protein kinase C (PKC) inhibitor. Since activation of PKC destroys IgAR activity, the possibility that PIPLC and trypsin might not cleave the receptor from the cell surface but instead cause activation of PKC was considered. Staurosporine reversed approximately 50% of the PIPLC effect suggesting that PKC activation accounts for part but not all of the effect of PIPLC. It plays little role in the effect of trypsin. The results suggest that the IgAR is a polypeptide about 30% of which is bound to the cell membrane via a PI linker. Recovery of IgAR activity after treatment of T560.2 cells with trypsin followed by washing and reculture is cycloheximide (CHX)- sensitive, whereas that occurring after PMA-treatment is CHX-resistant. Evidently, PKC activation with consequent phosphorylation of membrane proteins does not cause shedding of the IgAR but either a change in its affinity for IgA or interiorization. Supported by USPHS Grant AI 20786.

M 537 CLONAL DIVERSITY IN THE B CELL REPERTOIRE OF PATIENTS WITH X-LINKED AGAMMAGLOBULINEMIA, Brian A. Pollok, Roberto Anker, and Mary Ellen Conley*, Dept. of Microbiology and Immunology, Wake Forest University Medical Center, Winston-Salem, NC 27103 and *University of Tennessee College of Medicine, Memphis, TN 38103

Immunoglobulin protein and mRNA expression was examined in a collection of 18 monoclonal Epstein-Barr virus-transformed B cell lines derived from 5 patients with X-linked agammaglobulinemia (XLA). A diversity of heavy and light chain isotypes were synthesized by these lines: the majority (12 lines) expressed $\mu\kappa$ chains, while $\mu\lambda$ (2 lines), $\gamma\kappa$ (1), $\gamma\lambda$ (1), $\delta\lambda$ (1) and $\alpha\kappa$ (1) isotype expression was also observed. For all the $\mu\kappa$-producing XLA B cell lines, the μ and κ mRNA transcripts were of native size and sequence analysis across the regions of V_HDJ_H and $V_\kappa J_\kappa$ joining showed that immunoglobulin gene rearrangements occurred in a typical manner. A variety of V_HDJ_H and $V_\kappa J_\kappa$ gene rearrangements were observed, not only within the set of $\mu\kappa^+$ XLA B cells as a whole, but also among the cell lines derived from single patients. Southern blot analysis for genomic immunoglobulin heavy chain gene rearrangements was done to fully assess the extent of clonal heterogeneity among multiple $\mu\kappa^+$ XLA B cell lines derived from two patients; all the B cell lines possessed distinct gene rearrangement patterns demonstrating their clonal unrelatedness. Our findings indicate that the B cell repertoire in individual XLA patients is clonally diverse and that it is unlikely that the defect in B cell differentiation in XLA is the result of inefficient or ineffective rearrangement of immunoglobulin heavy or light chain genes. Rather, this study provides support for the idea that the XLA defect relates to a more generalized cellular function such as regulating the proliferation and/or clonal expansion of cells of the B lymphoid lineage.

M 538 HEAVY AND LIGHT CHAIN CONTRIBUTIONS TO DOUBLE STRANDED DNA BINDING BY ANTIBODIES, Marko Z. Radic, Mary Ann Mascelli, Jan Erikson, and Martin Weigert, Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, PA 19111

Antibodies to double stranded (ds) DNA arise spontaneously in patients with Systemic Lupus Erythematosus and in the MRL/lpr/lpr strain of mice. Murine anti-dsDNA antibodies show evidence for preferential V gene use and rearrangements leading to arginine-rich Vh CDR3 regions. Anti-dsDNA specificity, in most cases, also depends on the selection of suitable somatic mutations during clonal expansion.

The relative contribution of these factors for anti-dsDNA autospecificity was evaluated by transfection of the cloned anti-dsDNA heavy chain gene, 3H9, or site-directed mutants of 3H9, into a variety of hybridoma lines lacking endogenous heavy chains. Transfection of the 3H9 heavy chain into its clone members that previously were unable to bind dsDNA showed that somatic changes in this heavy chain lead to dsDNA specificity. Transfection of the 3H9 gene into heavy chain loss lines expressing light chains of different length-groups showed that dsDNA specificity is compatible with a wide range of light chain sequences, but that the relative affinity for dsDNA is modulated by light chain contributions.

M 539 NEONATAL T CELL TRANSFER RESCUES SCID B CELLS, James E. Riggs, Steve Stowers, and Donald E. Mosier; Division of Immunology; Medical Biology Institute; 11077 North Torrey Pines Road, La Jolla, CA 92037

We have recently discovered that SCID recipients of neonatal splenic (NSP) T cells or thymocytes (NTHY) cells from BALB/c mice congenic for the X chromosome-linked immune-deficiency (B.*xid*) produce normal (BALB/c) levels of IgM. The IgM produced was that of the SCID recipient (IgM^b) as shown by allotype-specific ELISA and low dose irradiation of the recipients prior to transfer. Nylon wool-passed B.*xid* NSP and BALB.*xid* NTHY promote SCID B cell breakthrough, whereas T cell depleted NSP do not, illustrating that T cells are requisite. The reduced capacity of XID B cells to secrete IgM is necessary to "permit" SCID B cell rescue as IgM^a production after BALB/c NSP or NTHY transfer masks IgM^b production. Transfer of adult BALB/c or adult BALB.*xid* thymocytes, lymph node or spleen T cells, and bone marrow fail to promote SCID B cell rescue. Allotype-specific spot-ELISA revealed that the SCID antibody secreting cells were evident in the spleen, peritoneal cavity, and bone marrow. These data will be discussed with respect to SCID B cell rescue by autoreactive T cells. (supported by NIH grants RO1 A1-22871 and PO1 A1-24526)

M 540 AUTOIMMUNE NZ MICE EXHIBIT DECREASED MITOTIC ACTIVITY AMONG B CELL PRECURSORS WITH AGE, Richard L. Riley and Mark G. Kruger, Department of Microbiology and Immunology, University of Miami School of Medicine, Miami, FL 33101

In autoimmune NZB and (NZB x NZW)F1 (BWF1) mice, the number of pre-B cells ($c\mu^+Ly5[220]^+$) and earlier B cell progenitors ($c\mu^-Ly5[220]^+$) demonstrate an accelerated and progressive decline with age (Riley R.L., et. al. 1989. Clin. Immunol. Immunopathol. 51:372). Since proliferation among B cell precursors is necessary for the maintenance of normal steady-state levels of B lineage cells, the mitotic activity of the $sIg^-Ly5(220)^+$ bone marrow cells was measured in NZB, and conventional strains of mice as a function of age. The proportion of $sIg^-Ly5(220)^+$ B cell precursors in (S + G2/M) phases of cell cycle were comparable in both young normal and NZ mice (3-16 weeks). However, at $\geq$20 weeks of age the mitotic activity of NZ B cell precurors was significantly reduced compared to age matched normal controls (Kruger, M.G. and Riley, R.L. 1990. J. Immunol., in press). The rate of accumulation of $sIg^-Ly5(220)^+$ bone marrow cells into G2/M phase was measured by vincristine induced metaphase arrest. Young NZB mice (11-16 weeks) and normal mice (10-35 weeks) displayed similar rates of accumulation in G2/M (3.9%-5.7%/hr.). However, upon vincristine induced metaphase arrest older NZB ($\geq$20 weeks) B cell precursors accumulated into G2/M phase at substantially lower rates (1.5%/hr.). Therefore, the reduction in B cell precursor mitotic activity may contribute to the age-related decline in B cell precursor numbers in autoimmune NZ mice. Supported by NIH grant AI23350 and a Lupus Foundation award to RLR.

M 541 CHROMATIN STRUCTURE AND TRANSCRIPTIONAL REGULATION OF FcERII/CD23 GENE EXPRESSION IN EBV-INFECTED B CELLS, W.H. Schubach, M. Holloway, J. Tierney Department of Medicine, SUNY Stony Brook, Stony Brook, NY 11794.

The B cell receptor for IgE, (FcERII or CD23) is an early appearing antigen on activated B cells. FcERII plays pivotal roles in B cell differentiation, Epstein-Barr virus (EBV)-induced B cell immortalization, and regulation of the IgE response. EBV-negative Burkitt lymphoma cell lines that have been converted *in vitro* with the B95-8 strain of EBV show a 20 to 200-fold increase in FcERII expression as a result of induced gene transcription. By contrast, P3HR-1 converted cells, [(which lack the EB nuclear antigen2(EBNA2)], fail to show enhanced FcERII transcription. In an effort to explore the basis for this differential expression we have examined the chromatin structure of the FcERII gene in transcriptionally active lymphoblastoid cell lines (LCLs) and in both active and inactive, EBV-converted BL cells by determining the pattern and distrubution of DNaseI hypersensistive sites (DHSs) over a 25kb domain encompassing the gene and its 5'and 3' flanking sequences.

B95-8 converted BL cells display a prominent DHS near the beginning of exon 2 and two unique DHS in intron 3. There is no difference in the pattern of DHS seen in EBV-negative and P3HR-1 converted cell lines. Two LCLs (139 and 171) have unique DHSs at -4.8kb and in intron 1, while another LCL (IB4) has unique DHSs in intron 9, 11, and at the 3' end of the gene. These results suggest that EBNA2 and/or latent membrane protein can induce FcERII transcription by several distinct mechanisms.

M 542 A HUMAN NATURAL AUTOANTIBODY IS ENCODED BY DEVELOPMENTALLY RESTRICTED HEAVY AND LIGHT CHAIN VARIABLE REGION GENES, Katherine A. Siminovitch, Virginia Misener, Pak Kwong, Qian-li Song, Pei-Ming Yang*, Laurence A. Rubin and Pojen P. Chen*, University of Toronto, Toronto, Ontario, M5T 2S8 and *Scripps Clinic, La Jolla, CA 92037. To investigate the genetic basis of natural autoimmune responses, we have recently characterized the VH and VL genes encoding a tonsillar derived monoclonal autoantibody, Kim13.1, which reacts with cardiolipin and Fc of human IgG. The Kim 13.1 VH and VL sequences display 99% nucleotide sequence identity with two V genes, 51P1 and 38K, respectively, which appear to be preferentially expressed during early B cell ontogeny. Sequence comparisons between the Kim13.1 VH, 51P1 and other VHI genes, suggest that the single nucleotide difference distinguishing the Kim13.1 and 51P1 VH genes may represent allelic variation. The Kim13.1 VK gene sequence is identical to that of a germ-line VKIII gene, Vg. Taken together with our previous finding that another natural autoantibody, Kim4.6, uses a germ-line VH gene closely resembling a foetal-related V gene sequence, these results indicate that natural autoantibodies can be encoded by nonmutated germ-line V genes which are preferentially expressed in the early pre-B cell repertoire. The overlap between autoantibody-associated and early ontogeny B cell V gene utilization suggests that autoreactivity not only occurs in healthy individuals, but may be instrumental to the development and maintenance of the normal immune repertoire.

M 543 ANALYSIS OF PRIMARY IMMUNODEFICIENCIES USING SCID/hu CHIMERAS. C. I. Edvard Smith, Khalid B. Islam, Mohammad R. Abedi, Birger Christensson and Lennart Hammarström, Department of Clinical Immunology and Center for Biotechnology, Karolinska Institute at Huddinge Hospital, S-141 86 Huddinge, Sweden.

Reconstitution of immunodeficient SCID mice with human lymphoid cells results in chimeras designated SCID/hu. We have employed this method for the analysis of human primary immunodeficiencies. However, since the technique was first reported by McCune, Weissman et al and by Mosier et al. in 1988, and still is subject to further modifications, we have also investigated the effect of reconstitution with cells from normal healthy individuals. In an initial series, using the peritoneal reconstitution protocol, mice received either 20 x 10^6 ficoll-purified lymphocytes or 0.5 ml of concentrated whole blood cells per animal. Measurable levels of human immunoglobulins were only found in mice reconstituted with 20 x 10^6 cells. In most animals, human IgM, IgG as well as IgA was produced. However, IgG normally reached the highest level (measured as percentage of isotype level in normal human serum). The immunoglobulin synthesis could persist for several months, but normally declined after a few months. When a limited number of individuals with primary immunoglobulin deficiency have been analyzed, we have sofar not found expression of the deficient isotype in chimeras reconstituted either with patient cells alone or together with purified T-lymphocytes from healthy donors. As has previously been reported, tumors with a lymphoblastoid morphology developed in some animals.

M 544 LY-1 LINEAGE B CELLS DO NOT EXPRESS THE FcεR II, Karen Richter Snapp, John Cowdery , Richard Lynch, Thomas Waldschmidt, University of Iowa College of Medicine, Department of Pathology, Iowa City, Iowa 52242

By means of multi-color flow cytometry, we have previously shown that murine B lymphocytes can be divided into 2 separate populations based on surface IgM and FcεR II expression. Splenic B cells which are IgM^{lo}/FcεR+ are members of the conventional B cell lineage, whereas a second population which may be derived from the Ly-1 lineage is IgM^{hi}/FcεR- as well as Ly-1-. We found these 2 populations to be easily detectable in the spleens of various strains of autoimmune mice and in the peritoneal cavities of normal mice. The B cells which are FcεR- could not be induced to express the FcεR even after incubation with IL-4. Additional characterization of the splenic IgM^{hi}/FcεR- cells from autoimmune mice revealed that they can be further distinguished from conventional B cells based on a panel of cell surface markers. These include LFA-1, pgp-1, J11d, BLA-1, BLA-2 and MEL-14. Finally, functional experiments demonstrated that in the spleens of autoimmune mice, the large majority of induced autoantibodies are derived from the IgM^{hi}/FcεR- B cell population. In summary, this data indicates that in autoimmune mice, the FcεR delineates two distinct populations, and may be a more reliable marker for distinguishing between conventional and Ly-1 lineage B cells.

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M 545 CLONOTYPIC ANALYSIS OF THE ANTI-ACETYLCHOLINE RECEPTOR ANTIBODY RESPONSE. Patricia A. Thompson and Keith A. Krolick, Department of Microbiology, University of Texas Health Science Center at San Antonio, San Antonio, TX 78284

Dysfunction at the neuromuscular junction in the autoimmune disease myasthenia gravis (MG) has been attributed to the auto-antibody response against the acetylcholine receptor(AChR) located on muscle membranes. Curiously however, it has been observed in patients that an imperfect correlation exists between anti-AChR antibody titer and disease severity. For example, a patient with very low antibody titer may present with very severe disease, whereas a patient with very high antibody titer may present with only minimal disease. Thus, we have addressed this paradox in a Lewis rat model system of MG. Our goal has been to dissect and characterize the disease-causing potential of the anti-AChR response at the level of individual antibody clonotypes. Taking advantage of preparative isoelectric focusing (IEF) techniques, we have been able to separate and purify individual clonal antibody products of AChR-reactive B cells; purified anti-AChR antibody clonotypes have been tested in passive transfer studies for their ability to interfere with AChR-dependent neuromuscular function. Furthermore, using an anti-idiotypic probe generated against anti-AChR antibodies we are able to examine idiotypic associations with individual anti-AChR antibody clonotypes. Using these two approaches, anti-Id analysis and IEF, we are therefore attempting to characterize, at the clonotypic and idiotypic levels, antibody responses against AChR that differ with regard to disease inducing potential. The aim of these studies includes the identification and purification of anti-idiotypes which can distinguish between disease causing and non-disease causing anti-AChR antibodies. Our eventual goal is to generate anti-idiotypic probes specific for disease causing clonotypes in order to design more specific immunotherapies and potentially to monitor more directly those antibodies important in disease induction in human patients.

M 546 HUMAN FOLLICULAR (B-TYPE) LYMPHOMA CELLS ARE INDUCED TO PROLIFERATE BY THE COGNATE INTERACTION WITH A CD4+ T CELL CLONE. D. T. Umetsu, L. Esserman, T. A. Donlan, R. H. DeKruyff, and R. Levy. Departments of Pediatrics, Medicine, Surgery and Pathology, Stanford University School of Medicine, Stanford, CA 94305

We examined stimuli which are required for the induction of in vitro proliferation of follicular lymphoma cells, a low grade non-Hodgkin's B cell lymphoma characterized by a specific chromosomal translocation, and by in vivo growth of the lymphoma cells in germinal center-like follicles infiltrated with CD4+ T cells. The purified follicular lymphoma cells, which are morphologically uniform, small and dense, did not respond to stimulation with soluble lymphokines in the absence of T cells. However, vigorous in vitro proliferation of follicular lymphoma cells was induced when the follicular lymphoma cells were cultured with a CD4+ T cell clone which recognized alloantigens expressed by the lymphoma cells. This response required B-T cell contact, and was inhibited by anti-class II but not by anti-class I MHC mAb, indicating that these neoplastic B cells behaved as normal B cells and responded to normal activation and differentiation signals from T cells.

These results, using a monoclonal and homogeneous population of B cells, affirm the idea that cognate interaction between B cells and helper T cells is required for the effective activation of resting B cells. Moreover, these results suggest that a critical host-tumor interaction occurs in vivo, and that the polyclonal CD4+ T cells that infiltrate follicular lymphomas play a role in sustaining rather than inhibiting tumor growth in vivo. If so, therapies directed not only against the neoplastic cell but also against specific T cells and their cognate interactions with tumor cells may have a rationale.

M 547 THE EFFECT OF THE AUTOIMMUNE ENVIRONMENT ON THE HUMORAL RESPONSE TO THE HAPTEN p-AZOPHENYLARSONATE, D. Very[1], B. Jacobson[1], T. Manser[2], S-Y Shaw[3], D. Weissman[1], L. Wysocki[4], M. Margolies[3], and A. Marshak-Rothstein[1], [1]Dept. of Microbioogy, Boston Univ. School of Medicine, Boston, MA 02118, [2]Dept. Biology, Princeton Univ., Princeton, NJ 08544, [3]Dept. Surgery and Medicine, Massachusetts General Hosptial, Harvard Medical School, Boston MA 02114, [4]Center for Immunology and Respiratory Medicine, National Jewish Hospital, Denver CO 80206 To assess the effect of the autoimmune environment on a defined anti-hapten humoral response, we have developed a strain of mice designated (MxA)Id/lpr (Id mice). These mice contain the A strain Ig genes within the context of the MRL/lpr autoimmune environment and readily produce a CRI-A positive response when immunized with p-azophenylarsonate (ARS) In order to follow the effect of the lpr genetic background on the anti-ARS response, Id mice were sequentially immunized with ARS-KLH. CRI-positive ARS binding and ARS non-binding hybridomas proteins were produced. Sequence analysis and autoantigen specificity of CRI-positive, somatically mutated, ARS non-binding antibodies can give important insights into the involvement of foreign antigen selected B cells on the generation of the autoreactive repertoire.

M 548 ISOLATION OF THE ALPHA SUBUNIT GENE OF THE HIGH AFFINITY RECEPTOR FOR IgE AND CONSTRUCTION OF DELETION MUTANTS FOR EXPRESSION, Bruce Witthuhn and Charles Faust, Department of Biochemistry and Molecular Biology, Texas Tech University Health Sciences Center, Lubbock, TX 79430. The high affinity receptor for the Fc region of IgE is found only on mast cells and basophils. This receptor is a tetramer, composed of one alpha, one beta and two gamma subunits. Cross-linking of this receptor via alpha subunit-bound IgE results in release of histamine and mediates an allergic reaction. Both cDNA and genomic DNA clones encoding the alpha subunit gene were isolated from rat DNA libraries, using synthetic oligonucleotide probes. The identities of these clones were established by restriction enzyme mapping and DNA sequencing. The genomic DNA clone was modified by deletion mutagenesis in order to remove the C-terminal exon, i.e., that coding for the putative transmembrane domain and cytoplasmic tail. This was replaced by alternative genetic information sufficient to encode regulatory regions for transcriptional termination and polyadenylation, as well as for translational termination. These constructions were electroporated into mast cells and B-cells for expression and secretion. The long-term objective is to produce a secreted form of this high affinity Fc receptor alpha subunit to study its interaction with Fc region of rat IgE. Details on the isolation and characterization of the cDNA and genomic DNA clones are reported, as well as the subsequent constructions and attempted expression resulting from the deletion and replacement mutagenesis. This work was supported in part by NIH grant, AI-23456.

M 549 ANALYSIS FOR THE MECHANISM OF IL-6 PRODUCTION IN CASTLEMAN'S DISEASE, A B-CELL PROLIFERATIVE DISORDER. Kazuyuki Yoshizaki, Kazuhiro Kondo, Norihiro Nishimoto, Hiromi Tagoh, Astushi Ogata, Atsusi Muraguchi, Taro Kuritani and Tadamitsu Kishimoto. The Third Department of Internal medicine. Osaka University Medical School, Osaka, Japan.

Castleman's disease is a syndrome consisting of giant lymph node hyperplasia with plasma cell infiltration, hyper-γ-globulinemia, increase of acute phase proteins, and increase number of platelets in the blood.Moreover the clinical abnormalities disappear after the resection of the affected lymph nodes, suggesting that products of lymph nodes may cause such clinical abnormalities. We reported that the germinal centers of hyper-plastic lymph nodes of patients produced large quantities of IL-6 without any significant production of other cytokines (Blood, 74, 1360;1989). It indicated that the generation of IL-6 by activated B cells in germinal centers is one of the pathogenic causes. However the causes of deregulated production of IL-6 are still unknown in Castleman's disease.

Previously it was reported that HIV virus induced the production of IL-6 in macrophage or EV-virus-trans formed B cell lines produced IL-6. Therefore cord blood cells or primate kidney epitherial cells (Vero cells) were co-cultured with the lymph node cells obtained from hyperplastic lymph nodes of the disease. Several weeks later, cytopathic effect was observed in Vero cells. In the culture supernatant of Vero cells with cytopathic sign, IL-6 activity was observed and IL-6 production in Vero cells was confirmed by immunohistochemical staining with anti-IL-6 antibody. The data suggested the presence of unknown viruses which stimulated the induction of IL-6 production in activated B cells in the hyperplastic lymph node of Castleman's disease.

M 550 HISTOLOGIC TRANSFORMATION OF FOLLICULAR LOW GRADE LYMPHOMA TO DIFFUSE INTERMEDIATE GRADE LYMPHOMA REPRESENTS A CLONAL EVOLUTION; A NEW SUBTRACTIVE HYBRIDIZATION STRATEGY MAY ELUCIDATE ITS MOLECULAR BASIS,

Andrew D. Zelenetz, Thomas Chen and Ronald Levy, Department of Medicine/Division of Oncology, Stanford Medical School, Stanford, CA 94305-5306

Patients with follicular low grade lymphoma are at risk of developing an aggressive diffuse lymphoma during the course of their disease. We have found that this histologic transformation represents a clonal evolution of the low grade follicular lymphoma to the intermediate grade diffuse lymphoma; this conclusion is based on two lines of evidence. First, anti-idiotypic monoclonal antibodies prepared to react with a low grade lymphoma invariably (10/10) react with the idiotype of the diffuse lymphoma arising in the same patient. Second, Southern blot analysis of the DNA from two matched pairs (i.e. from the same patient) of the follicular and diffuse lymphomas demonstrates clonal rearrangements using probes for the *bcl-2* and immunoglobulin gene rearrangements. DNA sequence analysis of the immunoglobulin variable regions suggests the diffuse lymphoma arises from a common ancestral clone.

To further investigate the basis for histologic transformation we have developed a subtractive hybridization scheme that allows the use of small tumor samples. cDNA derived from matched pairs of follicular and diffuse tumors was directionally cloned into a new pair of phagemid vectors, pLIB: AZ and pLIB:ZA. These vectors have the advantage that rescued single-stranded DNA differs only in the orientation of the the cDNA inserts. Rescued ssDNA from the follicular lymphoma was biotinylated to facilitate strand separation after hybridization. After two rounds of subtractive hybridization the resulting DNA was enriched 700-fold for sequences specific to the diffuse lymphoma. The subtracted library is being investigated for transformation specific genes.