B CELL DEVELOPMENT

Organizers: Owen Witte, Maureen Howard and Norman Klinman
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Keynote Address

G 001 STUDIES OF STRUCTURE AND REGULATION OF IgE. Seiji Haba and Alfred Nisonoff, Rosenstiel Research Center, Brandeis University, Waltham Massachusetts , 02154.

In collaboration with E.M.Rosen and K.Meek we have determined the primary structure of an A/J IgE mAb that is specific for phenylarsonate (Ar) and expresses a major idiotype. A comparison of nucleotide sequences with those of corresponding germline genes revealed only 3 substitutions in VH and 3 in VL. Very similar results were obtained with another IgE mAb that appears to utilize the same germline VH and VL. This small number of somatic mutations (after repeated immunization) may be consistent with evidence for a direct switch from IgM to IgE. Studies of regulation of IgE have been facilitated by development of a method for quantifying IgE anti-Ar in the presence of much larger concentrations of IgG anti-Ar. One study showed that synthesis of IgE anti-Ar is inhibited by excess secondary B cells specific for IgG anti-Ar ("clonal dominance").This suggest a possible approach to immunotherapeutic inhibition of IgE. With a KLH-IgE conjugate as immunogen,we have induced high titers of syngeneic ant-IgE and syngeneic anti-IgE mAbs (useful for studies of regulation).These observations suggest that adult mice are tolerant of isotypic determinants of autologous IgE at the T cell, but not B cell level. Using unconjugated syngeneic IgE, anti-IgE can be induced in neonatal but not adult mice.The loss of responsiveness corresponds with the appearance of IgE in serum. Cell transfer studies may determine whether adult T or B cells can induce tolerance to unconjugated IgE in neonatal mice.

Rearrangement and Expression of Immunoglobulin Genes

G 002 CONTROL OF GENOMIC REARRANGEMENT EVENTS DURING LYMPHOCYTE DIFFERENTIATION, Pierre Ferrier, Stuart Lutzker, Paul Rothman, Andrew Furley, Roberta Pollock, Bernie Krippl, Frank Costantini, Wendy Cook, and Frederick W. Alt, The Howard Hughes Medical Institute and Departments of Biochemistry, Microbiology and Human Genetics, College of Physicians and Surgeons of Columbia University, New York, New York, 10032

We have suggested that tissue- and stage-specific regulation of Ig and TCR variable region gene assembly is controlled by modulating accessibility of substrate gene segments to a common VDJ recombinase; accessibility could be correlated with transcription of the substrate gene segments. To elucidate controlling mechanisms, we introduced into transgenic mice a recombination substrate that consisted of a TCR β chain V, D, and J segments separated from a Cμ gene by a DNA segment which either did or did not contain the Ig heavy chain enhancer. Analyses of normal lymphocytes in multiple transgenic strains demonstrated that the heavy chain enhancer was necessary and sufficient to obtain DJβ rearrangements in normal B and T cells, but that Vβ to DJβ occurred only in T cells. This has been confirmed preliminarily by analyses of A-MuLV transformed B and T cell lines from the transgenic strains. In accord with implications of our previous cell culture studies, the enhancer may be necessary to achieve general access to the locus, but elements associated with the Vβ gene, perhaps the promoter, may provide tissue specificity of variable region gene assembly.

We have also proposed that heavy chain class-switching is regulated by controlling "accessibility" of the different heavy chain switch regions to a common switch recombinase and that such "accessibility" correlates with the presence of germline γ2b transcripts which initiate 5′ to the DNA region where switches to γ2b normally occur (Sγ2b); therefore transcription proceeds through the Sγ2b region but not through other switch regions. Bacterial lipopolysaccharide (LPS) induces germline γ2b transcripts in both A-MuLV-transformed pre-B cells and normal murine spleen cells; in both systems, this induction is followed by an increased number of cells that produce γ2b protein. The LPS induction appears to be transcriptional and is associated with the induction of a factor which specifically binds to a sequence in the γ2b promoter region. This data suggests that LPS treatment directs switching to γ2b by increasing the accessibility of the γ2b locus. The T cell factor IL-4 is known to suppress in vitro induction by LPS of swithing to γ2b production by murine spleen cells. Correspondingly, we find that IL-4 also inhibits the LPS induction of germline γ2b transcripts in such cultures, indicating that T cell factors may directly regulate class-switching in the context of an accessibility mechanism.

Interrelationships of B Cell Subsets

G 003 DIFFERENTIATION PATHWAY OF LY-1 B CELLS FROM STEM CELLS/PROGENITORS PRESENT IN NEWBORN LIVER. Kyoko Hayakawa and Richard R. Hardy, Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, PA 19111.
One B cell subset, $CD5^+$ B cells, although comprising a minor B cell population in the adult is conserved in such phylogenetically diverse species as mouse (Ly-1 B) and human (Leu-1 B) (1). In contrast to their relative paucity among B cells in the adult, they constitute a large fraction of the B cells that appear early in development. The deficiency of adult bone marrow in generating Ly-1 B and the ability of surface-Ig-expressing Ly-1 B (from newborn and adult mice) to maintain the Ly-1 B population raised the question whether B cells found in the adult might already be distinct at the progenitor level of development. The distinction between newborn liver and adult bone marrow in yielding Ly-1 B is found in the stem/progenitor population which lacks expression of surface proteins found on mature erythroid, myeloid, or lymphoid cells ("null fraction"). Furthermore, we have found an early B cell-committed population ($B220^-$) restricted uniquely to fetal and newborn liver. This population originally expresses determinant(s) recognized by an anti-asialoGM1 antiserum, but lacks Thy-1. These cells differentiate on a liver-derived stromal layer (ST2), yielding Ly-1 B (up to 50%) after 2-3 weeks of culture (2). Cells in the stage prior to this $aGM1^+$ B-committed population are likely to be those proliferating extensively on ST2 (originally $asialoGM1^-$ in the null fraction) since transfer of such cells into SCID mice has led to their differentiation, generating Ly-1 B. Therefore, we postulate that a distinct B cell differentiation pathway is allowed in early developmental hematopoietic tissues and that commitment to this B cell lineage occurs at the stem/progenitor level. Among such cells generated early in development, Ly-1 B are capable of self-maintenance and show restricted specificities, largely to autoantigens in the adult. Particularly, certain specificities (to bromelain-treated mouse erythrocytes and to mouse T cells) are characteristically enriched in this population found in the spleen and peritoneum of adult animals.

1. Hayakawa, K. and Hardy, R.R. (1988) Ann. Rev. Immunol. vol 6, in press.
2. Hardy, R.R., Kishimoto, T. and Hayakawa, K. (1987) Eur. J. Immunol., in press.

G 004 THE SECONDARY B CELL LINEAGE, Phyllis-Jean Linton, Gary L. Gilmore and Norman R. Klinman, Scripps Clinic & Research Foundation, La Jolla, CA 92037

Two competing hypotheses for the origin of secondary B cells are a) that memory cells arise as the product of unequal division of primary antibody forming cell (AFC) precursors vs b) that memory B cells are the progeny of a separate precursor cell pool (lineage ?). The latter postulate is most consistent with the disparate kinetics of AFC vs secondary B cell generation and the distinct biological properties of secondary vs primary B cells including: a) cell surface markers and isotypes, b) homing patterns, c) requisites for stimulation and d) resistance to anti-idiotypic suppression. In addition, evidence indicates that secondary B cells disproportionately accumulate somatic mutations.

The transfer of primed helper T cells along with limiting numbers of splenic B cells to irradiated carrier primed recipients has enabled the secondary *in vitro* antigenic stimulation of B cells in fragment culture two weeks after establishment of cultures and primary stimulation. Consistent with the existence of separate precursors for primary AFC and secondary B cells, three populations of precursor cells have been identified: a) B cells that give rise to AFC subsequent to primary stimulation, but are unaffected by secondary challenge (90-95%), b) B cells that yield no AFC subsequent to primary stimulation but vigorous antibody responses after secondary *in vitro* stimulation (3-5%) and c) B cells whose progeny transiently produce antibody after primary stimulation but respond vigorously to a secondary *in vitro* challenge (3-5%). FACS analyses indicated that all three populations express surface IgM and IgD; however, the majority of precursors to primary AFC (category a) expressed high levels of the surface glycoprotein recognized by the J11D monoclonal antibody ("$J11D^{hi}$"), whereas the majority of progenitors to secondary B cells (categories b and c) are "$J11D^{lo}$". FACS enriched "$J11D^{hi}$" B cells yielded only primary AFC responses in fragment culture whereas the majority of FACS enriched "$J11D^{lo}$" splenic precursor cells responded to secondary *in vitro* challenge. Analysis of enriched populations of "$J11D^{lo}$" secondary progenitors demonstrated that these cells are resistant to both anti-idiotypic suppression and tolerance induction. However, after primary *in vitro* stimulation, newly developing secondary B cells develop a marked susceptibility to *in vitro* tolerance induction. Our current analyses include the selective repopulation of SCID mice using "$J11D^{hi}$" vs "$J11D^{lo}$" splenic precursor cells and attempts to compare the accumulation of somatic mutations in the clonal progeny of these disparate precursor cell populations.

This work was supported by grant AI 15797 from the National Institutes of Health.

Diversification of the B Cell Repertoire

G 005 IDIOTYPE DIRECTED INTERACTIONS AND DEVELOPMENT OF THE B CELL REPERTOIRE, John Kearney, Andries Bloem and Meenal Vakil, Division of Developmental and Clinical Immunology, University of Alabama at Birmingham, Birmingham, AL 35294.

To determine factors that are involved in the development and establishment of the B cell repertoire, the spectrum of antibodies produced by early B cells was analyzed following rescue of perinatal B cells in the form of B cell hybridomas. The essential findings are that (i) Auto-anti-idiotypic B cells occur at a high frequency in the lymphoid organs of perinatal mice; (ii) IgM antibodies derived from some of these hybridomas are multispecific and serve as idiotypic links between B cells involved in distinct antibody responses; (iii) Many of these antibodies as well as having classical anti-idiotype activity appear to react with structures on B cells other than sIg and in vitro have high mitogenic activity for B cells; (iv) Certain of these anti-idiotypic antibodies appear to be involved in idiotype directed clonal expansion of B cells that appear later during ontogeny. (v) Interference with the proposed idiotype directed interactions in neonatal mice with the appropriate antigen, Ab1 or Ab2 results in a distortion in the developing repertoire leading to the loss of B cell clonal dominance in certain Ag responses. (vi) Inactivation of these perinatal B cells with anti-idiotypic activities results in an long lasting suppression of appropriate idiotypically connected B cells.

From these data we conclude that early B cells exhibit a set of IgM receptors with a high degree of autoreactivity which includes anti-idiotype activity. These B cells interact during development and produce a non-antigen driven clonal expansion in which certain idiotypes come to dominate certain antigen responses. These idiotype directed antigen independent clonal expansions appear to involve activation or proliferation involving surface receptors other than IgM and probable release of nonspecific growth factors. However, the idiotype directed nature of this developmental process observed in in vivo studies derives from the demonstrated ability of these antibodies to bind to clones of B cells by virtue of the V region determinants on the specific IgM receptors on targeted B cells. (NIH grants - CA 16673, CA 13148 and AI 14782)

G 006 SELECTION MECHANISMS OF ANTIBODY SPECIFICITY IN THE MOUSE - SOMATIC MUTATION AND B CELL MEMORY, Christine Kocks, Ana Cumano, Debbie Allen, Thomas Simon, Fred Sablitzky and Klaus Rajewsky, Institute for Genetics, University of Cologne, Cologne, F.R.G..

The B cell response to antigen may be grouped into different pathways, - primary response, memory induction and memory response; the latter two being observed with T cell dependent antigens. Memory induction is characterized by somatic hypermutation of antibody v-genes and the absence of terminal differentiation, while primary and memory response involve proliferation and differentiation, but little hypermutation. How does selection of antibody specificities relate to this scheme of B cell differentiation?

The germline encoded (thus evolutionary selected) repertoire is expressed in primary responses. In T cell dependent responses B cells expressing antibodies with higher affinity for antigen are generated from the pre-existing repertoire by somatic mutation and selection into the memory compartment. We have analysed the molecular basis for affinity maturation towards the hapten 4-hydroxy-3-nitrophenylacetyl (NP) in secondary response antibodies. Increase in affinity to the hapten NP can result from a single point mutation (pos 33 of the heavy chain, which is seen in the majority of secondary response antibodies). Other mutations seem to not affect affinity, simply representing "noise" in the system or being selected by other means such as idiotypic selection. We have evidence that mutants which no longer bind the immunizing antigen remain available for stimulation by an anti-idiotypic antibody. Does this mean, that somatic mutation contributes to the antibody repertoire by generating new specificities? We currently address the question whether in a particular somatic selection system, namely the selection of clonally related B cells producing high affinity anti-idiotypic antibodies, somatic mutation was a necessary requirement to create antigen binding specificity.

Development of B Cells

G 007 REGULATED PROGRESSION OF B LYMPHOCYTE DIFFERENTIATION, Kathleen A. Denis, Kenneth Dorshkind and Owen N. Witte, Howard Hughes Medical Institute and Department of Microbiology, UCLA, Los Angeles, 90024 and Department of Biomedical Sciences, UC Riverside, 92521.

Using a combination of long term culture systems and in vivo reconstitution we have been able to regulate and study the development of immunoglobulin secreting B lymphocytes from early precursor cells. B lymphocytes differentiate from a hematopoietic stem cell found in fetal liver and adult bone marrow. A culture system has been developed which isolates early B lymphocyte development from these murine tissues in vitro. Both lymphoid bone marrow cultures (LBMC) and lymphoid fetal liver cultures (LFLC) retain characteristics of their native tissues in vitro and do not differentiate beyond the pre B or early B cell stage. When placed in the environment of a severe combined immunodeficient (SCID) mouse however, further expansion and differentiation along the B lymphocyte lineage occurs. Both LBMC and LFLC cells reconstitute B lymphocytes and serum IgM in SCID mice. These reconstituted mice can respond well to immunization with TNP-ficoll, a T-independent antigen. Only LBMC cells reconstitute serum IgG and T-dependent serum responses in these mice however. When thymocytes, as a source of T lymphocytes, are coinjected with LFLC cells, the reconstituted B lymphocytes are able to class switch fully to all gamma subisotypes and are able to respond to T-dependent antigens. These serological responses are heterogeneous and derived from the LFLC cells. This experimental system allows separation of three B lymphocyte developmental stages: 1) early differentiation in vitro, 2) progression to IgM secretion in vivo, and 3) late differentiation dependent upon mature T lymphocytes in vivo. Both types of cultures are being utilized to study the early events in B lymphocyte committment and differentiation and hybridomas derived from a variety of reconstituted SCID mice are being analyzed for their regulation of immunoglobulin gene expression.

G 008 **CELLULAR AND MOLECULAR RELATIONSHIPS INVOLVED IN B LYMPHOCYTE FORMATION,** Paul W. Kincade, Grace Lee, Carolynn Pietrangeli, Shin-Ichi Hayashi, and Patricia S. Thomas, Oklahoma Medical Research Foundation, 825 N.E. 13th Street, Oklahoma City, OK 73104.

Soluble mediators can profoundly influence the progenitors of B lymphocytes in culture and this can be used to study the acquisition of functional receptors for particular substances during their differentiation. Such responses may also reflect some of the regulatory mechanisms which control B lymphocyte formation within hemopoietic tissues. For example, TGF-β is a potent inhibitor of pre-B cell maturation which has selective effects on gene expression. Known agonists which induce increased, or decreased, gene expression in B lineage cells include IL-1, IL-4, IFN-γ, and substances isolated from immunologically defective patients and animals.

Cells which presumably provide the inductive microenvironment for B lineage progenitors are themselves sensitive to exogenous factors. Cloned stromal cell lines were isolated from marrow and spleen of normal and genetically defective animals, and selected on the basis of their ability to support growth of cloned B lineage lymphocytes. In some respects, the phenotypes of individual clones differ markedly from stromal cells which have not been extensively subcultured and the lineage derivation of stromal cells is still unclear. Their proliferation and function in culture can be modulated by several known mesenchymal growth factors and current studies are focused on how they physically and functionally interact with lymphocytes.

Experiments with genetically defective animals indicate that all components of the bone marrow microenvironment are not represented in long term cultures. For example, defective SCID lymphocytes grow in culture, but not *in vivo*. In contrast, lymphopoiesis occurs *in vivo*, but not in culture with cells taken from mutant motheaten mice. In the latter model, a dominant suppression of lymphoid or myeloid cell growth was observed, but no soluble mediators which could account for this effect were demonstrable. One interpretation of these findings is that short range, and possibily membrane bound, signals may be important for normal physiologic regulation *in situ*. In this context, it is interesting that TGF-β is easily visualized (presumably in latent form) on the surface of pre-B cells which respond to the active molecule.

It is important to learn how differentiating cells in the B lymphocyte lineage are correctly positioned and moved through the bone marrow environment. Lymphocyte specific, as well as lymphocyte non-restricted cell surface proteins would presumably be involved and we have recently detected glycoproteins related to known families of adhesion molecules in long term lymphocyte cultures. For example, one major, and two minor species of N-CAM proteins are made by stromal cells, but not by lymphocytes or fibroblasts. Preliminary studies suggest that at least one other type of adhesion protein is expressed on lymphocytes, but not by stromal cells. It is now important to learn if these represent tissue specific isoforms and if they are functionally significant to normal lympho-hematopoiesis.

G 009 LINEAGE-SPECIFIC AND DEVELOPMENTAL STAGE-SPECIFIC GENES OF B LYMPHOCYTES, Gary Hermanson*, Michael Gilly*, Paul Kincade° and Randoph Wall*; *UCLA, Los Angeles, CA 90024 and °OMRF, Oklahoma City, OK 73104. Immunoglobulins are exclusively expressed in B lineage cells and comprise one of the most extensively characterized gene families. In contrast, little is known of other genes whose expression is restricted specifically to B cells. We have isolated a set of cell specific cDNA clones from a B - T subtracted cDNA library using an improved screening method. One of these B cell specific clones expressed at all stages in B cell development, beginning with the earliest pre-B cells undergoing heavy gene rearrangements, was selected and further characterized. This isolate encodes an integral membrane protein containing a single immunoglobulin-like extracellular domain with structural features most closely related to immunoglobulin κ light chain variable regions followed by accessory molecules on T cells involved in cellular recognition and interaction. These features along with a simple immunoglobulin-like exon/intron structure for the complete genomic gene indicate that this B cell specific gene corresponds to a new member of the immunoglobulin superfamily. The characteristics of other B cell specific genes will also be presented. A different molecular cloning strategy has been used for the isolation of B cell stage-specific genes activated along with κ light chain in LPS- and γ-interferon induced 70Z/3 pre-B cells. In the context of ongoing studies on the molecular features in κ gene transcription, these clones provide a unique set of coordinately regulated genes for determining the mechanisms in stage specific gene activation in B lymphocytes.

B Cell Growth and Differentiation Factors

G 010 BSF2/IL6 AND ITS RECEPTOR, Tadamitsu Kishimoto, Toshio Hirano, Shizuo Akira, Tetsuya Taga, Tadashi Matsuda and Katsuhiko Yamasaki, Inst. for Molecular and Cellular Biology, Osaka University, 1-3, Yamada-Oka, Suita City, Osaka 565, Japan.

BSF2-IL6 is involved in the final differentiation of activated B cells into high-rate antibody producing cells. The study with recombinant BSF2 shows that BSF2 acts directly on activated B cells to induce antibody production. Anti-BSF2 antibody almost completely inhibited PWM-induced Ig-production in peripheral lymphocytes, indicating that BSF2 is one of the essential molecules in the antibody induction. BSF2 did not show any grwoth activity in activated B cells. However, BSF2 is a potent growth factor for myeloma cells.

BSF2 has a wide variety of biological functions, not only on B cells but also on various other cells. Actually, BSF2 functions as hepatocyte stimulating factor and induces acute phase proteins. BSF2 is inducible in glia cells by IL-1 stimulation, suggesting that BSF2 may have a certain physiological function in brain. A preliminary study demonstrates that BSF2 induces differentiation of PC12 cells into neuron cells. Although BSF2 is identical with the molecule called IFNβ2, the study with rBSF2 demonstrates that BSF2 does not have any antiviral activity and is functionally and structurally not related with the IFN family.

Several studies suggested that deregulation of the BSF2 expression might be involved in the pathogenesis of autoimmune diseases and myelomas. Synovial fluids from the affected joints of rheumatoid arthritis (RA) patients contained large amounts of BSF2, suggesting that BSF2 might be involved in the pathogenesis of RA. Myeloma cells express BSF2 receptor and produce BSF2. Anti-BSF2 antibody inhibits the growth of myeloma cells, suggesting that the autocrine mechanism might operate in the pathogenesis of myelomas. Therefore, the study on the regulation of the expression of the BSF2 gene will provide essential informations with regards to the pathogenesis of autoimmune diseases and myelomas at the molecular level.

In this presentation, the regulation of the BSF2 gene expression and the receptors for BSF2 will be discussed.

G 011 ISOLATION OF A cDNA FOR MURINE LYMPHOPOIETIN-1, A PRE-B CELL GROWTH FACTOR.
Anthony Namen, Stephen Lupton, Ann Schmierier, Kathryn Hjerrild, David Cosman, Janis Wignall, Diane Mochizuki, Carl March, Robert Overell, Linda Park, David Urdal, Steven Gillis and Raymond Goodwin. Immunex Corp., 51 University Street, Seattle, WA 98101.

We have utilized long term bone marrow cultures as a source of lymphocyte progenitors to establish a rapid and specific proliferation assay. This assay detects a pre-B cell growth promoting activity which we have designated as lymphopoietin-1 (LP-1). This previously uncharacterized factor stimulates the proliferation of lymphoid progenitors and is capable of the extended maintenance of these progenitors in the absence of exogenous stromal or feeder cells. In addition, a clonal cell line secreting LP-1 (designated (IxN/A6) is derived from the stromal elements of bone marrow cultures utilizing the transforming sequences of SV40. LP-1 from IxN/A6 supernatants was purified some 10 million-fold and the biological activity was shown to reside in a single protein of 25 kd. N-terminal sequence analysis confirmed that LP-1 is a unique, previously undescribed growth factor. Additionally, the IxN/A6 cell line has served as a source of specific RNA transcripts coding for LP-1. Polyadenylated RNA was isolated and used to prepare a double-stranded cDNA library for use in direct expression cloning. Pools of cDNA fragments were screened in monkey COS-7 cells using a mammalian expression vector (pDC201). Bacterial clones resulting from the transformation of this cDNA-containing vector were pooled and transfected into COS-7 cells and the culture supernatants assayed for LP-1 activity. A single cDNA clone was isolated which encoded biologically active LP-1. Nucleic acid sequence analysis revealed that this cDNA contained an open reading frame capable of coding for a protein of 154 amino acids with (including) a leader sequence of 25 amino acids. Further studies on the transcriptional expression of LP-1 will also be presented.

G 012 REGULATION OF B CELL GROWTH AND DIFFERENTIATION BY INTERLEUKIN 5,
Kiyoshi Takatsu, Akira Tominaga, Nobuyuki Harada and Seiji Mita, Dept. Biol., Inst. Med. Immunol., Kumamoto University Medical School, Kumamoto 860, JAPAN.

Since the original discovery of T and B cell interaction in the antibody response, the mechanism of the regulatory function(s) of T cells in the B cell activation is one of the central issues in cellular immunology. It is now well accepted that a number of factors produced by T cells is involved in the regulation of B cell growth and differentiation. T-cell-replacing factor (TRF), which, when secreted by the murine T cell hybridoma B151K12, is defined by two activities: induction of IgM secretion by BCL_1 leukemic B-cell line; and induction of secondary anti-DNP IgG synthesis by DNP-primed B cells. Although TRF from B151K12 was categorized as a B cell differentiation factor, purified TRF has B-cell growth factor II (BCGFII) activity. We isolated cDNA encoding for TRF from the 2.19 T-cell line. The recombinant TRF also exerts BCGF II activity, indicating that TRF is identical to BCGF II. Therefore, we proposed that TRF or BCGF II will be called interleukin 5 (IL-5). IL-5 acts on B cell for their terminal differentiation into IgM, IgA, and IgG secreting cells, and proliferation of some types of cells on B-cell lineage. Moreover, it induces expression of functional IL-2 receptors on B cells and on antigen-stimulated thymocytes and supports growth and differentiation of eosinophil from bone marrow cells. Secreted forms of IL-5 (apparent m.w. of 46 kd) is a glycoprotein which has 113 amino acid residues and comprises of homodimers. IL-5 mRNA is expressed in IL-5-producing T cell hybridoma, mitogen-stimulated T cell lines and antigen-stimulated T cells. We will discuss the role of IL-5 and its receptor in the T cell-dependent B cell growth and differentiation.

Antigen Presentation by B Cells

G 013 MECHANISMS UNDERLYING B CELL ANTIGEN PRESENTATION, Lisa A. Casten, Ellen K. Lakey and Susan K. Pierce, Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, IL 60208.

Helper T cell recognition of soluble globular protein antigens requires the processing and presentation of antigen by an Ia expressing antigen presenting cell (APC). While most Ia expressing cells function as APC, B cells which express surface Ig specific for the antigen are very effective APC, capable of maximally activating antigen-specific T cells when provided with 1/1000th the antigen required of nonspecific cells. The time dependence of processing and presentation of a peptide and a native protein antigen and the retention of processed antigen and peptides by B cells has been investigated, as well as the role of the surface Ig in facilitating antigen processing. A panel of antigens capable of binding all B cells has been synthesized by coupling the well characterized soluble globular protein antigen pigeon cytochrome c (Pc) to monoclonal antibodies directed toward various B cell surface structures including Ig, Ia and class I. B cells require 6-8 hours incubation with native Pc to process and present it to an I-E^k restricted Pc-specific T cell hybrid, and the time required is similar whether antigen is taken up by nonspecific fluid phase pinocytosis or by binding to surface Ig. Processed antigen is lost from the B cell surface by 8 to 12 hours. The association of the peptide fragment Pc 81-104 with B cells is relatively rapid, requiring only 1 to 2 hours, and this time is similar for live and paraformaldehyde fixed B cells which cannot internalize the antigen. B cells retain peptide on their surface in a stimulatory fashion for 8 to 12 hours. However, prolonged incubation of B cells with peptide, but not the native protein, triggers a dramatic and selective loss of the peptide or peptide associated complex from the B cell surface and a consequent loss of APC function. B cell surface Ig, Ia and class I are each capable of initiating the transport of Pc to cytoplasmic vesicles where proteolysis occurs, as shown by the B cell's effective presentation of Pc covalently coupled to antibodies directed toward Ig, I-A^k and K^k, requiring up to 1000 fold less Pc as compared to Pc alone or to Pc coupled to nonspecific immunoglobulin. Pc coupled to monovalent fragments of Ig-specific antibodies are nearly as effective as Pc coupled to divalent antibodies, indicating that the phenomena associated with bivalent binding, such as patching and capping of the surface Ig, are not required for effective antigen processing. This approach is being extended to assess the ability of other cell surface proteins to internalize antigens for processing, including surface Ig of the μ, γ and δ isotypes, F and transferrin receptors and virally encoded proteins.

G 014 B CELL INTERACTION WITH HELPER T CELLS: CHARACTERIZATION OF TWO Th SUBSETS WHICH REGULATE B CELL RESPONSE, Susan L. Swain, Douglas Rowe, Richard W. Dutton, Douglas T. McKenzie, Susan Tonkonogy, Wendy Hancock, Joseph Voland and Andrew Weinberg, University of California, San Diego, La Jolla, CA 92093.

For B cells to receive the help they require to differentiate into Ab-secreting cells, they must present specific Ag to helper T cells. In a model system using T cell lines and B cell hybridomas, we have shown that the resultant T-B interaction consists of the formation of stable T cell-B cell conjugates of considerable avidity. In earlier studies, we and our colleagues showed that there is a rapid redistribution of T cell molecules involved in cell-cell recognition and adhesion so that they become concentrated at the area of T-B contact. The golgi apparatus in the T cell becomes oriented to face the B cell (Kupfer et al., 1987). These facts suggest that lymphokines may well be delivered by the helper T cell in a directional fashion to specific B cells.

Two kinds of helper T cells can be shown to participate in B cell responses and these can be distinguished on the basis of the lymphokines they produce. Thus, only Th1 produce IL2 and IFNγ while Th2 produce IL4 and IL5. These two sets of lymphokines appear to drive the production of different Ig isotypes. We have begun a characterization of Th1 and Th2 cells from normal animals. We find that Th1 cells exist in a pool of long-lived recirculating T cells and secrete lymphokines within 24 hours of stimulation. In contrast, Th2 cells are absent from that pool and secrete IL4 and IL5 only after priming. The two subsets also appear to require distinct lymphokines for growth.

Autoimmunity and Immunodeficiency

G 015 THE scid MOUSE MUTANT. Melvin Bosma, Walter Schuler, Norman Ruetsch, Ann Carroll, Amelie Schuler, Michal Fried and Gayle Bosma, Fox Chase Cancer Center, Institute for Cancer Research, 7701 Burholme Avenue, Philadelphia, PA 19111.

The autosomal recessive mutation, scid, occurred in the C.B-17 inbred strain, an Igh congenic partner strain of BALB/c. Mice homozygous for the scid mutation (C.B-17scid) are severely deficient in B and T lymphocytes whereas other hematopoietic cell types such as granulocytes, monocytes, erythrocytes and megakaryocytes (all members of the myeloid series) are present in normal number.

Our recent results concerning the scid locus and its possible role in lymphocyte development indicate the following:

1) scid maps to the centromeric end of chromosome 16 (work done in collaboration with M. Davisson, H. Sweet and L. Shultz of the Jackson Laboratory, Bar Harbor, ME).
2) the effect of scid becomes manifest early in lymphopoiesis after the transcriptional activation of Ig and TCR loci in developing B and T cells, respectively. Transcripts of unrearranged (but not functionally rearranged) Ig and TCR genes are clearly evident in the lymphoid tissues of scid mice.
3) recombination of V, D and J elements in developing scid B and T cells is highly error-prone. The resulting nonfunctional lymphocytes presumably turn-over rapidly.

and 4) scid is "leaky" in that productive VDJ and VJ rearrangements occasionally occur as some scid mice (∿15%) develop oligoclonal Ig-producing B cells and limited numbers of functional T cells.

The above points will be discussed with emphasis on the last point.

G 016 MHC-LINKED B-B CELL INTERACTION AND ITS RELATION TO AUTOIMMUNITY, Toshiyuki Hamaoka, Shinya Murakami, Kunio Dobashi, Yousuke Takahama, Fumiya Hirayama, Katsuhiko Ishihara, Shiro Ono, Inst. for Cancer Res., Osaka University Medical School, Osaka, Japan. The B cell differentiation factor B151-TRF2 derived from murine B151K12 T cell hybridoma directly acts on unstimulated murine B cells to induce a polyclonal differentiation into IgM-producing cells. To be noted, B-B cell interaction via recognition of self-Ia molecules, which is inhibitable by anti-Ia monoclonal antibody (mAb), is involved in B151-TRF2-mediated polyclonal B cell activation process. The self-Ia recognition by B cells is shown to be mediated by yet unidentified molecules(s) other than immunoglobulin and Ia molecules. In addition, we have recently demonstrated that B151-TRF2 is capable of inducing in vivo and in vitro autoantibody production to ssDNA and Bromelain-treated mouse red blood cells(BrMRBC). The present study investigated immunogenetic control of autoantibody production induced by B151-TRF2 in view of Ia-restricted B-B cell interaction. The experiments utilizing H-2 congenic mice revealed that the B cells from mice bearing I^s haplotype exhibited low anti-BrMRBC IgM plaque forming cells(PFC) response, whereas they gave rise to reverse IgM PFC, anti-trinitrophenyl (TNP) IgM PFC and anti-ssDNA antibody responses comparable in magnitude to those of the other high responder strains. This selective low anti-BrMRBC PFC response was shown to be resulted from low frequency of BrMRBC-specific B cell clones by limiting dilution analysis. Interestingly, (high responder B10 x low responder B10.S)F1 ($H\text{-}2^{b/s}$) B cells exhibited intermediate anti-BrMRBC PFC response, and their response was preferentially inhibited by anti-Ia^b mAb but not by anti-Ia^s mAb. These results raise a possibility that (B10 x B10.S)F1 B cells with recognition specificity for self-Ia^s molecules contain less BrMRBC-specific B cell clones than those bearing self-Ia^b recognition structures. In fact, it was demonstrated that when (B10 x B10.S)F1 B cells were fractionated into two populations by their ability to bind to parental B cell monolayer, the F1 B cells adherent to B10 ($H\text{-}2^b$) monolayer gave rise to anti-BrMRBC and anti-TNP PFC responses comparable to high responder B10 B cells, whereas those adherent to B10.S($H\text{-}2^s$) monolayer exhibited selective low anti-BrMRBC PFC response like as low responder B10.S B cells. Taken together, these results provide evidence showing that self-Ia recognition specificity expressed by B cells importantly influences generation of BrMRBC-specific B cell clones. Thus, this finding has important implication to the problems of the MHC-linked Ir-gene control of B cell response, especially generation of antibody repertoire. Moreover, the present results provide a new insight into basic mechanism underlying autoimmune disease, because we have shown that the immunologic properties of B151-TRF2 are very akin to B cell differentiation factor produced by T cells from autoimmune MRL/lpr mice and mice undergoing chronic graft-vs-host reaction.

Oncogenes and B Cell Transformation

G 017 TRANSGENIC MICE AND LYMPHOID NEOPLASIA, Suzanne Cory, W.S. Alexander, I. Hariharan, A.W. Harris, W.Y. Langdon and J.M. Adams, Walter and Eliza Hall Institute of Medical Research, P.O. Royal Melbourne Hospital, Victoria 3050, Australia.

Many lymphoid tumors carry a deregulated c-myc gene. The demonstration that transgenic mice carrying c-myc subjugated to the immunoglobulin heavy chain enhancer (Eμ) invariably develop pre-B or B lymphomas was dramatic proof that constitutive c-myc expression can play a causative role in lymphoid neoplasia (1). Nevertheless, deregulation of c-myc is apparently insufficient for malignancy, since B lineage cells taken from Eμ-myc mice exhibiting no overt sign of disease are not tumorigenic on transplantation(2). B lymphoid growth and differentiation within these mice is nevertheless perturbed by the constitutive expression of myc, since the proportion of pre-B cells is considerably increased and B cells develop in reduced numbers(2). Furthermore, the B lineage cells are larger than normal and a greater fraction are in cycle(2). Enforced myc expression thus seems to favor proliferation over maturation and provides a population of cells susceptible to changes that lead to tumor development. Evidence is presented here that such changes include loss of growth factor requirements and activation of additional oncogenes.

Eμ-myc mice provide a unique opportunity to investigate possible synergy between c-myc and other oncogenes for B lymphoid neoplasia. We are currently using retroviruses to deliver additional oncogenes to Eμ-myc B lineage cells and are comparing the growth properties in vitro and in vivo of cells carrying two oncogenes with those carrying just one. We have also made transgenic mice bearing other oncogenes subjugated to the immunoglobulin enhancer and are analysing tumors developing both in these mice and in mice bred with the Eμ-myc lineage. These studies should shed new light on the role of specific oncogenes in lymphoid neoplasia.

1. Adams, J.M., Harris, A.W., Pinkert, C.A., Corcoran, L.M., Alexander, W.S., Cory, S., Palmiter, R.D. and Brinster, R. (1985) Nature 318, 533-538.
2. Langdon, W.Y., Harris, A.W., Cory, S. and Adams, J.M. (1986) Cell 47, 11-18.

G 018 THE MOLECULAR BASIS OF B-CELL NEOPLASIA, Frank G. Haluska, Yoshihide Tsujimoto and Carlo M. Croce, The Wistar Institute, 3601 Spruce Street, Philadelphia, PA 19104.

The B-cell is an especially useful system for the study of the development of human malignancy. The majority of B-cell neoplasms carry characteristic, non-random chromosome abnormalities, particularly translocations, which provide important clues regarding the locations of genes involved in B-cell oncogenesis. We have taken advantage of these translocations to characterize these proven and putative oncogenes at the molecular level of analysis.

The best understood B-cell malignancy is Burkitt's lymphoma. Burkitt's lymphomas carry translocations which juxtapose the c-myc oncogene, on chromosome 8, with one of the immunoglobulin loci. The most prevalent translocation, occurring in about 80% of Burkitt's lymphoma cases, is the t(8;14). Extensive analyses of these translocations in our laboratory, utilizing somatic cell hybrid and molecular techniques, have demonstrated that the consequence of this translocation is the inappropriate expression of c-myc in a B-cell-specific, and differentiation-specific, manner, and that differences exist in the nature of the t(8;14) translocations in endemic, African Burkitt's lymphomas and sporadic cases. We have extended our study of B-cell neoplasia to tumors with other translocations as well. Follicular lymphomas carrying the t(14;18) translocation are the most common B-cell malignancies. We have isolated a putative oncogene from the region of chromosome 18 consistently involved in these translocations and designated it bcl-2 (for B-cell leukemia/lymphoma 2). This gene is deregulated in a manner similar to c-myc by chromosome translocation. The t(11;14) translocation is another chromosome abnormality common to B-cell tumors. This translocation occurs in chronic lymphocytic leukemia, various diffuse lymphomas, and multiple myeloma. We have cloned the region on chromosome 11 consistently implicated in these translocations and denoted it bcl-1. Molecular analysis of the t(11;14) and t(14;18) translocations also has led to important insights concerning the mechanism whereby translocations particular to lymphoid malignancies occur. The nucleotide sequences surrounding the translocation breakpoints exhibit features which suggest the action of the lymphoid V-D-J recombinase in their genesis. In Burkitt's lymphoma, similar features are found near the t(8;14) translocations. However, these features correlate with the immature, endemic Burkitt's lymphoma phenotype and not with the sporadic type of tumor.

In summary, these studies provide access to a clearer comprehension of the genes and processes crucial to normal and malignant B-cell ontogeny. They also suggest the formulation of concepts which can be applied to other hematopoietic neoplasms, especially those of T-cell origin.

G 019 MECHANISM AND ROLE OF C-MYC ONCOGENE ACTIVATION IN B-CELL TRANSFORMATION. Riccardo Dalla Favera, Dept. of Pathology, Kaplan Cancer Center, New York University, New York, N.Y. 10016

Chromosomal translocations involving the c-myc and different immunoglobulin (Ig) loci represent specific pathogenetic features of different types of B-cell neoplasms, including the endemic (eBL) and sporadic (sBL) forms of Burkitt lymphomas (BL), and most AIDS-associated non-Hodgkin lymphoma (AIDS-NHL). We analyzed the structure of chromosomal breakpoints in several eBL, sBL and AIDS-NHL and found that different regions of the Ig_H and c-myc loci are involved in the translocations in eBL vs. sBL and AIDS-NHL. The implications of these differences for the mechanism(s) of translocations and time of occurrence during B-cell development in different types of BL will be discussed.

Structural analysis of translocated c-myc alleles has also contributed to elucidating the mechanism of c-myc oncogene activation. In 29 of 29BL cases analyzed, structural alterations, namely truncations (sBL) or mutations (eBL) of the 3' portion of the first exon of c-myc have been found. These alterations correlate with: i) the removal of a block of transcript elongation which maps to the same area and ii), in most cases, with the absence or mutation of the larger (65kd) c-myc protein product.

Regarding the role of c-myc activation in B-cell transformation, we have demonstrated that the expression of activated c-myc genes leads to in vitro tumorigenic conversion of human lymphoblast immortalized by EBV, suggesting that the same two pathogenetic steps may be involved in the development of BL. We have investigated the expression of genes and proteins influenced by myc-mediated transformation in B-cells. We found that constitutive c-myc expression is accompanied by consistent changes in the expression of a number of cell-surface molecules including members of the integrin/adhesion-receptor superfamily which are involved in cell-cell interaction and immune recognition by T-cells.

G 020 ALTERED FORMS OF THE abl ONCOGENE IN HUMAN MYELOID AND LYMPHOID LEUKEMIAS, Owen N. Witte, Howard Hughes Medical Institute, Department of Microbiology, and Molecular Biology Institute, University of California, Los Angeles CA 90024.
Recent studies from several laboratories have demonstrated the expression of altered forms of the Abelson oncogene in chronic myelogenous leukemia and a subset of patients with acute lymphocytic leukemia. In both cases, the common cytogenetic abnormality known as the Philadelphia chromosome is present. This reciprocal translocation between chromosomes 9 and 22 results in the formation of chimeric genes, messenger RNAs, and proteins which contain the abl tyrosine kinase sequences. In each case the chimeric gene structure results in new sequences from a gene called bcr or Phl being added to the amino or 5' end of the abl gene coincident with a small deletion of exonic information from the abl gene. CML and ALL have different forms of the mRNAs and proteins. In CML, the mRNA is approximately 8.5 kb in size and the gene product is 210,000 M.W. (called P210), while in Ph^+ acute lymphocytic leukemia the mRNA is approximately 7 kb in size and the gene product 185,000 M.W. (called P185). The presence of such messenger RNA structures and proteins can be used as an accurate and sensitive diagnostic tool for these diseases by either biochemical analysis for the protein or enzyme activity, or, alternatively, for the unique chimeric structure of the RNA using nuclease protection assays or polymerase chain reaction amplification of the chimeric structure.

We have concentrated much of our effort on demonstrating that these molecules are important participants in the pathogenetic process for these diseases by creating transmissible retroviral vectors which express either the P210 or P185 protein. Our current results demonstrate that the P210 protein, although not a transforming gene for mouse fibroblasts, can transform immature hematopoietic precursor cells using an in vitro system. We plan to utilize these systems and constructs to evaluate the progression of both chronic myelogenous and acute lymphocytic leukemias using mouse model systems.

Mechanisms of B Cell Activation

G 021 DIFFERENTIAL AND INTERACTIVE ASPECTS OF TRANSMEMBRANE SIGNAL TRANSDUCTION CASCADES ACTIVATED FOLLOWING LIGATION OF B CELL mIg AND Ia MOLECULES AND RECEPTORS FOR IL4, IFN and immunoglobulin Fc. John C. Cambier, Dept. of Pediatrics, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO 80206.

The biology of quiescent B lymphocytes is regulated by a variety of species including antigen, T cell born Ia binding ligands, IL4, Ifnγ, immunoglobulin Fc and others. Interaction of these species with their receptors on B cells leads to transmembrane signal transduction, signal integration, and appropriate biological responses. The B cell presumably must employ distinct signal transduction cascades to maintain the fidelity of signaling by each of the regulatory species. Accumulating evidence indicates that mIgM and mIgD transduce signals via activation of phosphatidylinositol 4,5-bisphosphate hydrolysis generating second messenger diacylglycerol, which activates protein kinase C and inositol 1,4,5-trisphosphate, which mediates Ca^{++} mobilization. Recent studies suggest that ligation of Fc receptors leads to activation of an inositol polyphosphate and a phosphatidylinositol phospholipase C. Ia molecules and receptors for Ifnγ appear to utilize cAMP generating systems in signal transduction. This presentation will focus on molecular aspects of these signaling mechanisms and how the respective cascades may interact to determine the ultimate biologic response of the B cell.

G 022 TRANSMEMBRANE SIGNALING IN RESPONSE TO ANTI-IgM AND BACTERIAL LIPOPOLYSACCHARIDE, Anthony L. DeFranco, Michael R. Gold, and Dawne M. Page, Department of Microbiology and Immunology, University of California, San Francisco, CA 94143-0552.

Cross-linking the antigen receptors, membrane forms of immunoglobulins, on B lymphocytes induces breakdown of a plasma membrane phospholipid, phosphatidylinositol 4,5-bisphosphate (PIP_2), resulting in the production of diacylglycerol, which activates protein kinase C, and inositol trisphosphate, which causes the release of Ca^{++} into the cytoplasm. These events have been observed in resting B cells isolated from mouse spleen and in several B lymphoma cell lines, including WEHI-231, which ceases growth in the presence of anti-IgM. As this cell line has a phenotype that resembles that of immature B cells, this growth arrest may be analogous to antigen-induced tolerance of immature B cells.

We have used WEHI-231 cells to study the mechanism by which mIgM induces phosphoinositide breakdown, as well as the roles of the phosphoinositide second messengers in the regulation of B cell growth. Anti-IgM-stimulated PIP_2 breakdown was achieved in cells permeabilized with the mild detergent, saponin. Under these conditions, it was possible to control the concentrations of small molecules. At physiological calcium concentrations, PIP_2 breakdown was substantially enhanced by the addition of non-hydrolyzable analogs of GTP and inhibited by analogs of GDP that cannot be converted to GTP. These results demonstrate that mIgM signaling involves a GTP-dependent component analogous to the G protein family of signal transducing proteins, which play a central role in mediating receptor stimulation of adenylate cyclase, ion channels, etc. The putative G protein responsible for mediating the action of mIgM was found to be insensitive to two bacterial toxins, cholera toxin and pertussis toxin, which modify a number of the G proteins. Interestingly, the action of bacterial lipopolysaccharide (LPS) in WEHI-231 cells was abolished by treatment of the cells with pertussis toxin. This was also found to be the case with $P388D_1$ macrophage cells. This suggests that a distinct G protein mediates the action of LPS in these two cell lines.

We have also probed the roles of the phosphoinositide-derived second messengers calcium and diacylglycerol in mediating the growth inhibition seen in WEHI-231 cells treated with anti-IgM. The calcium ionophore ionomycin was used to elevate calcium appropriately and phorbol esters were used to mimic diacylglycerol in its ability to activate protein kinase C. Used appropriately, these agents were able to largely, but not completely, mimic the effect of anti-IgM on cell growth.

G 023 RECEPTOR SIGNALLING IN B LYMPHOCYTES, Gerry G.B. Klaus, Margaret M. Harnett and Kevin P. Rigley, Division of Immunology, National Institute for Medical Research, Mill Hill, London NW7 1AA, UK.

Crosslinking of surface immunoglobulin (sIgM and sIgD) receptors on B lymphocytes by potentially mitogenic forms of anti-Ig antibodies (e.g. $F(ab')_2$ fragments of rabbit anti-Ig) leads to rapid, and prolonged hydrolysis of phosphatidylinositol 4,5 biphosphate (PIP_2), with consequent elevation of intracellular Ca^{2+} and activation of protein kinase C. Both of these two second messengers are apparently required for optimal activation of resting B lymphocytes following crosslinking of their Ig receptors. Many Ca^{2+}-mobilizing receptors on non-lymphoid cells are known to be coupled to the polyphosphoinositide-specific phosphodiesterase (PPI-PDE) via guanine nucleotide regulatory (G) proteins, termed G_p. Our experiments with permeabilized B cells have shown that both sIgM and sIgD receptors are coupled to the PPI-PDE by one or more pertussis toxin-insensitive forms of G_p, which have yet to be identified.

Intact (IgG) rabbit anti-Ig antibodies are not mitogenic for mouse B cells. This is because these antibodies co-crosslink Fc receptors and sIg on B cells: this results in rapid abrogation of PIP_2 hydrolysis, and presumbly explains why such antibodies only induce abortive B cell activation. Our recent studies with GTP analogues in permeabilized B cells suggest that crosslinking of sIg and Fc receptors leads to uncoupling of G_p from the antigen receptors. These results will be discussed in the context of G protein involvement in the transduction of both positive and negative signals controlling B lymphocyte activation.

Transgenic Mouse Models for Immune Regulation

G 024 V-RAF CONVERTS Eμ-MYC TRANSGENIC B CELLS INTO MACROPHAGES

S. Peter Klinken, Warren S. Alexander, and Jerry M. Adams.
The Walter and Eliza Hall Institute of Medical Research, Post Office, Royal Melbourne Hospital, Parkville, Victoria 3050, Australia.

Hemopoietic lineage commitment is shown to be breached by concomitant expression of the c-myc and v-raf oncogenes. Switching to the myeloid lineage occurred frequently when B lineage cells, from either lymphomas or pre-leukemia bone marrow cells of Eu-myc transgenic mice, were infected with a v-raf retrovirus. Cloned pre-B and B cell lines changed into either mature or immature macrophages as assessed by their morphology, adherence, phagocytic activity, surface markers and lysozyme production, but retained clonotypic immunoglobulin gene rearrangements. Although expression of the Eμ-myc transgene was reduced, or abolished, in the more differentiated lines, they remained tumorigenic. The converted lines produced myeloid growth factors and most had karyotypic alterations. These results suggest that constitutive myc plus raf expression can provoke genetic reprogramming in lymphocytes.

G 025 ALLELIC EXCLUSION IN IMMUNOGLOBULIN HEAVY CHAIN GENE TRANSGENIC MICE, Georges Köhler, Marinus Lamers and Antonio Iglesias, Max-Planck-Institut für Immunbiologie, Stübeweg 51, 7800 Freiburg, FRG.

Expression of transgenic immunoglobulin (Ig) μ and ƒchains leads to roughly a 2-fold reduction of B-cells in the spleens of transgenic animals and to a similarly reduced number of precursor cells in bone marrow. Only 20%-30% of the splenic B-cells express endogenous heavy (H) and light (L) chains together with the transgenic H chain. About 70%-80% express the trans-H chain alone (together with endogenous L chains). These B-cells show immature joining configurations of the endogenous variable H (V_H) chain locus. We conclude that both μ and ƒchains inhibit the rearrangement of endogenous V_H gene elements, but have no inhibitory effect on the V_L gene rearrangement and expression.

It has been shown that only the membrane, but not the secretory μ-chain inhibits rearrangement. In order to further analyse the structural requirements of μ-chains in this process, transgenic mice expressing μ-chains lacking the first constant domain ($C_\mu 1$) were studied. We noticed a 10-fold reduction of the number of B-cells in the spleen and a similar reduction of precursor-cells in bone marrow. Most B-cells coexpressed endogenous and mutant μ-chains. Therefore, a similar depletion of peripheral B-cells expressing endogenous μ chains is observed with all three constructs indicating that the $C_\mu 1$ domain is dispensable for this effect. Nevertheless, a major B-cell population expressing the transgenic heavy chain only (with endogenous L) is missing in the mutant μ transgenic mice. This altered phenotype indicates a role of $C_\mu 1$ in B-cell maturation.

G 026 REARRANGEMENT AND EXPRESSION OF IMMUNOGLOBULIN GENES, Ursula Storb, Katherine Gollahon, Joanna Manz, Peter Engler and James Hagman, Dept. of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL 60637; Kathleen Denis, UCLA, Los Angeles, CA 90024; Ralph Brinster, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104.

During B lymphocyte development the rearrangement of immunoglobulin genes proceeds in an ordered sequence, first H, then L genes rearrange. Apparently the same recombinase is responsible for all rearrangements and the sequential order is determined by the sequential activation of the target genes for rearrangement. Furthermore, productive rearrangement of H genes can apparently be sensed by the developing preB cells and leads to the stop of further H gene rearrangements. This feedback inhibition is only exerted by membrane bound μ chains, not by secreted μ, nor by γ-2b in any form.

κ gene rearrangement is likewise inhibited after productive rearrangement of a κ gene. The combined presence of κ and membrane μ chains is required for this feedback, κ alone or in combination with secreted μ chains does not cause feedback inhibition.

Finally, the rearrangement of λ genes appears to be controlled in a different way. A separate line of B lymphocytes seems to undergo both λ and κ gene rearrangement. This postulated λ/κ B cell lineage does not respond to feedback inhibition at the preB cell stage. Instead, gene rearrangement continues and may lead to elimination of κ genes by rearrangement of a recombining sequence (RS, described previously by E. Selsing and K. Siminovitch and their collaborators) located originally downstream of the C-κ gene. In this way, a λ/κ cell may convert to a pure λ producer. This cell may later undergo an arrest of recombinase activity by a mechanism different from the feedback inhibition of H and κ gene rearrangement.

Late Addition

G 027 ISOLATION AND IDENTIFICATION OF THREE CLASSES OF BONE MARROW PROGENITORS: THE HEMATOPOIETIC STEM CELL, MULTI-POTENTIAL NON-STEM CELLS, AND AN EARLY B LINEAGE COMMITTED PRECURSOR, Gerald J. Spangrude, Shelly Heimfeld, George Tidmarsh, Jan Klein, Christa Muller-Sieburg, Jeff Friedman, and Irving Weissman, Laboratory for Experimental Oncology, Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305.

We have identified the mouse hematopoietic stem cell. Phenotypically, it is Thy-1^{lo}, Sca-1^{+}, Lin^{-} (Lin^{-} means lacking markers for any of the known hematolymphoid lineages such as B220, Gr-1, Mac-1, L3T4, Lyt-2, etc.). Upon i.v. injection into lethally irradiated animals it gives rise mainly to one 12 day spleen colony for every 10-12 cells injected, compared to 1/6,700 unfractionated bone marrow cells. (The seeding efficiency of i.v. CFU-S is 1/6 to 1/10 cells.) The limiting number for thymic colonies following intrathymic injection is 1 per 5 cells injected, compared to 1/8000 bone marrow cells. The dose of these stem cells required to restore fully lethally irradiated animals is 20-50 cells to rescue half of the injected animals, as opposed to $>10^{4}$ whole bone marrow cells; all hematolymphoid lineages (B, T, erythroid, myeloid, etc.) are reconstituted.

A second multi-potent hematopoietic progenitor is Thy-1^{lo} $B220^{+}$ Mac-1^{+} (Gr-1^{+}). It gives rise almost exclusively to 7 day spleen colonies when injected i.v. into lethally irradiated animals. Approximately 1 spleen colony is found for every 250 cells injected. Injection of these cells into lethally irradiated Ly5 congenic animals along with host stem cells leads to (at least) short-term repopulation of donor-derived cells of all hematolymphoid lineages bearing the Ly5 marker (T, B, macrophage, granulocyte). Animals which were lethally irradiated and injected with over 1500 of these cells die at approximately 20 days following irradiation, presumably because this population lacks self-renewal capacity. We are currently testing the hypothesis that this multi-potent poorly self-renewing hematopoietic progenitor is a necessary intermediate between the hematopoietic stem cell and committed lineage precursors; and whether it is each cell (vs the population) which is multipotent.

The committed early B lineage precursor is Thy-1^{lo} $B220^{+}$ Mac-1^{-} Gr-1^{-}. These cells are poorly self-renewing <u>in vitro</u>. <u>In vivo</u>, however, as few as 2-7,000 cells are as potent as 500,000 whole bone marrow cells in giving rise to B$\mu\delta$ cells by 6 weeks following irradiation. The Thy-1^{lo} $B220^{+}$ committed B lineage precursor does not give rise to T cells or myelomonocytic cells, and represents the most highly enriched target cell population yet defined for Abelson leukemia virus <u>in vitro</u> leukemogenesis (1400 per 10^{6} plated compared to 60 per 10^{6} whole bone marrow cells).

Ig Genes, Diversity

G 100 ISOTYPE SWITCHING BY CELLS OF A LY-1^+ B CELL LYMPHOMA GROWN IN VITRO. Larry W. Arnold, N. Keith Collins, and Geoffrey Haughton. Univ. of North Carolina, Chapel Hill, NC 27514. CH12 is a B cell lymphoma derived from the Ly-1^+ B cells of B10·H-2^aH-4^bp mice. It produces and can be induced to secrete IgM reactive with phosphatidyl choline, hemolytic for SRbc and BrMRbc and expressing a defined idiotype. CH12.LX is a subline of CH12 which has been adapted to growth in culture; it has a cloning efficiency of about 25%. We have selected and cloned variants of CH12.LX which produce IgM and IgA, IgG_3, IgG_3 and IgA, IgG_{2b}, IgG_{2b} and IgA or IgA. We have not found any variant which produces IgG_1 or IgG_{2a}. Two color immunofluorescence and subcloning analyses demonstrate that individual cells of the double producer clones are producing two isotypes simultaneously. All variant immunoglobulins display the same idiotype and antigen binding specificity as the original IgM and utilize the same $V_HD_HJ_H$ assembly as evidenced by Southern blot analysis and by primer extension sequence analysis of the mRNA encoding the μ and α heavy chains. The double producers are unstable, undergo further isotype switching and are all tetraploid. One intrepretation of these findings is that each duplicate chromosome is rearranging independently to produce one each of the expressed Ig heavy chains. The data demonstrate that isotype switching in B cells does not require signals derived from any other type of cell. They further suggest that isotype switching may be a multistage process and that the range of isotypes that may be produced may be limited by programming of the B cell prior to initiation of switching. We are currently analyzing the Ig gene rearrangement staus of these variants.

G 101 A MICRORECOMBINATION EVENT GENERATES A IgG2b-IgG2a-IgG2b HEAVY CHAIN GENE IN A MOUSE MYELOMA CELL LINE, Jonathan A. Bard, Gary Gilmore and Barbara K. Birshtein, Albert Einstein College of Medicine, Bronx, New York 10461.
A variant, E5.7A12, of the MPC11 mouse myeloma cell line (IgG2b, K) makes a short immunoglobulin (Ig) heavy chain of 52,000 MW, in contrast to the parental 2b heavy chain of 55,000 MW. Primer extension sequencing of the H chain mRNA and genomic Southern analysis documented the presence of a tract of 2a-derived sequences, extending from the 3' end of the CH2 domain into the CH2-CH3 intervening sequence (IVS). Both 5' and 3' ends of the microrecombination event are flanked by regions of identity between 2b and 2a sequences: thus, it is likely that either a double cross-over or a gene conversion event accounts for the 2b- 2a- 2b gene formation. Contiguous to the 3' end of the microrecombination, there is a 200bp deletion that spans the 3' end of the CH2-CH3 IVS--including the 3' acceptor splice site--and DNA corresponding to the N-terminal 39 amino acids of the CH3 domain. E5.7A12 fails to assemble H-H dimers, halting the Ig assembly process at the HL stage, and does not secrete H chain. We speculate that these defects in H chain assembly and secretion may result in an inherent instability in the Ig gene rearrangement machinery. This instability may permit further rearrangment, as evidenced by the isolation of E5.7A14 a heavy chain non-producing sister clone of E5.7A12. In addition to the 3' microrecombination/deletion event described above, the heavy chain gene of E5.714 contains a novel restriction map 5' to the enhancer region. Until the origin of this novel DNA is determined through cloning, we speculate that it may represent an abberrant attempt at V region replacement or a chromosomal translocation.

G 102 HUMAN VpreB: A PRE-B CELL LINE SPECIFIC MEMBER OF THE IMMUNOGLOBULIN GENE FAMILY, Steven R. Bauer, Akira Kudo and Fritz Melchers, Basel Institute for Immunology, CH-4005 Basel, Switzerland.
A newly identified member of the human immunoglobulin gene family has been isolated by utilizing cross-species hybridization of a mouse VpreB1 probe (Kudo et al., 1987, EMBO J. 6:2267) to a human genomic library. This gene, human VpreB, is 76% homologous with mouse VpreB1 and has one stretch of both amino acid and nucleotide identity covering 45 nucleotides encoding the entire putative second framework region. Human VpreB has several features typical of Ig V region genes including: a 19 amino acid leader coded in 2 exons with the first nucleotide of a glycine codon split from the remainder of the codon by an 86 bp intron, conserved cysteine residues at positions amenable to disulfate bond formation typical of Ig variable region domains, and a conserved trytophan residue at the boundary of CDR1 and framework region 2. Like mouse VpreB1, the 3' end of human VpreB is not related to any reported gene sequences. Messenger RNA expression of human VpreB is to date observed only in human pre B cell lines and not in mature B, B lymphoblastoid, myeloma, myeloid, erthyroblastoid and T cells lines. Rearrangement or deletion of the gene has not been seen in any of these lineages.

G 103 THE REGULATION OF k IMMUNOGLOBULIN ENHANCER BY ADENOVIRUS E1APRODUCTS. A POSSIBLE INVOLVEMENT OF A CELLULAR FACTOR, Yehudit Bergman, Ludmila Shurman and Ranjan Sen*. The Hebrew University, Jerusalem, Israel, and *Brandeis University, Waltham, MA 02254.

The regulation of k chain immunoglobulin gene by the adenovirus E1A gene products has been studied. The E1A proteins have a pleiotropic effect on the regulation of the k chain gene enhancer. In lymphoid cells, the k light chain enhancer is repressed by the E1A products. In fibroblasts, by contrast, that enhancer is activated by the E1A proteins irrespective of whether the E1A is in an extrachromosomal location or is stably integrated in the genome. This activation is mediated by cellular factors that interact with the k chain enhancer. The k3 enhancer fragment encompassing the NF-kB transcription factor binding site, that was found to be critical for the in vivo function of k chain enhancer in lymphocytes, was also found to be involved in the activation by E1A products. The level of nuclear factors, which bind to the k3 fragment, increases dramatically in fibroblasts expressing E1A products. Presently, we do not know whether it is the actual amount that increases or the binding activity which does. We suggest that the increase in those factors is responsible in part for the stimulation of the k chain enhancer by the E1A in nonlymphoid cells.

G 104 CONTENT AND ORGANIZATION OF THE HUMAN IG V_H LOCUS, J.E. Berman, S.J. Mellis, R. Pollock, K. Nickerson, C. Smith, H. Suh, B. Heinke, L. Chess, C. Cantor and F.W. Alt. Howard Hughes Med. Inst. and Depts. of Biochem., Micro., Human Genet. and Med., Coll. of Phys. and Surg., Columbia Univ., New York, N.Y. 10032. We have performed a detailed analysis of the content and organization of the human immunoglobulin V_H locus. Human V_H genes were isolated by two strategies. First, a panel of probes consisting of representative members of selected murine V_H gene families was used to isolate related genes from a human genomic library. Secondly, V_HDJ_H rearrangements were isolated from genomic libraries of human B lineage tumors. These two approaches yielded V_H genes representing five distinct families, including novel members belonging to two out of three of the known V_H gene families (V_H1 and V_H3) as well as representatives of three new families (V_H4, V_H5, and V_H6). We report the nucleotide sequence of 21 novel human V_H genes, many of which belong to the three new V_H gene families. In addition, we have performed a preliminary analysis of the organization of these gene segments over the full extent of the locus. We find that the five multi-segment families (V_H1-5) have members interspersed over nearly the full 1500-2000 kb of the V_H locus, and estimate that the entire heavy chain locus covers 2500kb or less. We have also shown the first physical linkage of the variable and constant loci of a mammalian Ig gene family by demonstrating that the most proximal human V_H segments are found within 100kb of the constant region locus. Using the above information, we have begun studies of V_H gene usage. Preliminary results suggest differences in the patterns of V_H gene usage in normal fetal and adult tissues.

G 105 RECOMBINATION POTENTIAL OF A-MULV-TRANSFORMANTS DERIVED FROM SCID MICE. B. Malynn, P. Ferrier, G. Fullop, T. K. Blackwell, A. Furley, G. Rathbun, M. Morrow, B. Heinke, R. Phillips, F. Alt. Howard Hughes Med. Inst. & Columbia Univ., N.Y., N.Y. Severe combined immunodeficiency (SCID), an autosomal recessive trait which causes a deficiency of mature lymphocytes, was shown by Schuler and Bosma to result in abnormal rearrangements of genes encoding T and B cell antigen receptors, suggesting that the SCID mutation adversely affects the recombinase system. To further characterize the defect, we are analyzing A-MuLV transformants from SCID mice with respect to RNA expression of relevant genes, status of endogenous Ig and TCR gene rearrangements, and potential of these lines to rearrange introduced recombination constructs. We find that SCID A-MuLV-transformants rearrange endogenous Ig H chain genes at a low frequency, have grossly aberrant rearrangements, or both. Although all rearrangements sequenced thus far completely delete the coding regions of either the D-segment, J_H-segment, or both, one third of the rearrangements are precise at either the 5' or 3' end, and several rearrangements contained N-regions, suggesting that certain recombinase functions--ie. specific recognition of heptamer-nonamer signal sequences, DNA cutting, and TdT activity--may be intact. These lines also express germline V_HJ558 and sterile C_u transcripts, suggesting appropriate regions are "accessible" to the recombinase system. An inversion recombination construct which was introduced into SCID lines resulted in neomycin-resistent lines at a frequency several orders of magnitude lower than that observed in A-MuLV-transformants from normal mice, and occurred by either aberrant rearrangement or amplification of the construct. Together these results suggest a defect in either ligation of cleaved ends or in exonuclease activity.

G 106 BIASED USAGE OF CERTAIN V_K GENE FAMILIES BY AUTOANTIBODIES AND THEIR POLYMORPHISM IN AUTOIMMUNE MICE, Kuppuswamy N. Kasturi, Marc Monestier, Raoul Mayer and Constantin A. Bona, Mount Sinai Medical Center, New York, NY 10029

Germline genes of V_H and V_K loci coding for the variable regions of immunoglobulins have been classified into families on the basis of their sequence homology and serological reactivity. Combinatorial association of light and heavy chains plays an important role in the generation of antibody diversity. However, a restricted association of certain V_H and V_L gene families has been observed among antibodies specific for polysaccharides and some haptens. Furthermore, the 3' V_H gene families have been found to be used frequently among autoantibodies. In a recent study we also observed that the vast majority of hybridomas producing autoantibodies obtained from motheaten mice, an autoimmune prone strain, use only a restricted number of V_K gene families as do murine rheumatoid factors. We report here that over 75% of the hybridomas derived from stimulated or unstimulated autoimmune disease prone mouse strains, secreting autoantibodies of various specifities (anti-DNA,Sm,MBP,IF, TG,coll,Thy,RBC and GBM) show a bias towards the use of V-genes belonging to V_K families V_K1,V_K4,V_K8,V_K10, and V_K19. Most interestingly, RFLP analysis of genomic DNAs from TSK,NZB and SJL, three autoimmune disease prone mouse strains, also reveals marked differences in the polymorphism of those V_K gene families used by autoantibodies. The role of these germline genes in the development of autoreactive B cell clones merits attention.

G 107 THE INFLUENCE OF ANTIGENIC STIMULATION ON VH GENE USAGE. Nico A. Bos, Corine G. Meeuwsen and Robbert Benner. Dept. of Cell Biology, Immunology and Genetics, Erasmus University, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands.

The VH-gene usage was investigated in panels of hybridomas made from LPS-activated splenic B cells from adult germfree BALB/c mice fed a chemically defined synthetic diet (GF-CD) and in panels of hybridomas made from unstimulated neonatal spleen cells from conventional BALB/c mice. This was done with the RNA-dot-blot assay using probes for the VH-gene families Vh36-60, VhJ606, VhJ558, VhS107, VhQ52 and Vh7183. Both groups of mice resemble situations with virtually no exogenous antigenic stimulation.
After hybridization of the RNA from 100 at random chosen hybridomas from both groups approximately 50% showed a clear signal with one of the probes. More than 50% of the positive hybridomas derived from neonatal spleen cells reacted with probes from the 7183 and Q52 gene families, while this was 36% in the hybridomas from adult GF-CD mice. In hybridomas from adult conventional BALB/c mice much lower percentages have been found. These data suggest that in the absence of exogenous antigenic stimulation B cells preferentially use the most 3' VH genes, while after antigenic stimulation more 5' VH genes are used.
This investigation was supported by the Netherlands Foundation for Medical Research (MEDIGON).

G 108 VH AND VL GENE SEQUENCE ANALYSIS OF NATURAL ANTIBODIES, Leif Carlsson & Dan Holmberg Unit of applied Cell and Molecular Biology, University of Umeå, 90187 Umeå, Sweden

Using collections of B cell hybridomas derived from normal nonimmunized mice, we have previously shown that idiotypic complementarities occur in a very high frequency in the internally activated pool of B cells in newborn mice. A fraction of these clones have also been demonstrated to be multireactive, a characteristic, which in newborn individuals appears to correlate with usage of the PC 7183 and QUPC 52 VH gene families. In contrast, hybridomas derived from adult individuals do not show this correlation.

In order to investigate the structural basis for this discrepancy we have determined VH and VL sequences of a number of hybridomas representing either of these two groups. The most striking finding was that all the multireactive antibodies had unusally short heavy chain CDR3 regions, only three codons.

G 109 CHANGES IN GENE EXPRESSION IN DIFFERENTIATING LY-1+ B LYMPHOCYTES. R.B. Corley, S. Sharma, and L.B. King, Duke University Medical Center, Durham, NC 27710.
The molecular events responsible for the differentiation of B cells into antibody secreting cells remain poorly understood. Along with the well documented increase in immunoglobulin (Ig) transcripts, the expression of many other genes must be regulated during B cell differentiation. We are identifying genes which change in abundance in differentiating B cells so that we can then determine their role in this process, and ask whether there are differences between Ly-1+ and Ly-1- B cells in this regard. As a model system, we are using the inducible Ly-1+ B cell lymphoma, CH12, which can be stimulated by lipopolysaccharide (LPS) to differentiate to high rate IgM secretion. Steady state levels of Ig increase during CH12 differentiation in a manner analogous with those changes seen in normal B lymphocytes. We are analyzing the expression of other genes in two ways. First, specific cDNA probes and antibodies are used in the analysis of "known" genes, including membrane proteins and proto-oncogenes. Second, we are identifying unknown genes whose abundance changes during differentiation by screening a cDNA library from LPS-stimulated CH12 cells with a subtractive probe specifically enriched for cDNA expressed in these cells. Positive clones have been isolated and are being characterized with respect to the mRNA species they detect, the kinetics of expression of the mRNA species, and frequencies within cDNA libraries from both non-stimulated and stimulated CH12 cells. Clones of interest have been sequenced. Results of this study will provide not only a phenotypic analysis of B cell differentiation, but also insights into the function of genes expressed in B cell subsets.

G 110 POSTTRANSCRIPTIONAL CONTROL OF IMMUNOGLOBULIN GENE EXPRESSION, Angela Cox, Celltech Limited, Slough, Berkshire, England.

Various lines of evidence indicate that the large increase in steady state u mRNA levels that occurs when resting B cells differentiate to plasma cells is due mainly to posttranscriptional regulation. A pulse chase protocol has been used to analyse the half life of u mRNA in transformed cell lines representing these stages of development. Experiments indicate that u mRNA in a hybridoma cell line (representing the plasma cell stage) has a longer half life than u mRNA in a B cell lymphoma cell line representing the resting B cell stage. Transfection experiments are in progress to identify sequences required for posttranscriptional regulation of u mRNA expression.

G 111 DYNAMICS OF ANTIGEN-DRIVEN, CLONAL EXPANSION OF PC-SPECIFIC B CELLS TO *P. morganii*. C. Dell, Y. Lu, and L. Claflin, Department of Microbiology and Immunology, The University of Michigan, Ann Arbor, Mi 48109-0620.

Sequence analysis of VH and VL regions has shown that antibodies from individual mice, specific for the PC moiety of *Proteus morganii*, are normally derived by somatic mutation from a single precursor B cell. Binding studies indicate that specificity of these antibodies is dependent upon somatic point mutations and also that continued selection of mutants is antigen driven. Mutations are nonrandomly distributed; critical mutations occur in CDR2 and CDR3 of VH. Further work (J. Immunol. 138:3060) found five, independently selected secondary response antibodies from one fusion which had identically mutated VH and VL sequences. The B cells represented by these five hybridomas comprise a large and dominant portion of the PC specific pool in the secondary response. For this to be the case, one must conclude that the hypermutation mechanism is not continuously activated during clonal expansion of a B cell clone.

To establish whether the hypermutation mechanism is continously active during clonal expansion, we have used the serial transfer system of B cell propagation of Askonas. Spleen cells from mice immunized with *P. morganii* were adoptively transferred into paired irradiated recipients. Recipients were boosted and fused at a later date. Sequence analysis of hybridomas from these paired fusions showed examples of identically mutated VH and VL regions, indicating that extensive somatic mutation is no longer occurring in the transferred B cell population. This provides strong evidence that the hypermutation mechanism is a regulated event occurring during clonal expansion.

G 112 **IN PLASMA CELLS, ALTERNATIVE POLYADENYLATION CONTROLS THE LEVELS OF μ_S AND μ_m mRNA**, Nicolas Fasel, M. Rousseaux, H. Govan*, M. Briskin*, R. Law*, C. Bron and R. Wall*, University of Lausanne, 1066 Epalinges, Switzerland, and UCLA, Los Angeles, CA 90021.

The expression of different immunoglobulin μ and δ heavy chain mRNA species from the μ and δ complex transcription unit changes radically during B cell development. Post-transcription RNA processing controls the levels of secreted (μ_S) and membrane (μ_m) mRNA that differ only in their 3' termini. Alternative splicing or polyadenylation could be responsible for this differential processing. By deleting the region between the two poly (A) regions including the splice signals we demonstrate that the usage of the second poly (A) site (μ_m) is favored in plasma cells and in L cells. This result suggests that the choice of polyadenylation site is influenced by the distance between the two poly (A) sites. If we invert the two poly (A) sites, the μ_m poly (A) site is used. This poly A region contain DNA sequences which have been shown to block transcription of the δ gene. We suggest that in this DNA construct the second poly (A) site is not used because sequences downstream of the μ_m poly (A) site are not transcribed.

G 113 CLONAL EVOLUTION OF THE ANTIBODY RESPONSE TO PC-KLH, A.J. Feeney, D.E. Mosier, Medical Biology Institute, 11077 North Torrey Pines Road, La Jolla, CA 92037.

The antibody response of phosphorylcholine (PC) is comprised of three families of antibodies (T15, 511 and 603) which use the same Vh gene, and differ in Vk usage. The primary antibody response to PC-KLH is almost entirely comprised of IgM antibodies of the T15 clonotype. The secondary response, in contrast, is much more heterogeneous. T15 antibodies still dominate the IgM response, but there is now a large IgG response, some of which are 511$^+$. To assess the role of somatic mutation and affinity maturation in the secondary response, we analyzed several secondary anti-PC hybridoma antibodies. We showed that 511 antibodies were somatically mutated, many had significantly higher affinity for NPPC than T15 antibodies, and interestingly, all had Vh-D junctional sequences which encode at least one extra amino acid compared to T15 antibodies. An analysis of all the published sequences of anti-PC antibodies showed this to be a consistent finding. In addition, 603 antibodies also have a family-specific junctional sequence, namely a change from aspartic acid to asparagine at position 95. These observations led us to propose that specific Vh-D junctional diversity is required for non-T15 antibodies to be triggered by PC upon first exposure to antigen. Assuming that the Vh-D-Jh joining resulting in the prototypic T15 H chain is a much more frequent event than the events which appear necessary to generate 511 or 603 PC-binding antibodies, the result would be that T15 PC-precursors outnumber 511 or 603 PC-precursors prior to immunization. After immunization, somatic mutation can act upon these non-T15 precursors with the requisite junctional diversity to yield high affinity secondary antibodies.

G 114 GENE CONVERSION AMONG T15 V_H GENES IN CBA/J MICE. Stacy E. Ferguson[1], Stuart Rudikoff[2] and Barbara A. Osborne[1], [1]University of Massachusetts, Amherst, MA and [2]Laboratory of Genetics, National Institutes of Health, Bethesda, MD.

The T15 V_H gene family consists of four members in most strains of inbred mice. These genes are designated V1, V11, V13 and V3. We have determined the nucleotide sequences of the four T15 genes of the CBA/J mouse. Comparison of these sequences with the published BALB/c and C57/BL10 homologues suggests that gene conversion is occurring frequently among members of this multigene family. Nucleotide sequence differences found between corresponding alleles are found in other family members. We suggest that gene conversion may contribute to the evolution and germline diversification of this multigene family. We have also determined the DNA sequence of the 5'-flanking region of the CBA/J V1 family member. This sequence contains a long GT dinucleotide repeat between the coding region of the leader peptide and the mature peptide . This sequence is conserved among inbreds examined thus far. The possible effects of this repeat structure on V1 gene expression will be addressed.

G 115 USAGE OF VH AND Vκ GENES UPON ALLOGENEIC MANIPULATION OF THE GAT IDIOTYPIC CASCADE. Sylvie Corbet, Michel Hirn, Claudine Schiff, and Michel Fougereau. Centre d'Immunologie de Marseille-Luminy, Case 906, 13288 Marseille Cédex 9, France.

Ab1 and most Ab3 of the GAT idiotypic cascade express similar idiotypic determinants in BALB/c and C57BL/6 strains. These idiotopes depend upon the presence of both the H and the L chains. The VH of the BALB/c Ab1 (germ-line gene VH 10) and that of the C57BL/6 Ab1 (germ-line gene V 186-2) are only 75% homologous whereas Vκ are much more conserved. C57BL/6 mice were immunized with BALB/c Ab2 (anti-idiotypic set) and monoclonal Ab3 were derived after fusion of immunized spleen cells with the non secreting hybridoma cell-line Sp/2.0-Ag. From 13 cell-lines, 5 clones were isolated and the mRNA variable regions sequenced. Immunization with BALB/c anti-idiotypes elicits expression of the same C57BL/6 VH and Vκ genes as when C57BL/6 mice were immunized with GAT, although functional VH BALB/c equivalents have been identified in the B6 strain. Our results suggest that manipulation of the repertoire via antigenic or idiotypic stimulation both lead to the expression of different genes in different strains, but of identical genes within a given strain. They further confirm that the immune system is largely degenerate, for both idiotype expression and antigen recognition.

G 116 ONSET OF CLONAL COMMITMENT TO PC (*P. morganii*), Julia George and Latham Claflin, University of Michigan, Ann Arbor, MI 48109.

The PC-specific antibody response to *P. morganii* is dependent upon somatic hypermutation in a single VK/VH pair. Within individual mice the response is often generated from one precursor B cell; a mature response is therefore a clonally expanded population of related but nonidentical B cells. Initial studies on the mutational process showed that it is not continuously active during the life of a B cell. A critical question then is: when is the mutational process active and what role does antigen play in initiating the mechanism? Also why is the secondary response dominated by progeny of only a single B cell precursor? To explore these questions we examined clonal commitment of primary response B cells. Initial studies of hybridomas obtained four to five days after primary immunization indicated that clonal commitment and antigen-specific mutations occurred prior to immunization. This might be anticipated because *P. morganii* is part of the intestinal flora of the mouse, and mice are probably colonized soon after birth with this organism. Therefore, experiments with germ-free mice were conducted. Mice were left uncolonized or selectively colonized with *P. morganii* for 2 to 4 wk. Initial results showed that colonization of mice was sufficient to stimulate a serum antibody response. These mice were then used as donors in a series of adoptive transfer experiments in which one donor spleen was transferred to 2 recipient mice. IEF analysis of recipient mouse sera supports our hypothesis that intestinal exposure to antigen is sufficient to induce clonal commitment.

G 117 IDENTIFICATION AND CHARACTERIZATION OF A PROTEIN THAT SPECIFICALLY BINDS NONAMER RECOMBINATIONAL SIGNAL SEQUENCES OF IG GENES, Brian D. Halligan and Stephen V. Desiderio, Howard Hughes Medical Institute Laboratory of Genetics and Department of Molecular Biology and Genetics, The Johns Hopkins University, School of Medicine, Baltimore, MD 21205.

We have identified and characterized a protein that recognizes the conserved nonamer element of Ig recombinational signal sequences. The protein binds specifically to nonamer sequences from heavy and light chain gene segments, and to a synthetic 23 bp double-stranded oligonucleotide containing the nonamer sequence. The protein does not bind to the heptamer portion of the Ig rearrangement signal sequences. The binding activity has been detected in several lymphoid cell lines and in thymus, but not in non-lymphoid cell lines. Calf thymus has been used as a source of starting material for the partial purification of the protein. The activity elutes as a single peak during chromatography on BioRex70, non-specific DNA-cellulose, and heparin agarose. The size of the protein has been established by size exclusion chromatography and sucrose gradient sedimentation to be about 120 kDa.

Halligan, B. D. and Desiderio, S. V. "Identification of a DNA binding protein that recognizes the nonamer recombinational signal sequence of immunoglobulin genes" Proc. Acad. Sci. USA, in press.

G 118 IDENTIFICATION AND CHARACTERIZATION OF λ-LIKE PROTEINS IN HUMAN PRE-B CELL LINES. *L.M. Hendershot, W.G. Kerr, H. Kubagawa, L. Feng, M.D. Cooper, and P.D. Burrows, Univ, of Alabama at Birmingham, Birmingham, AL 35294.*

Proteins of 18 and 22 kD have been identified in human pre-B acute lymphoblastic leukemia (ALL) cell lines by immunoprecipitation with polyclonal anti-human λ antibodies. These λ-like proteins, which are smaller than conventional λ chains, were not identified in EBV-transformed cell lines or in surface Ig^+ B cell lymphoma lines, nor could they be immunoprecipitated with anti-κ antibodies. A small fraction of the μ heavy chains in the pre-B cell lines was associated with the λ-like proteins as demonstrated by the ability of anti-λ antibodies to co-precipitate some of the μ heavy chains. However, the majority of the μ chains were not associated with the λ-like proteins, remained endo H sensitive and were associated with heavy chain binding protein (BiP). When surface stained with fluorescinated anti-μ antibodies, the pre-B cell lines exhibited minute patches of immunofluorescence suggesting that association of the λ-like proteins may allow the expression of surface IgM in small amounts. Characterization of one of these lines by Southern blot analysis demonstrated germ-line configuration of λ genes . Analysis of poly A^+ mRNA from the cell lines revealed the presence of an ~1.2 kb transcript that hybridized with a human Cλ probe. These findings suggest that the λ-like proteins found in ALL-derived pre-B cell lines may be the human homologue of the murine λ_5 /ω gene product. Supported by CA16673, CA13148, AI23526-02 and AI23694.

G 119 LINKAGE STUDIES USING GENETIC MARKERS FOR RABBIT KAPPA LIGHT CHAIN AND T CELL RECEPTOR BETA CHAIN GENES. N.J.K. Hole, E. Lamoyi, M. Komatsu, N. Harindranath, G.O. Young-Cooper, and R.G. Mage, Laboratory of Immunology, NIAID, NIH, Bethesda, MD 20892

In order to investigate linkage we used serum allotypes of rabbit Cκ isotypes and restriction fragment length polymorphisms (RFLPs) of the genes for Vκ, Jκ, Cκ1, Cκ2 and T-cell receptor Cβ. The inheritance of these genetic markers was studied through backcross matings between a male $K1b^9$, $K2bas^2/K1b^{bas}$, $K2bas^1$ rabbit and several $K1b^9$, $K2bas^2/K1b^9$, $K2bas^2$ females, and F2 matings between $K1b^9$, $K2bas^2/K1b^{bas}$, $K2bas^1$ rabbits. Southern analysis and hybridization of genomic DNA with a probe for the C region of the K2 isotype detected a 5 Kb PstI fragment linked to the presence of the $K1b^{bas}$ gene and expression of K2bas1 allotype and a 6.6 Kb PstI fragment linked to the expression of the K1b9 allotype and K2bas2 (lack of expression of K2bas1). A V_κ probe detected a 1.3 Kb EcoRI fragment linked to the K1bbas and K2bas1 allotypes. In contrast, the 9 or 14 Kb EcoRI RFLP detected with a TCR β chain probe segregated independently from C_κ allotypes and RFLPs. In man Cκ and Cβ are also unlinked whereas in the mouse they are linked at a distance of ~8 centimorgans. Work is now in progress on the RFLP seen in the Jκ region. In addition, pulsed field gel electrophoresis is currently being used to further investigate various linkage relationships.

G 120 THE EXPRESSION OF V_H GENE FAMILIES IN LPS INDUCED, UNTRANSFORMED FETAL B CELLS, Hyun Do Jeong, Jack L. Komisar and Judy M. Teale, University of Texas Health Science Center at San Antonio, San Antonio, TX 78284

The V_H gene segments that partially encode the variable regions of Ig heavy chains have been categorized into families based on nucleotide sequence similarity. Evidence from studies of hybridomas and virally transformed cells suggests that there is a nonrandom usage of V_H gene families in the fetus with the vast majority utilizing the most 3' family, V_H 7183. By using in situ hybridization, we have studied V_H gene expression at the single cell level in B cells from different stages in ontogeny. This protocol eliminates any potential bias from transformation protocols. Our results indicate that V_H gene expression as a result of LPS stimulation is clearly different in fetal and early neonatal B cells compared with adult B cells. Thus, LPS induced fetal B cells compared with adult B cells express a significantly greater proportion of V_H genes from the 7183 (25% vs 17%) and Q52 (34% vs 20%) families and a lesser proportion of V_H genes from J558 (20% vs 34%). The remainder of the V_H families appear to be expressed at similiar frequencies in the adult and fetus. Interestingly, the differences in the expression of V_H gene families 7183, Q52, and J558 were somewhat diminished in the day 4 neonate and absent in the day 7 neonate with no differences in V_H gene expression found between adult vs day 7 neonates. Also, the extensive bias for the utilization of V_H 7183 previously reported for transformed fetal B cells (>80%) was not observed in this analysis of normal fetal B cells. Our results indicate that there are significant differences in V_H gene family expression during ontogeny and suggest a genetic developmental regulation of B cell repertoire generation.

G 121 IMMUNOGLOBULIN V_H REPERTOIRE BY IN SITU HYBRIDIZATION, Daniel L. Kastner, Tina M. McIntyre, and Alfred D. Steinberg, National Institutes of Health, Bethesda, Maryland 20892.

Murine immunoglobulin heavy chain variable region (V_H) genes have been grouped into nine distinct families by Southern hybridization. Using hybridomas and Abelson virus-transformed cell lines, other investigators have found an apparent skewing of the repertoire toward more 3' V_H families early in ontogeny and among autoantibody-secreting plasma cells. The interpretation of such studies is limited, however, by potential selection bias and sampling error in producing these cell lines. To meet these concerns, we have recently developed a system for examining the V_H utilization of individual, unselected B cells by in situ hybridization. We have subcloned probes for the 7183, Q52, S107, J558, 36-60, X24, J606, and 3609 V_H families into the PGEM3Z plasmid, and we have generated ^{35}S-labeled probes by in vitro transcription reactions. By a modification of the technique of Harper et al. (PNAS 83:772-776), labeled probes have been hybridized to cytocentrifuge preparations, and positive hybridization has been detected by autoradiography. When tested on a panel of hybridomas and myelomas of known V_H families, these probes hybridized only to the cells of the appropriate family. We will present data on V_H utilization in normal and autoimmune mice as detected by this technique.

G 122 DIFFERENTIATION STAGE-SPECIFIC TRANSCRIPTION OF HUMAN Cγ AND Cα LOCI IN IgM PRODUCING B CELL LEUKEMIAS AND CELL LINES. *William G. Kerr and Peter D. Burrows, Univ. of Alabama at Birmingham, Birmingham, AL 35294 .*

We examined the transcriptional activity of the Cγ, Cα and Cε loci in leukemic B lineage cells and cell lines stable for either μ chain or IgM expression, and that lack detectable expression of γ or α heavy chains by surface and cytoplasmic immunofluorescence. Transcription of the Cγ loci was demonstrated by nuclear run-ons in all leukemias and cell lines representative of mature sIg^+ B cells, but not in μ+ pre-B cells or in IgM-secreting plasmacytoid cells. This differentiation stage-specific transcription was initially identified with a Cγ4 probe that cross-hybridizes with all Cγ subclass genes. Analysis with a subclass-specific Cγ3 probe revealed that most Cγ transcription derives from loci other than Cγ3, the most 5' Cγ gene. In one IgM lymphoma, however, transcription of Cγ3 was significant. Transcription of the Cα loci was detected in all sIg^+ B cell lines and leukemias, but not in pre-B cell lines. No transcription was observed for Cε . Analysis with the Cγ4 probe of poly(A)+ RNA from the cell lines and leukemias revealed a profile of 4-6 RNAs ranging in size from 1.6 to 3.7 kb in cell lines transcribing Cγ. Two transcripts (3.7 kb,γm; 2.0 kb,γs) were seen in a control IgG producing myeloma cell line. Profiles of the "sterile" RNA species were not identical among the different cell lines and leukemias. The results suggest that different Cγ loci may be transcribed in different human B cell clones possibly reflecting a molecular mechanism for precommittment to switch to a particular isotype.

G 123 REGULATION OF IMMUNOGLOBULIN GAMMA 2b mRNA PROCESSING. Charles Lassman and Christine Milcarek, University of Pittsburgh, School of Medicine, Pgh., PA 15261

We have previously observed equimolar transcription across the entire immunoglobulin gamma 2b locus in myeloma and lymphoma cells which produce membrane- to secretory-specific mRNA ratios of 0.1 mb:1 sec and 1 mb: 1 sec, respectively (Milcarek & Hall, 1985, Mol. Cell. Biol. 5:2514). When sequences encoding the sec polyA site and downstream elements were removed from the gene, stably transfected myeloma cells produced mb-mRNA in abundance (Kobrin et al., 1986, Mol. Cell. Biol. 6:1687), indicating that the myeloma cell is capable of efficiently splicing CH3 to M1 exons and that sequences downstream of the sec polyA site are important for regulation. We have recently transfected new constructs into cells to address the question of tissue specific regulation of Ig mRNA processing. Myeloma cells containing a construct with the mb polyA site as the only polyadenylation region produce gamma 2b mRNA in the same abundance as myeloma cells transfected with the entire gamma 2b gene, thereby demonstrating that the mb polyA site is efficiently recognized in myeloma cells. When the normal order of sec...mb polyA sites was reversed, the now promoter proximal mb site was used greater than ten times more often than the promoter distal sec site in both the myeloma and lymphoma cells, demonstrating that normal developmental control of utilization of the two polyA sites was abolished. We conclude that splicing and selective polyadenylation are not strictly tissue specific and that the position of the polyA sites is critical.

G 124 ANALYSIS OF SOMATIC MUTATION IN Ig V REGION GENES OF HUMAN FOLLICULAR LYMPHOMAS, Shoshana Levy, Eileen Mendel, Shinichiro Kon, Zafrira Avnur and Ronald Levy. Stanford University School of Medicine, Stanford, California 94305.

Human B cell lymphomas represent certain stages of B cell differentiation which have been clonally expanded after the malignant transformation. When V_H genes of individual tumor cells from two patients with follicular lymphoma were analyzed, each set was found to be derived from one original cell and to have undergone extensive somatic mutation during clonal evolution (Cleary et al. Cell 44:97, 1986; Kon et al. Proc Natl Acad Sci 84:5053, 1987). We have now proceeded to analyze the V_L genes expressed in these same sets of tumor cells. We determined that the V_κ genes derived from 4 cells of one patient and the V_λ genes derived from 6 cells of a second patient had also extensively mutated. Many mutations within each set of V_H or V_L genes were shared among members of the set. Because of this there was an apparent genealogic relationship between the mutated V_H or the V_L genes within each tumor. However, no simple genealogy could be derived for the cells of the tumors taking into account data from both the V_H and V_L genes. Thus the mechanism of mutation in these clonal B cell populations may involve hot spots within the V gene segments.

G 125 STUDIES OF V(D)J RECOMBINATION WITH THE EXTRACHROMOSOMAL SUBSTRATE. M. Lieber, J. Hesse, M. Mizuuchi, M. Gellert, National Institute of Diabetes, Digestive and Kidney Disease, NIH, Bethesda, MD 20892.

We have developed a sensitive and rapid assay for V(D)J recombination that uses plasmid DNA transiently introduced into transformed pre-B cells. We have been able to show that recombination requires no specific sequence beyond that contained in a pair of signal sequences, each of which is composed of a heptamer, a nonamer, and either a 12 or 23-base pair spacer. We find that deletional and inversional V(D)J recombination between signals occur at comparable frequencies. Therefore, V(D)J recombination may be relatively insensitive to the topological arrangement of sites, and events at the two novel junctions produced by the reaction may be coupled.

We have examined the level of V(D)J recombination activity in a number of cell lines derived from lymphoid or non-lymphoid lineages. The recombination activity is highest at the earliest stages of committed B cell differentiation and then falls progressively, reaching undetectable levels at the mature B cell stage. The activity is also present in multi-potential progenitors of myeloid cells, and in pre-T cells but not mature T cells. No activity was found in several non-hematopoietic cell lines.

G 126 MITOGEN- AND IL-4-REGULATED EXPRESSION OF GERMLINE IMMUNOGLOBULIN γ2b TRANSCRIPTS: EVIDENCE FOR DIRECTED HEAVY CHAIN CLASS-SWITCHING, Stuart Lutzker, Paul Rothman, Roberta Pollock, *Robert Coffman and Frederick W. Alt, Columbia University, NY, NY 10032 and *DNAX, Palo Alto, CA 94304

It has been proposed that heavy chain class-switching is regulated by controlling "accessibility" of the different heavy chain switch regions to a common switch recombinase; this "accessibility" may correlate with the presence of transcripts containing heavy chain constant (C_H) regions but not variable regions. To examine the relationship between these putative germline C_H region transcripts and heavy chain class-switching, we isolated a cDNA copy of a germline γ2b transcript produced by A-MuLV transformants that have a predisposition to switch to γ2b. We mapped the transcription initiation sites of this transcript to a region 5' S_γ2b; therefore transcription proceeds through the S_γ2b region but not through other switch regions. We show that treatment with bacterial lipopolysacharide (LPS) induces germline γ2b transcripts in both A-MuLV-transformed pre-B cells and normal murine spleen cells; in both systems, this induction is followed by an increase in the number of cells that produce γ2b protein. The T cell factor interleukin-4 (IL-4) is known to suppress the in vitro induction by LPS of the appearance of increased numbers of γ2b protein-producing murine spleen cells. Correspondingly, we find that IL-4 also inhibits the LPS induction of germline γ2b transcripts. This data indicates that mitogens and T cell factors may directly regulate class-switching in the context of an accessibility mechanism.

G 127 ANALYSIS OF DNA SEQUENCES INVOLVED IN THE ASSEMBLY OF IG GENES, Ewa Morzycka-Wroblewska, John Niederhuber, Karen Wolff and Stephen Desiderio, Howard Hughes Medical Institute Laboratory of Genetics and Department of Molecular Biology and Genetics, The Johns Hopkins School of Medicine, Baltimore, MD 21205.

To define the nucleotide sequence requirements for immunoglobulin gene rearrangement, model substrates for the joining of immunoglobulin (Ig) V_H and DJ_H gene segments were constructed in the retroviral vector pDOL and introduced into progenitor B-cell lines by infection. Recombination was monitored by a DNA hybridization assay. V_H-to-DJ_H joining was observed in cell lines representative of three distinct stages in early B-cell differentiation. We have used degenerate, complementary oligonucleotides to mutate the nonamer recombinational signal sequence of the V_H segment present in the substrate. Changes at highly conserved positions in the nonamer sequence failed to abolish V_H-to-DJ_H joining, suggesting that an intact nonamer sequence is not absolutely required for rearrangement. When V_H, DJ_H and J_H segments were included within the same substrate, the V_H segment recombined with the DJ_H segment but not to the J_H segment; thus, rearrangement of wild type sequences was found to obey the 12/23 bp spacer rule. We are currently determining the effects of spacer and nonamer deletions on the targeting of these Ig gene segments for one another.

G 128 RECOMBINATION POTENTIAL OF A-MULV-TRANSFORMANTS DERIVED FROM SCID MICE. B. Malynn, P. Ferrier, G. Fullop, T. K. Blackwell, A. Furley, G. Rathbun, M. Morrow, B. Heinke, R. Phillips, F. Alt. Howard Hughes Med. Inst. & Columbia Univ., N.Y., N.Y. Severe combined immunodeficiency (SCID), an autosomal recessive trait which causes a deficiency of mature lymphocytes, was shown by Schuler and Bosma to result in abnormal rearrangements of genes encoding T and B cell antigen receptors, suggesting that the SCID mutation adversely affects the recombinase system. To further characterize the defect, we are analyzing A-MuLV transformants from SCID mice with respect to RNA expression of relevant genes, status of endogenous Ig and TCR gene rearrangements, and potential of these lines to rearrange introduced recombination constructs. We find that SCID A-MuLV-transformants rearrange endogenous Ig H chain genes at a low frequency, have grossly aberrant rearrangements, or both. Although all rearrangements sequenced thus far completely delete the coding regions of either the D-segment, J_H-segment, or both, one third of the rearrangements are precise at either the 5' or 3' end, and several rearrangements contained N-regions, suggesting that certain recombinase functions--ie. specific recognition of heptamer-nonamer signal sequences, DNA cutting, and TdT activity--may be intact. These lines also express germline V_HJ558 and sterile C_u transcripts, suggesting appropriate regions are "accessible" to the recombinase system. An inversion recombination construct which was introduced into SCID lines resulted in neomycin-resistent lines at a frequency several orders of magnitude lower than that observed in A-MuLV-transformants from normal mice, and occurred by either aberrant rearrangement or amplification of the construct. Together these results suggest a defect in either ligation of cleaved ends or in exonuclease activity.

G 129 MODULAR CONSTRUCTION AND ASSEMBLY OF RAT IgE HEAVY CHAIN GENES AND THEIR EXPRESSION IN B-CELLS, Randy McMillan, Shin-Shay Tian and Charles Faust, Texas Tech University Health Sciences Center, Lubbock, TX 79430.
IgE is a most interesting immunoglobulin (Ig), both because of its central role in allergy, as well as its involvement in antiparasitic immunity. As an initial step in understanding some of the molecular aspects of its involvement in these processes, we have isolated a functionally rearranged gene for the epsilon heavy chain of IgE from the IgE-secreting immunocytoma, IR162, of the LOU rat strain (Molec. Cell. Biol. 7:2614-2619, 1987). This gene was characterized by restriction enzyme mapping, Southern blotting and partial DNA sequence analysis. Its individual variable and constant region exons were isolated and subcloned as discrete modules into the polylinker region of a plasmid vector to provide for simple modular reassembly in any desired permutation of domains, i.e., to facilitate multiple chimeric Ig gene fabrications. The structures of the individual genetically engineered modules were confirmed by further restriction enzyme mapping or limited DNA sequence analysis. These modules were then reassambled into a normally organized epsilon heavy chain equivalent to the authentic one found in the IR162 immunocytoma, and the recombinant DNA construction verified. This modular reconstruction was introduced into a myeloma cell with a eukaryotic selection marker to verify expression and authenticity of product produced. Details of the module design, assembly, construction and expression of the synthetic IgE heavy chain will be described.

G 130 IN VITRO RECOMBINATION OF HUMAN IMMUNOGLOBULIN SWITCH SEQUENCES.

F.C. Mills, J.S. Brooker, P.R. Pittman, and R.D. Camerini-Otero.
Genetics and Biochemistry Branch, NIDDK, NIH, Bethesda, Maryland.

We have observed that a human recombinase characterized in our laboratory (Hsieh et al., Cell 44,885-894) carries out in vitro strand exchange reactions between the human Sμ heavy chain switch site and the Sε, Sγ3 and Sγ4 switch sites. This recombination activity apparently requires only small segments of similar sequence, as there are at most 60% matches in 25 basepairs between our single-strand ε substrate and the reactive end of the double-strand μ substrate, yet this reaction goes as well as the reaction of Sμ with itself. Moreover, the Sγ sequences seem to contain very weak matches with Sμ, yet the Sμ-Sγ3 and Sμ-Sγ4 reactions are reduced only 2 to 3 fold compared to the Sμ-Sμ and Sμ-Sε reactions. The fact that our recombinase will mediate a reaction between Sμ and the most similar switch site (Sε), as well as the least similar switch site (Sγ4) strongly argues that this activity plays a role in the step(s) of class switching involving recognition of switch site sequence similarity.

G 131 ACTIVATION OF THE IGλ LOCUS IN C-MYC TRANSFORMED ABELSON CELLS, B. Müller and M. Reth, University of Cologne, D-5000 Köln 41, FRG.
During B cell development V gene rearrangements occur at the three Ig loci in an ordered fashion. VH gene rearrangements occur prior to Vk rearrangements. Only if no functional k chain is produced, the Vλ genes may become activated. We transfected the Abelson line 300-19 with a murine c-myc gene and we obtained transfectants which rearrange V segments of the λ locus. All transfectants with VJλ rearrangements had previously destroyed the Ck locus of both alleles via RS recombination. Although RS recombinations have been shown in λ bearing cells before we could show that they are a requirement for the onset of λ gene recombination. Only 4/18 rearranged VJλ complexes were expressed immediately after rearrangement.

G 132 MOLECULAR EVOLUTION OF THE IgA GENE IN THE GENUS MUS, Barbara A. Osborne. University of Massachusetts, Amherst, MA 01003.
IgA is the principle immunoglobulin of the secretory immune system. In humans, there are two isotypes of IgA, IgA1 and IgA2, while in BALB/c, it occurs as a single class. In order to more closely examine the precise mechanisms involved in the evolution of this gene, we have undertaken an examination of the Cα gene from a number of different representatives of the genus Mus. The Cα gene from Mus pahari was isolated and its DNA sequence determined. Mus pahari, a member of the subgenus Coelomys, is believed to be one of the most ancient mouse species existing today. A detailed sequence analysis of the Mus pahari Cα gene revealed a number of interesting features. The 1st and 2nd domain appear to accumulate substitutions at a more rapid rate than the 3rd domain. while the hinge is quite different from either the BALB/c or human Cα hinges. Our data have also revealed that the predominant number of substitutions accumulated between BALB/c and Mus pahari Cα sequences are those leading to amino acid changes. Replacement site substitutions are very rare in most proteins however, the immunoglobulins appear to accumulate this type of substitution at a high rate. A model that suggests the mechanism by which replacement site changes are favored over silent substitutions will be presented.

G 133 MECHANISMS OF SWITCH RECOMBINATION. John Petrini, Cynthia Schultz, Mary Hummel, Briton Shell and Wesley Dunnick. University of Michigan Medical School, Ann Arbor, MI 48109.

In order to define the possible mechanisms of the switch recombination process, we have undertaken the characterization of unusual switch region rearrangements from two IgG3-producing cell lines, 470.25 and 198.5C8. These lines are unique in that they contain γ1 switch region rearrangements, yet they express the γ3 locus which is normally deleted in a conventional Sγ1 rearrangement. Southern blot analysis has shown that two of the Sγ1 rearrangements in these cell lines reflect a net insertion of sequences. In 470.25, the insertion results from a Sγ1/Sγ1 interchromosomal recombination which involves the expressed chromosome, yet leaves the expressed γ3 locus intact. A molecular clone from 470.25 reveals that successive rearrangement of Sμ/Sγ3/Sγ1 has also occurred in this cell, presumably on a non-expressed chromosome. Studies presently underway will involve analysis of molecular clones from 198.5C8 to determine if similar mechanisms are responsible for the unusual pattern of switch region rearrangements in this cell.

G 134 MOLECULAR BASIS FOR SURFACE μ CHAIN EXPRESSION WITHOUT LIGHT CHAIN IN HUMAN B LYMPHOCYTES, B. Pollok[1], R. Anker[1], D. Levitt[1], L. Hendershot[2], Guthrie Research Institute[1], Sayre, PA and UAB[2], Birmingham, Alabama.

Four distinct human B-lymphoid cell lines possess the ability to circumvent the mechanism regulating intracellular transport of immunoglobulin protein. These cells do not produce light chains yet express μ heavy chains on the cell surface at comparable levels to B cell lines that produce native forms of both proteins. The μ chain mRNA produced in all four cell lines was found to contain an identical deletion of most of the Vh region (75% of the 3' portion), with no apparent alteration in constant region structure. The truncated μ (μ*) chain mRNA in these cells was created through the use of a cryptic splice donor site found within the human Vh gene(s) utilized by these B cell lines. The truncated μ* chains exhibited a decreased ability to associate with the intracellular transport regulatory protein, heavy chain binding protein (BiP). This result indicates that Vh region structure, in addition to Ch1 region structure, influences the formation of the BiP recognition site on heavy chains. Further, it suggests that the mechanism allowing for cell surface expression of the μ* chains in the absence of light chain pairing is the inability of BiP to bind to the μ* chains and hence prevent their intracellular transport. The high frequency with which the μ-only sIg^+ phenotype is present in our collection of human B cell lines and the isolation of one of the cell lines from a healthy individual also suggests that B cells of this type may represent a significant subpopulation among the normal human B cell repertoire.

G 135 THE REMOVAL OF V_H GENE DOWNSTREAM SEQUENCES IS REQUIRED FOR ITS EXPRESSION IN MATURE B-CELLS - HYPOTHESIS. Gideon Rechavi, Institute of Hematology, The Chaim Sheba Medical Center, Tel-Hashomer and Sackler School of Medicine, Tel-Aviv, Israel.

In immunoglobulin (Ig)-producing cells only the rearranged V_H gene is expressed, while all the other germline V_H genes are silent. On the other hand, it was shown that unrearranged V_H gene segments are expressed at a high level in the early stages of B-lymphocyte differentiation. This expression is independent of the Ig heavy chain enhancer element. It seems, therefore, that unrearranged gene expression is repressed in mature B-cells by a mechanism that will not operate on the rearranged functional heavy chain gene. We have shown that a highly conserved DNA segment is present unpstream to several mouse and human V_H genes. DNA sequencing downstream to germline genes revealed a DNA element related to that described at the 5' of the V_H genes. A model is proposed in which the 5' and 3' conserved segments are functional in the regulation of V_H gene expression. The model suggests the existence of a protein in mature B-cells which recognizes the 5' and 3' conserved DNA elements and prevents germline V_H gene expression by binding these segments. V_H-D-J_H joining results in the deletion of the 3' flanking sequences of the rearranged V_H gene but not of other upstream V_H genes. This would result in the release of promoter suppression in the case of the rearranged gene, while all other V_H genes would remain transcriptionally silent.

G 136 CONTROL OF IMMUNOGLOBULIN GENE REARRANGEMENT BY MEMBRANE-BOUND IMMUNOGLOBULIN CHAINS, M. Reth, Eva Petrac, Olaf Sauermann and Petra Wiese, University of Cologne, D-5000 Köln 41, FRG.
We have studied the control of Ig gene rearrangement in Abelson pre-B cells which were transfected with expression vectors specific for either the secreted (μs) or membrane-bound (μm) form of the μ chain. These experiments demonstrated that in pre-B cells the μm chain but not the μs chain is the regulatory active molecule. Pre-B cells expressing the μm chain activated Vκ gene rearrangements at the Igκ locus and stopped further VH gene assembly at the IgH locus. Thus in pre-B cells rearrangements at both loci seem to be controlled by the expressed μm chain which presumably generates a transmembrane signal. The molecular requirements for this signal are presently studied in a new series of transformation experiments.

G 137 SELECTIVE ASSOCIATION OF IMMUNOGLOBULIN H AND L CHAINS, Oscar V.Rokhlin, Alexandr R.Ibraghimov, Leonid Z.Jackubov and Vladimir V.Cherepakhin, Cardiology Research Center, 121552 Moscow, U.S.S.R
Random pairing of Ig H and L chains is usually considered to be an important component of Ig diversity. Our results obtained in chain reassociation experiments in vitro imply that only a fraction of H (or L) chains is capable of forming initial olygomeric structure with a given monoclonal chain. For L chains this fraction is about 30%. We have also examined Ig polypeptides association in a set of 21 selected neonatal and adult rat hybridomas producing two rat kappa chain allotypes in one cell. Similarly, only in about 30% cases random pairing of H and L chains was observed. In other cases the "second" kappa allotype was either secreted in free form or it associated with H chain in such a way that a given IgM or IgG molecule contained L chains of only one allelic variant. Thus antigen-independent selection of B cells with associable H and L chains rigorously influences Ig-producing cells repertoire.

G 138 **THE CHROMATIN STRUCTURE OF TRANSGENIC Igκ GENES DOES NOT MIMIC THE ENDOGENOUS GENES STRUCTURES: IMPLICATIONS FOR GENE REGULATION.**
Stephen M. Rose, University of New Mexico,Albuquerque,N.M. 87131
My research is focused at determining the role of chromatin alterations in the regulation of immunoglobulin gene transcription. Recently, with cell lines provided by Dr. Ursula Storb I have been able to define specific chromatin changes associated with this process. Cell lines from transgenic mice have allowed my laboratory to define transcription rate dependent chromatin alterations. The Igκ transgene in the 4E10.2 hybridoma and in the 7.3 pre-B and 7.3H4 hybridoma does not exhibit the same altered chromatin structure as seen along endogenous Igκ genes in plasmacytomas. The chromatin along the transgenes **does** exhibit an increased DNaseI sensitivity and the κ enhancer has the expected DNaseI hypersensitive site centered over it. However, the transgenic κ gene **does not** exhibit the same disrupted nucleosome arrangement or subnuclear compartmentalization as endogenous κ genes show. *In vitro* nuclear transcription experiments have demonstrated that the transgene is transcribed at approximately 20-40% of the rate that the endogenous κ gene. This result along with other observations lead me to propose that the altered chromatin structure observed along the myeloma κ genes is dependant on the transcription rate of the gene and that while transgenic mouse experiments are important for the understanding of gene regulation, the results obtained in these systems must be viewed with caution when proposing molecular mechanisms of gene activation and regulation.

G 139 RABBIT LATENT VHa1 ALLOTYPE SEQUENCES: COMPARISON OF AMINO ACID AND cDNA SEQUENCES, Wayne T. McCormack and Kenneth H. Roux, Dept. Biol. Sci., Florida State University, Tallahassee, FL, 32306

The genetic basis for latent VH allotype expression was investigated. VH region cDNA libraries were produced in lambda gtll from spleen mRNA of a homozygous $\underline{a^2a^2}$ rabbit expressing an induced latent a1 allotype. The sequences derived from this library were compared to those from a homozygous $\underline{a^1a^1}$ rabbit. VHa1-homologies were identified by hybridization with oligonucleotide probes for VHa1 allotype-specific segments of FR1 and FR3. The sequence of the 15 N-terminal amino acids of latent VHa1 heavy chain was also determined and found to be identical to the published nominal VHa1 amino acid sequence. cDNA sequence comparisons reveal that some latent VHa1 genes encode a FR1 which is completely homologous to that of the nominal VHa1 allotype. Others have blocks of sequences identical to nominal VHa1 in FR1 or FR3 but are flanked by VHa2 or VHa-negative sequences. These results suggest that latent allotypes may be encoded by nominal-like VH genes and by allotype-composite VH genes. These genes may be germline encoded or be the product of somatically-generated recombination or gene conversion. The latent sequences do not appear to be the result of extensive modification by somatic point mutation.

G 140 STABLE EXPRESSION OF IMMUNOGLOBULIN GENE V(D)J RECOMBINASE ACTIVITY IN A FIBROBLAST USING GENE TRANSFER, David G. Schatz and David Baltimore, Whitehead Institute for Biomedical Research and Massachusettes Institute of Technology, Cambridge, MA 02142.

The genes encoding the antigen specific immune receptors are assembled in developing lymphocytes by a process of somatic recombination that joins widely separated gene segments to form a complete variable region. Retroviral recombination substrates that are recombined in murine pre-B cells are not recombined in fibroblasts. Using DNA transfer, we have successfully transferred V(D)J recombinase activity into an NIH 3T3 cell stably infected with a recombination substrate. Recombinase activity could be transferred in a second round of transfection. A secondary transfectant has been isolated that stably expresses recombinase activity at a level comparable to that of a recombinationally active pre-B cell. It is likely that expression of a single, lymphoid-specific gene in a fibroblast is sufficient to confer V(D)J recombinase activity on that cell.

G 141 A NUCLEAR FACTOR PRESENT IN IG NON-EXPRESSING CELLS BINDS TO MULTIPLE SITES FLANKING THE IG HEAVY CHAIN ENHANCER, Richard H. Scheuermann, Basel Institute for Immunology, CH-4005 Basel, Switzerland.

An important aspect of B cell development is the regulation of immunoglobulin gene expression. One of the elements controlling expression is the tissue specific enhancer located within the J-C immunoglobulin heavy chain intron. Competition experiments indicate that enhancer function is mediated by trans-acting factors which interact with specific segments within the enhancer. Using mobility shift and protection assays a number of nuclear factors have been identified which bind to the enhancer in vivo and in vitro. However, a model for tissue-specific enhancer function has been elusive since most of these factors are ubiquitously present. Recently, Imler et al. (Mol. Cell. Biol., 7:2558, 1987) have defined sequences flanking the minimal "core" enhancer which appear to inhibit enhancer function in non-expressing cells. Using mobility shift assays, I have identified a nuclear factor which binds to these negative regulatory regions. Based on DNase protection, sequence comparisons and competition experiments, this nuclear factor, termed NF-μNR for "Nuclear Factor-μ Negative Regulator", binds to multiple, similar sites both 5' and 3' of the minimal "core" enhancer. NF-μNR is present in most cells which do not express immunoglobulin heavy chain, and absent from cells which express heavy chain. These results suggest that tissue-specific heavy chain enhancer function may, in part, be regulated by a trans-acting negative factor.

G 142 USAGE OF THE Ig GERM-LINE REPERTOIRE : ECONOMY OR WASTAGE ?
Claudine Schiff and Michel Fougereau, Centre d'Immunologie de Marseille-Luminy, Case 906, 13288 Marseille Cédex 9, France.

Antibody diversity stems from a variety of mechanisms, from which random rearrangement of discrete gene segments (VH-D-JH, and VL-JL) and random pairing of the resulting chains account for over 10^7 different Ig molecules. This relies on the assumption that each event has an equal probability to occur. In studying the GAT idiotypic cascade, we have isolated 19 VH genes and 3 Vκ genes that pertain to the J558 family and to the VκI subgroup, respectively. One VH and 2 Vκ are used in the Ab1 and Ab3 antibodies of the GAT cascade, either in their germ-line configuration or with a few somatic replacements. By computing published antibody sequences, we observed that both the VH "GAT" and the Vκ "GAT" were frequently used in antibodies of discrete specificities. We propose that only a few number of genes are directly functionaly active, either in their germ-line configuration, or after slight modifications by somatic events, (such as conversion using the large pool provided by the other genes - including the "pseudo-genes" - of the corresponding family). This way of generating diversity might represent a generalization of that used in the Vλ system of the chicken, and might be considered an adaptation of the "master and slave" model. This hypothesis would thus reconcile the apparent "wastage versus economy" dilemma.

G 143 TRANSCRIPTION OF UNREARRANGED ANTIGEN RECEPTOR GENES IN scid MICE, Walter Schuler, Amelie Schuler, Gayle Bosma, Gregory Lennon and Melvin J. Bosma, Institute for Cancer Research, Philadelphia, PA 19111.

Cells with rearranged antigen-receptor genes cannot be demonstrated in mice homozygous for the scid mutation (scid mice) (1). To evaluate the developmental stage at which the effects of the scid mutation become manifest, scid lymphoid organs were analyzed for expression of T- and B-lymphocyte specific genes. Igh-Cμ, Igh-VH558 and Igl-Cκ in germ-line configuration were found to be transcribed in scid fetal liver and adult bone marrow derived cells. Likewise, unrearranged TCRβ and TCRγ genes were transcribed in cells obtained from thymus glands of young adult scid mice, whereas no TCRα transcripts could be demonstrated. The T cell specific T3-δ gene was also expressed in scid thymocytes. These results suggest that the scid mutation impairs lymphopoiesis at or about the stage (after commitment to the B and T cell lineages) at which antigen receptor genes are normally rearranged; i.e., after these genes presumably become accessible to factors responsible for the recombination. This gives further support to our hypothesis that the scid mutation adversely affects the mechanism of antigen receptor gene recombination.

1. Schuler, W., Weiler, I. J., Schuler, A., Phillips, R. A., Rosenberg, N., Mak, T. W., Kearney, J. F., Perry, R. P. and Bosma, M. J., 1986 CELL 46: 963-972.

G 144 ISOTYPE SPECIFICITY OF MURINE IMMUNOGLOBULIN HEAVY CHAIN SWITCH RECOMBINASE MACHINERY AND CHROMATIN STRUCTURE OF SWITCH REGION DNA,
C.Schultz, M.Hummel and W.Dunnick, Univ. of Michigan, Ann Arbor, MI 48109.

The enzymatic machinery responsible for the heavy chain switch in mice may be specific, i.e. capable of recognizing and distinguishing the different switch regions and directing the DNA deletion event, resulting in a correlation of the isotype of the expressed and unexpressed alleles. Alternatively, the switch may be a non-specific event, resulting in two alleles which have switched to different isotypes. We can distinguish between these two possibilities by examining the isotype content of hybridomas made from spleens of immunized mice. Data accumulated thus far in our laboratory strongly suggest isotype specificity in the case of the gamma-1 and gamma-3 genes. We are currently immunizing mice to enhance the gamma-2 response and thus acquire data on this isotype as well. Specificity suggests that there may be switch region specific DNA-binding proteins. DNase hypersensitive sites have been shown to represent sites of protein binding. Using a DNase hypersensitivity assay, we have found several strong hypersensitive sites in and around the 5′ region of the gamma-1 switch region of an Ig-producing hybridoma. We are currently looking for the presence or absence of these sites in other lymphoid and non-lymphoid cells.

G 145 ANTIGEN SPECIFICITY OF Ig PRODUCED BY $CD5^+$ HUMAN B CELL CLONES
A.E.M. Schutte, J.H. van Es, H. Aanstoot, F.H.J. Gmelig Meyling and T. Logtenberg, Dept. Clinical Immunology, University Hospital, Utrecht, The Netherlands
$CD5^+$ B cells represent a minor B cell subpopulation (1-3%) in adults. In cord blood and fetal tissue, however, their frequency is much higher (30-50%). Furthermore, their frequency is elevated (up to 20%) in a proportion of patients with rheumatoid arthritis; in patients with B cell chronic lymphocytic leukemia almost all B cells express this marker. It has been suggested that $CD5^+$ B cells are the precursors of cells producing autoantibodies as well as multispecific antibodies. In an attempt to clarify the repertoire and the function of $CD5^+$ B cells, we transformed lymphocytes with Epstein Barr Virus under limiting dilution conditions. Lymphocytes were isolated from adult peripheral blood, cord blood and fetal tissues. We obtained a thousand B cell clones, from which ten clones expressed the CD5 marker. These clones were analyzed for antigen reactivity against a panel of 15 antigens (xeno-, auto-ag, haptens). All these clones produced IgM molecules, with single specificity against haptens, or human IgG; Ig from 6 clones did not react with one of the tested antigens. Multispecific antibodies were not found. Implications of these findings will be discussed.

G 146 PARTICULATE MYCOBACTERIUM LEPRAE ANTIGEN PRESENTATION BY HUMAN B CELLS. M. Selvakumaran, T. Jayaraman, H.K. Prasad, I. Nath, All-India Institute of Medical Sciences, New Delhi, India.
Epstein Barr Virus transformed B cells (EBV-B) are useful sources of antigen presenting cells in T cell clone technology. In order to understand B cell ability to present complex particulate antigen such as intact M.leprae to T cells we have carried out lymphoproliferation testes by culturing irradiated EBV-B cells with resting as well as activated T cells in presence of varying concentrations of antigen. B cells were found to be inefficient in presenting antigens to nylon wool passed, anti-Ia antibodies and low toxic rabbit complement treated T cells.However B cells were involved in antigen presentation to lesser extend with purified T cells obtained prior to anti-Ia antibody and complement treatment. Despite the lack of accessory cell function to autologous T cells ,B cell presented antigen very well in a dose dependent manner to ConA, PPD,M.leprae activated short term and long term maintained T cell lines.

G 147 IMMUNOGLOBULIN HEAVY CHAIN SWITCH DNA: SPECIFIC PROTEIN BINDING, AND UNIQUE PROPERTIES OF THE DNA, Dipankar Sen and Walter Gilbert, Dept. of Cellular and Developmental Biology, Harvard University, Cambridge, MA 02138.

The switch-regions of the immunoglobulin heavy chain genes, GC-rich stretches of DNA found 5' to constant region genes for the secondary isotypes, participate in switch recombination of those genes. The switch sequences are conserved between different CH genes in the same species, as well as between different species. In mouse, one strand is highly guanine-rich while the other is cytosine-rich.

We find that short stretches (40-50 bases) of single stranded DNA from the different switch-regions specifically bind proteins from crude nuclear extracts from B cells stimulated to differentiation with LPS. The two strands bind different proteins, and there is some specificity for different switch regions.

In addition, the unusual sequence and nucleotide content of the switch-region single strands permits the guanosine-rich 'coding strand' to generate self-multimers at physiological salt, that are nevertheless capable of substantial Watson-Crick base pairing with their complementary strands. Self-association appears to take place specifically at a highly-conserved guanosine-rich stretch and raises the possibility of non-Watson-Crick auto-recognition by switch-regions.

G 148 MIDGESTATION HEMATOPOIETIC DEVELOPMENT: COMPARISON OF NORMAL PRECURSORS AND ABELSON MURINE LEUKEMIA VIRUS (Ab-MuLV)-TRANSFORMED CELLS M. Siegel, C. Brown and E. Siden* Univ. of Florida, College of Medicine and *Mt. Sinai School of Medicine, division of clinical immunology, New York , NY 10029
We have analyzed the earliest stages of murine immunologic development by quantitation and characterization of Ab-MuLV transformants and their normal counterparts in the embryo. In contrast to the primative lymphoid cell lines isolated from the fetal liver after 12 days of gestation, cell lines derived from 9-11 days of gestation express multiple characteristics of mucosal mast cells as well as certain B cell traits including B 220 surface protein, transcription of unrearranged heavy chain variable region genes, and inducible expression of T cell receptor mRNA. Lymphokine-dependent mast cell precursors which are most abundant at 12 days of gestation, appear two days later than Ab-MuLV-sensitive cells and authentic B cell precursors detected by a paternal allotype-specific ELISA.

G 149 **ASSESSMENT OF MECHANISMS REGULATING δ CHAIN GENE EXPRESSION.** Roland Tisch, Naomi Kondo and Nobumichi Hozumi, Mt. Sinai Hospital Research Institute, Toronto, Ontario M5G 1X5
Recent studies have shown that the μ and δ heavy chain genes form a complex transcriptional unit. Transcription of this unit initiates within the variable heavy gene segment (V_H), tranverses the μ chain gene and enters the δ chain gene locus. The μ-δ intron region appears to contain sequences that are critical for the regulation of the highly cell stage specific expression of δ mRNA. Depending on the particular B cell stage of development, either post-transcriptional cleavage/polyadenylation site selection or regulation of transcriptional termination within the μ-δ intron region are believed to be the mechanisms governing δ specific mRNA expression. In an attempt to elucidate specific sequences required for the different modes of regulation of δ mRNA expression, we have constructed vectors in which portions of the μ-δ intron region have been inserted betweeen V_H and δ chain encoding genes. These constructs have been transferred into B cell lines representing different stages of development and regulation of δ chain gene has been evaluated. We have isolated a region of approximately 200 base pairs within the μ-δ intron that appears to be essential for the regulation of cleavage/polyadenylation and transcriptional termination site selection. In addition we have evidence that suggests splicing and conformation of the primary transcript have a role in the observed regulation. These data will be presented.

G 150 UTILIZATION OF IMMUNOGLOBULIN V_h GENE FAMILIES IN B CELL COLONIES FROM AGED MICE, Dorith Zharhary* Gillian Wu+ and Christopher Paige#, *Weizmann Institute of Science, Rehovot, Israel; +University of Toronto, Toronto, Canada; #Ontario Cancer Institute, Toronto, Canada.
The frequency of splenic B cells responsive to most antigens is reduced in aged mice while that responsive to PC is increased several fold as compared to young mice. Moreover, the frequency of newly generated PC-specific B cells in the bone marrow (BW) of aged BALB/c mice is increased as well, with a preferential increase in precursors which do not express the predominant T15 idiotype. These observations suggest that changes in immunoglobulin (Ig) V region gene expression may occure with advanced age. In order to test this possibility, we determined V_H gene utilization in colonies of B cells proliferating in response to LPS in the CFU-B assay, using the RNA colony blot assay. For this purpose, BM splenic B cells from old (26 mo) and young (3 mo) BALB/c and C57BL/6 mice were stimulated to form colonies in agar. The agar discs were blotted on GeneScreenPlus papers and probed subsequently with probes representing 6 of the 9 known V_H gene families. We found that B cell colonies derived from spleen and BM of aged mice utilize V_H genes of all families. No major differences in the frequency of V_H family expression was detected between aged and young mice. We note that the clonal B cell population samples 25-30% of all surface Ig^+ cells in C57BL/6 mice and 5-10% in BALB/c mice. These observations indicate that in B cells that can form colonies in agar the potential to utilize different V_H gene families does not change with age.

B Cell Subsets, Development, Growth and Differentiation

G 200 **B CELL DEVELOPMENT: ROLE OF THE mIgD RECEPTOR,** **José E. Alés-Martínez, Phil W. Tucker* and David W. Scott, University of Rochester Cancer Center, Rochester, NY 14642 and *UTHSC, Dallas.**

Like neonatal B cells, some members of the CH family of murine B cell lymphomas are functionally immature. Due to the exquisite sensitivity of the CH33 derivative to growth inhibition by anti-IgM crosslinking, and its low expression of mIgD, we consider it a suitable model to investigate the possibility that mIgD and mIgM can mediate different signals. We also wanted to know what influence the acquisition of a more mature phenotype could have on the functional profile of these cells. We have demonstrated that: 1) IgD can be successfully transfected into and expressed by IgD$^-$ CH33 cells, 2) Crosslinking of IgD does not inhibit cell growth, 3) Combined crosslinking of both Ig receptors still results in growth inhibition, 4) Pretreatment with anti-IgD antibodies abolishes anti-IgM mediated Ca++ mobilization without preventing anti-IgM effect on growth inhibition 5) mIgD receptors are functionally capable structures as shown by their ability to undergo capping and to mediate Ca++ mobilization to the same extent as mIgM. From the above results it follows that mIgD can mediate signals that are qualitatively different from mIgM and that mIgD expression is not a sufficient condition to change the functional phenotype of neonatal-like B cells (Supported by CTR #1840 and a Wilmot Fellowship).

G 201 THE INFLUENCE OF CD23 EXPRESSION AND MODULATION ON B-CELL FUNCTION, R.J.Armitage, L.K.Goff and P.C.L.Beverley, I.C.R.F. Human Tumour Immunology Group, London W1P 8PT, England.

The B-cell surface antigen detected by monoclonal antibodies (MAb) within CD23 is present on a subpopulation of freshly isolated peripheral blood and tonsil B cells with the level of expression increasing in the presence of IL4, TPA and a IgM. We have separated tonsil B cells into CD23$^+$ and CD23$^-$ populations using the MAb MHM6 and have found that while the intensity of CD23 staining increases on activated MHM6 $^+$ cells no reactivity is seen among similarly activated MHM6$^-$ lymphocytes. Furthermore, MHM6-depleted tonsil B cells show little or no proliferative response to TPA, a IgM and IL4 or a IgM and G28-5(CDw40) and a markedly reduced response to Staphylococcus aureus Cowan I (SAC). However, if supernatant from unseparated tonsil B cells cultured with these factors is added to the MHM6$^-$ lymphocytes the proliferative response is largely restored.Although the level of CD23 expression increases on activated tonsil and chronic lymphocytic leukaemia B cells, no increase is seen among the mature B cells found in the prolymphocytic leukaemia (PLL). Unlike tonsil B cells, MHM6$^-$ PLL B lymphocytes alone are able to respond as strongly to various mitogenic stimuli as MHM6$^+$ or unseparated cells.One striking feature seen with tonsil and PLL B cells is that the costimulatory effect of MHM6 and TPA is greatly enhanced by cross linking the CD23 antigen. Such cross linking also augments proliferation induced by SAC, a IgM and IL4 and a IgM and G28-5 whereas the addition of soluble MHM6 has no effect on the response to these factors. These results show that among normal and some leukaemic B lymphocytes CD23$^+$ cells play a crucial role in the proliferative responses of the majority CD23$^-$ population and modulation of the CD23 antigen greatly affects the subsequent response to various B-cell mitogens.

G 202 CLONAL IMMUNOGLOBULIN GENE REARRANGEMENT IN PRIMARY MEDIASTINAL CLEAR CELL LYMPHOMAS, Laura Borgonovo-Brandter, Birger Christensson, Lennart Hammarström, Christina Lindemalm and C.I. Edvard Smith, Center for BioTechnology and Dept. of Clinical Immunology, Karolinska Institute, Huddinge University Hospital, S-141 86 Huddinge, Sweden.

We here report on two cases of primary mediastinal tumors of clear cell type and their characterization by immunological and molecular biological techniques. In both cases a B-cell immunophenotype was suggested by the positive staining for the B1, B4, Leu 14 and pan-B monoclonal antibodies by immunohistochemistry. However, no definitive evidence for the expression of Ig light or heavy chains was found by immunological methods. Southern blot analysis of Ig heavy chain gene rearrangement revealed clonal B-cell populations in both cases. There was no indication of a somatic joining of T cell receptor genes using probes for T-beta and/or T-gamma genes. Our results give evidence for a clonal B-cell origin of clear cell lymphomas of the mediastinum in spite of the lack of Ig expression in most of these tumors and is in accordance with their expression of B-cell antigens.

G 203 THE ROLE OF GROWTH RECEPTORS IN THE PATHOGENESIS OF B LYMPHOCYTE CHRONIC LYMPHOCYTIC LEUKAEMIA. David Barnett, Gillian A. Wilson, Arthur C.K. Lawrence, Arundathy N. Bartlett, George A. Buckley, Department of Haematology, Northern General Hospital, Sheffield U.K.

Chronic lymphocytic leukaemias which are of B lymphocyte in origin (B-CLL) are the commonest form of leukaemia in the Western world. This disorder can show a benign course over a number of years. However, some cases transform to a prolymphoctoid stage (B-CLL pro). This involves the appearance of large nucleolated cells and may represent an intermediate stage between B-CLL and typical prolymphocytic leukaemia (1). We have studied 49 cases of B-CLL with relation to their expression of the receptors for interleukin-2 (IL-2R) and transferrin (TfR). The IL-2R and TfR are known to be specific growth receptors (2,3). The appearance of these receptors correlates with certain stages of a cells RNA/DNA cell cycle (4). We have shown that the expression of the IL-2R and TfR correlates with morphological, and an increase in the RNA/DNA cell cycle changes found during the transformation to B-CLL pro. Furthermore we have stimulated cases of B-CLL to produce the receptors in the order we predicted (appearance of IL-2R alone, followed by IL-2R and TfR and finally TfR alone). It is proposed that this mechanism may be involved in the evolutionary changes which occurs within B-CLL and that assessment of the receptor status may have diagnostic and prognostic importance. (1) Scott C.S., Limbert H.J., Roberts, B.E., Stark A.N., Leukaemia Research 1987 (in press). (2) Barnett D., Wilson G.A., Lawrence A.C.K., Buckley G.A., Disease Markers (submitted). (3) Barnett D., Wilson G.A., Lawrence A.C.K., Buckley G.A., Clin. Lab Haem. (in press). (4) Neckers L.M., Cossman J., Proc. Natl. Acad. Sci. USA 1983:80:3494-3498.

G 204 FUNCTIONAL SIGNIFICANCE OF DIFFERENT CLONAL B LYMPHOID CELLS IN PLASMA CELL MALIGNANCIES

Bert J.E.G. Bast, Andries C. Bloem, Henk M. Lokhorst, Saskia E. Boom and Rudy E. Ballieux, Dept. Clinical Immunology, University Hospital, Utrecht, The Netherlands

Phenotypic analyses have revealed the presence of idiotypic (clonally related?) B lymphoid cells (e.g. pre B cells, B cells) in multiple myeloma. Using various assays (mitogenic stimulation, EBV transformation, clonogenic assays, short term culture in the presence of thymidine analogues) we have studied the functional significance of these and other putative precursor cells present in blood or bone marrow of patients with malignancy. In that way we demonstrated (a) the present but impaired proliferative and differentiative response of Id B cells under various mitogenic conditions; (b) the raising of Id EBV transformed IgM^+ cell lines, which may not be clonally related; (c) the proliferative potential of Id^+ Transferrin receptor $(Trf\ Rec)^+$ and $Trf\ Rec^-$ lymphoplasmacytoid cells under various clonogenic conditions and (d) the identification of a novel proliferating lymphoplasmacytoid cell (the spotted cell). Characteristics of this latter cell include: expression of Trf Rec and the B cell differentiation antigens HB4 and HB6; absence of other B cell CD and plasmacellular antigens; presence of cytoplasmic Ig, located in the RER; presence of the nuclear antigen detected by Ki-67. These results may add to the notion that Myeloma is a multistep process rather than a pure plasma cellular malignancy.

G 205 **Multispecific anti-idiotypic IgM antibodies are mitogenic for B cells.**

Andries Bloem, Meenal Vakil and John Kearney, UAB, Birmingham, AL, 35294.

We have previously shown that idiotype (Id) directed interactions among B cells during ontogeny are important in the development of the adult repertoire. To determine the mechanisms involved a panel of IgM monoclonal antibodies (ab), many of which were derived from perinatal B cells, were tested for mitogenicity. Most of these also display multispecific anti-Id specificities. From the 49 IgM ab tested 36 were able to induce polyclonal proliferation and differentiation in splenic B cells from germfree nu/nu mice. A correlation exists between mitogenicity and binding to previously characterized monoclonal anti-M460, anti-T15 and anti-J558 ab. Furthermore $Ly\text{-}1^+$ B cells were stained by mitogenic ab. To examine this relationship splenic B cells from male or female CBA/NxBALB/C F1 mice were stimulated with the mitogenic ab. Cells from male mice carrying the xid defect (and lack $Ly\text{-}1^+$ B cells) failed to respond while those from females gave normal responses. Splenic and peritoneal cells from BALB/C nu/nu mice were then separated on basis of Ly-1 expression and cultured in vitro with mitogenic ab. More IgM secreting B cells could be induced within the $Ly\text{-}1^+$ than in the $Ly\text{-}1^-$ B cell subset. Furthermore $Ly\text{-}1^+$ B cells are biased towards the secretion of anti-PC, anti-Dextran, anti-DNP and anti-Id ab. Ab with such specificities have been shown in previous in vivo studies to play an important role in the establishment of the early and adult B cell repertoire. We propose that anti-Id ab with these mitogenic properties may be derived from $Ly\text{-}1^+$ B cells and play an important role in the generation of B cell diversity early in ontogeny. (NIH grants-CA16673, CA13148 and AI14782)

G 206 CELL-CELL AND CELL-MATRIX ADHESION STRUCTURES INFLUENCE THE GROWTH PATTERN OF B-CELL LYMPHOID MALIGNANCIES, F. Caligaris-Cappio, L. Bergui, M. Schena, M. Chilosi and P. C. Marchisio, Dip. Sc. Biomediche e Oncologia Umana, Sez. Clinica e di Istologia, Universita' di Torino, ITALY.

The cytoskeletal organization of B-chronic lymphocytic leukemia (B-CLL) cells differs from that of normal B lymphocytes. B-CLL cells concentrate the bulk of F-Actin into dot-shaped close contact sites that mediate the adhesion to substrates in vitro and are similar to the podosomes observed in class I oncogene transformed cells and in macrophages. B-CLL podosomes have a core of F-actin corresponding to the localization of β1 integrin surrounded by a ring where vinculin, talin and the integrin βchain CD18 (shared by LFA1 -CD11a-, MAC 1 -CD11b- and p150,95 -CD11c-) concentrate and co-localize. Thus, four components of the complex linking the extracellular matrix to the cytoskeleton are specifically organized in or around the F-actin core of podosomes. CD11a is either absent or very weakly expressed in B-CLL cells and never appears to be related to specific cytoskeletal structures. The malignant cells of follicular lymphomas have different features. Monoclonal B cells strongly and uniformly react with CD11a and homogeneously distribute F-actin beneath the plasma memebrane failing to organize it into podosomes. When lymphomas develop a leukemic phase, the circulating malignant cells show features undistinguishable from B-CLL cells. CD11A is either negative or very weakly expressed and F-actin becomes clustered into podosomes with a B-CLL-like organization and distribution. These data suggest that the pattern of growth of malignant B cells as lymphoma or as leukemia may be influenced by the organization of cell-cell and cell-matrix adhesion structures. Supported by P.F. Oncologia, CNR and by A.I.R.C.

G 207 Suppressor T cell recongition of a recurrent idiotype expressed on self erythrocyte-specific autoantibody forming cells, Michael J. Caulfield,* Sally Cochran, Robert D. Miller, and Catherine E. Calkins. *Research Institute of the Cleveland Clinic Fndn., Cleveland, OH 44106 and Thomas Jefferson University, Philadelphia, PA 19107.

A monoclonal antibody was prepared from an unimmunized Coombs-positive NZB mouse. This autoantibody (G-8) reacts specifically with mouse RBCs and appears to express a recurrent idiotype found on naturally occurring autoantibodies extracted from the RBCs of Coombs-positive NZB mice. Using a monoclonal anti-Id antibody (E-8), we demonstrated that the G-8 idiotype is expressed by mouse RBC-specific antibody forming cells (AFC) obtained from old Coombs-positive NZB mice. Previous studies by Moore and Calkins demonstrating that autoantibody forming cells (AFC) could be generated during culture of spleen cells from old (Coombs-positive) NZB mice stimulated with MRBC in vitro formed the basis of the current investigation. This autoantibody response could be suppressed with the addition of splenic T cells from young (Coombs-negative) NZB mice. Treatment of spleen cells from young NZB mice with the IgM autoantibody (G-8) plus complement (C) or with anti-Ly2 antibody plus C resulted in elimination of the suppressor cells and production of AFC specific for MRBC. Treatment with a control NZB IgM mAb (G-4) plus C or with a rabbit anti-MRBC antiserum plus C had no effect on the AFC response to MRBC. Splenic T cells from young NZB mice, when pretreated with G-8 plus C, also lost the ability to suppress the anti-MRBC response of spleen cells from old (Coombs-positive) NZB mice. The results indicate that clones of potentially autoreactive B cells preexist in young NZB mice and that this autoimmune response is suppressed by anti-idiotypic T cells. Thus, idiotypic regulation is important in controlling a spontaneously developing autoimmune disease. (Supported by NIH Grants AI-21178, AR-37093, and CA-37438.

G 208 THREE DIMENSIONAL STRUCTURAL ANALYSIS OF A SINGLE AMINO ACID CHANGE IN A NON-ANTIGEN BINDING MUTANT, Nadine C. Chien, Victoria A. Roberts*, Angela Guisti, Matthew D. Scharff and Elizabeth D. Getzoff*, Albert Einstein College of Medicine, Bronx, NY and Scripps Clinic and Research Foundation, La Jolla, CA.*

Antibodies that differ from their germline sequences by single amino acids provide useful tools for understanding the structural basis of antigen binding. We have generated such somatic mutants from the S107 cell line, which produces a phosphorylcholine (PC) binding antibody that is encoded by the T15 VH1 gene. One such mutant, U10, has completely lost its ability to bind PC. Sequence data of the heavy and light chain VDJ regions and the CH1 domain revealed a single base change resulting in the substitution of an alanine (Ala) for an aspartic acid (Asp) at 101 in the fifth residue of JH1. There was a loss of reactivity with both monoclonal and polyclonal binding site specific antibodies indicating that this mutation caused a local conformational change in the binding site. To better understand how a residue not located in a complimentarity determining region (CDR) could so dramatically disrupt antigen binding, computer modeling was carried out. A model of the S107 antibody binding site was built using the crystal structure of the PC binding antibody McPC 603. This was done by substituting 32 amino acids in the light chain and 4 in the heavy chain variable regions, and by inserting a tyrosine in CDR 3 between residues Arg 94 and Asp 101. From this, the mutant binding site of U10 was simulated in an Arg-in and an Arg-out model. It was found that Arg 94 of the heavy chain would no longer interact with the substituted Ala 101 and that the Arg 94 is most likely located in close association with the glutamic acid at residue 35 which resides in the PC binding site.

G 209 Regulation of Expression of Murine Membrane IgM, IgD and Ia Antigens by N-linked Oligosaccharide Processing.

William Cushley, Teresa Burke, [2]Alison Wood[1] and E. Charles Snow[2]. 1) Department of Biochemistry, University of Glasgow, GLASGOW G12 8QQ, Scotland, U.K. and 2) Department of Microbiology and Immunology, University of Kentucky Medical Center, LEXINGTON, KY 40536.

The enzymes mediating processing of protein-bound N-linked oligosaccharides are responsible for the diversity of glycan structures of glycoproteins. The precise role of oligosaccharide structure in relation to glycoprotein function remains unresolved, but interference with intracellular processing activities has been shown to inhibit the transport of certain proteins. In the case of secretory Igs, all isotypes except IgD are secreted normally when N-linked oligosaccharide processing is blocked. Considerably less data are available with regard to transport of membrane Igs.

The effect of interruption of N-linked oligosaccharide processing upon expression of murine membrane IgM (mIgM), mIgD and Ia antigens has been evaluated using a selection of specific processing inhibitors. The experimental procedures involved proteolytic removal of cell surface structures, re-expression of proteins in the presence or absence of inhibitors during an 18hr culture, and analysis of the expressed mIgs and Ia antigens. Flow cytometric analysis indicated that the levels of expression of mIgM on inhibitor-treated cells were comparable to those of cells cultured in the absence of inhibitors. However, the expression of mIgD was impaired by the presence of inhibitors of glucosidase, but not mannosidase, activities suggesting that transit of mIgD from the rough edoplasmic reticulum to the golgi was rate limiting with respect to kinetics of transport to the plasma membrane.

G 210 B-CELL AND T-CELL GROWTH PROMOTING ACTIVITIES OF RECOMBINANT HUMAN INTERLEUKIN 4. **Rene Devos, Bettadapura Jayaram* and Walter Fiers*, Biogent, J. Plateaustraat 22, B-9000 GENT, Belgium, *Laboratory of Molecular Biology, State University Gent, 9000 GENT, Belgium.**

Activation of B-cells from the resting state can be induced by crosslinking of surface Ig-receptors with anti-Ig or by stimulation with Staphylococcus aureus Cowan strain I. The proliferation and differentiation of activated B-cells can be brought about by B-cell factors produced by T-cells. At least three distinct B cell lymphokines have been described in the human system : BCGF I or BSF-1 or IL4, BCGF II or IL5 and BSF-2 or IL6 (26K protein). IL4 is a secreted glycoprotein expressed in T-helper lymphocytes after mitogenic stimulation. To define its biological activities, we expressed the recombinant human IL4 protein by translation in Xenopus laevis oocytes of RNA transcripts made in vitro on human IL4 cDNA-containing SP65 plasmids. SDS-gel-electrophoresis of ^{35}S - cysteine labeled proteins secreted by the oocytes revealed an additional band of molecular weight 20 Kd. Compared to recombinant human IL2, the oocyte derived supernatant induced a low (10%) proliferative response in human T lymphocytes preactivated with ConA or PHA. The reverse was observed when using a B-cell proliferation assay involving cultures of resting small human B-cells (isolated either from peripheral blood or from tonsils), in the presence of TPA. Here the oocyte derived human IL4 scored very well, while rIL2 gave a marginal response.

G 211 REGULATION OF LYMPHOKINE GROWTH AND RECEPTOR EXPRESSION BY INSULIN AND INSULIN-LIKE GROWTH FACTOR-1.

Diane D. Eardley, and William Hempel, Department of Bio. Sci., University of California, Santa Barbara, 93106. Insulin and insulin-like growth factor-1 (IGF-1) are growth factors for lymphocytes, but superphysiologic doses of insulin and the high range of physiological doses of IGF-1 inhibit proliferation of T and B lymphocytes induced by Interleukin-2 (IL-2). Since the growth inhibitory effect requires much higher doses of insulin than IGF-1, we have postulated that the inhibitory effect of insulin acts through the IGF-1 receptor. We now show that insulin/IGF-1 inhibit IL-2 production but not IL-2 receptor expression by T lymphocytes. This observation is significant because it separates the events of lymphokine production and receptor expression which are two closely linked events that occur shortly after T cell activation. We are currently measuring the effects of insulin/IGF-1 on insulin/IGF-1 receptor expression on T and B lymphocytes to determine whether down-regulation of these receptors may account for the inhibition of IL-2 induced proliferation. These results suggest that up-regulation of IL-2 receptors and initiation of lymphokine production by T cells are independent events and are sensitive to different inhibitory signals. This research was supported by a grant from the Concern Foundation.

G 212 INDUCTION OF DIFFERENTIATION IN HUMAN LEUKEMIC B CELLS BY IL2 ALONE: DIFFERENTIAL EFFECT ON THE EXPRESSION OF MU AND J GENES. Dominique Emilie, S. Karray, H. Merle-Beral, P. Debre and P. Galanaud, INSERM U 131, 32 rue des Carnets, 92140, Glamart, France and Department d'Immuno-Hematologie, CHU Petie-Salpetriere, Paris.
In order to separately analyze the effects of IL2 on the proliferation and differentiation of B cells we selected two patients suffering from B type Chronic Lymphocytic Leukemia (B-CLL). The monoclonal B cells from these patients exhibited an opposite pattern of responsiveness upon in vitro culture with IL2 in the absence of other stimulus. In the first patient, IL2 alone was able to induce DNA synthesis and no Ig production. In the second patient, although no DNA synthesis was detected, B lymphocytes synthesized IgM upon stimulation with IL2 alone. Analysis of mRNA levels were performed on the cells of this latter patient after culture without or with IL2. We observed a strong enhancement of C mu gene expression associated with an increase of the ratio between the secreted form and the membrane bound form of mu mRNA. In contrast IL2 induced no enhancement of J chain mRNA. Thus terminal B cell differentiation can be obtained in the absence of DNA synthesis and IL2 alone can mediate this process. Moreover IL2 can act at selective steps of the molecular events associated with IgM production. These results document the multiple effects of a given interleukin on the events leading to antibody production and strongly suggest that they can be conditionned by the maturation stage of a given responding cell.

G 213 CHARACTERIZATION AND AFFINITY-PURIFICATION OF AUTOCRINE BCGF MOLECULES FROM HUMAN B CELL LYMPHOMAS. Richard J Ford, Linda Yoshimura, Mark Pershouse, CG Sahasrabuddhe. U Tx MD Anderson Hospital and Tumor Inst.,Houston,Tx 77030
Of the group of lymphokines that putatively stimulate human B cell proliferation, the B cell growth factor(BCGF) group of B lineage-specific lymphkines, consists of a low M_r (12-20 K) and a high M_r(50-60K) forms, which have been either purified or cloned. The high M_r form is secreted by various neoplastic T and B cells, and also by some normal human lymphocytes under certain conditions. We have demonstrated the presence of a 60K BCGF in the cytoplasmic extracts from normal T cells(IC-BCGF), as well as neoplastic (non-Hodgkin's lymphoma) B cells. The question of the relationship of these BCGFs, which have a similar spectrum of proliferative activity on normal as well as neoplastic B cells, was undertaken, using polyclonal anti-IC-BCFG antibodies made against the normal T cell product. The antibodies were shown to recognize the 60K BCGFs from both the normal and neoplastic B cells, demonstrating at least some epitope homology. Western immunoblot analysis showed that some neoplastic B cells also secrete both 60K and 12-14K BCGF molecules. When affinity-purified, these molecules stimulated growth in anti-μ-activated normal human B cells . Binding of the BCGF molecules to the cell surface of the neoplastic B cells was demonstrated by flow cytometry, suggesting the presence of BCGF receptors. These studies support the concept of neoplastic B cell growth mediated by autocrine BCGF secretion.
Supported by NIH grants CA 31479 and GM 35483.

G 214 PHENOTYPIC AND FUNCTIONAL CHARACTERIZATION OF NORMAL AND MALIGNANT CD5+ B CELLS. Nancy Gadol, Becton Dickinson Immunocytometry Systems, Mountain View, CA 94039
CD5+ (Leu 1+) B cells comprise the total B cell population in most chronic lymphocytic leukemia (CLL) patients and may be the principal cell subset producing autoantibodies in these patients and in patients with various autoimmune disorders. Leu 1+ B cells are relatively unresponsive to most B cell mitogens. We have examined the functional response of Leu 1+ B cells from normal controls and from patients with CLL to various stimuli. Cell proliferation was measured by incorporation of ^{3}H-thymidine. Cells from 6 of 7 CLL patients proliferated in response to supernatant from T cells stimulated with PHA (PHA sup). Surprisingly, sorted normal Leu 1+ B cells did not respond to PHA sup, but Leu 1- B cells did respond. Cells from 7 of 8 CLL patients did not proliferate in response to a monoclonal mouse anti-human IgM bound to sepharose (MSEPH). Similarly, Leu 1+ B cells sorted from the peripheral blood of 5 normal individuals never responded to MSEPH. However, Leu 1- B cells from the same individuals proliferated 4X to 53X above background in response to MSEPH. The intensity of surface IgM and surface IgD expression was comparable in Leu 1+ and in Leu 1- B cells as determined by three color analysis of these cells from normal peripheral blood lymphocytes. In preliminary experiments, Leu 1+ B cells and Leu 1- B cells showed no difference in the expression of differentiation and activation antigens. Differences in the phenotypes of Leu 1+ and Leu 1- B cells are being examined as a possible explanation for the observed functional differences.

G 215 PROGRESSIVE HETEROGENEITY OF ANTIBODY SECRETING CELLS FOLLOWING MULTIPLE ANTIGEN ADMINISTRATIONS

Edmond A. Goidl and Susan J. Martin McEvoy. Dept. of Microbiology and Immunology, School of Medicine, University of Maryland at Baltimore, Baltimore, Maryland 21201.

There is an increase in affinity (Kav) with respect to time after administration of a thymic dependent antigen. This maturation of the immune response continues progressively until late in the primary response when a drop in Kav is seen. We have evidence that this drop in Kav may in part be due to potential high affinity antibody producing B cells which are blocked from secretion by auto-anti-idiotypic (auto-anti-id) antibody. "Families" of Ids and auto-anti-ids seem to be "moving -up" the maturation curve with respect to time after immunization. Such oscillatory patterns seem to indicate that the pauciclonal antibody response to 2,4,6-trinitrophenyl-lysyl-Ficoll is represented by 2-3 such families. Theoretical thermodynamic modeling seems to support this interpretation.

(Supported by USPHS grant AG-04042)

G 216 ROLE OF INTERFERON AND OTHER CYTOKINES IN SYNERGY WITH 8-MERCAPTOGUANOSINE.

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In previous studies, it has been demonstrated that a T cell-like differentiation signal is transmitted by C8-substituted guanine ribonucleosides such as 8-mercaptoguanosine (8MGuo) to antigen-stimulated B cells. A large subset of potentially reactive B cells remains unresponsive to antigen even in the presence of signals provided by 8MGuo except when this signal is preceded by a soluble activity present in mixed lymphocyte culture supernatants. Studies with purified preparations of interleukin (IL)-1, IL-2, IL-3, GM-CSF, BSF-1 (IL-4) and BCGF-II (IL-5) indicate that none of these activities synergizes with 8MGuo to augment B cell responsiveness to antigen. Therefore, supernatants from cloned cell lines were examined for activity that synergizes with 8MGuo, to determine its cellular source. Soluble products secreted by cloned 24/G1 T cells act synergistically with 8MGuo to evoke enhanced antibody responses to specific antigen from purified B cells. Because activated 24/G1 cells produce large quantities of interferon γ (IFNγ), the possibility that interferons might mediate synergy with 8MGuo was investigated. Purified murine IFNγ does not interact synergistically with 8MGuo; moreover, treatment of 24/G1 supernatants with monoclonal anti-IFNγ antibodies or at pH2 fails to abrogate their ability to synergize. In contrast to IFNγ, when B cells were supplemented with either IFNα or IFNβ, antigen-dependent synergy with 8MGuo was observed. However, abrogation of IFN activity with anti-IFN antibodies fails to interfere with synergy between 8MGuo and T cell supernatants. Thus, although IFNα and IFNβ are not the synergizing principles in activated T cell supernatants, they nonetheless represent a previously unrecognized source of synergizing activity.

G 217 ISOLATION OF A cDNA FOR MURINE LYMPHOPOIETIN-1, A PRE-B CELL GROWTH FACTOR.

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We have developed a quantitative proliferation assay using purified pre-B cells to detect a pre-B cell growth promoting activity which we have designated lymphopoietin-1 (LP-1). As a source for both protein and RNA transcripts coding for LP-1 we have utilized a clonal cell line established by transformation of murine bone marrow stromal cells. Polyadenylated RNA isolated from these cells was used to prepare double-stranded cDNA which was inserted into a mammalian expression vector. Bacterial clones resulting from the transformation of this cDNA-containing vector were pooled and transfected into COS cells and screened by assaying the culture supernatants for the presence of LP-1 activity. After several hundred thousand clones were screened, we were able to isolate a cDNA-containing plasmid which expressed LP-1. Nucleic acid sequence analysis revealed that this cDNA was derived from a mRNA capable of coding for a protein of 154 amino acids with a leader sequence of 25 amino acids. The N-terminus of the mature protein was determined by amino acid sequence analysis of the purified protein. Further studies on the transcriptional expression of LP-1 will be presented.

G 218 DIFFERENTIATION OF B CELL PROGENITORS *IN VITRO*: GENERATION OF SURFACE IGM+ B CELLS, INCLUDING LY-1 B, FROM THY-1-ASIALO GM_1+ CELLS IN NEWBORN LIVER. Richard R. Hardy and Kyoko Hayakawa. Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, PA 19111.

We have established a stromal adherent cell line (ST2) from fetal liver that promotes growth and differentiation of early B lineage cells. Many cells in the "null population" (3-5%) from newborn liver that lack antigens found on mature erythroid, myeloid or lymphoid cells proliferate extensively on this ST2 layer. Further division of this cell fraction on the basis of Thy-1 and asialoGM_1 (aGM_1) expression discriminates cells that predominantly proliferate from those that differentiate on the ST2 layer. Among four populations, Thy-1+aGM_1- cells proliferate most but yield few B220+ cells. In contrast, Thy-1- aGM_1+ cells proliferate to a very limited extent, but most (>90%) start to express B220 and a large fraction (up to 50%) become surface IgM+ after 2 weeks of culture. These B cells include cells expressing the pan-T cell molecule Ly-1, that is, Ly-1 B cells. Curiously, this Thy-1-aGM_1+ cell population is largely absent from bone marrow of adult mice (which fails to reconstitute Ly-1 B when transferred into irradiated recipients). Further characterization of the Thy-1+aGM_1- fraction by cell transfer into SCID mice demonstrates that these cells are capable of giving rise to Ly-1 B cells *in vivo*. Comparison of this fraction with a phenotypically similar population in bone marrow provides further evidence that Ly-1 B cells diverge from the predominant B cell lineage at the progenitor stage.

G 219 ENRICHMENT AND CHARACTERIZATION OF B LINEAGE PROGENITOR CELLS. Yvonne L. Harrison, Department of Microbiology and Immunology, West Virginia University Medical Center, Morgantown, West Virginia 26506

Hemopoietic progenitor cells for myeloid lineages are enriched in the marrow of mice recovering from in vivo cytotoxic drug administration. We found that progenitors of B lymphocytes were also elevated following in vivo treatment with 5-fluorouracil (5FU), cyclophosphamide (Cy) or hydroxyurea (HU). Pre-B cells (14.8+, cμ+, sIg-), and cells responsive to pre-B cell differentiation factors were enumerated for 2 weeks following drug treatment. Pre-B cell generation from 14.8- cells was determined in vitro in the presence of CN-urinary derived pre-B cell generating activity. B lineage progenitor cells and pre-B cells were enriched at 6,7, and 4 days following treatment with 5FU, Cy and HU, respectively. We used 4 day post-HU bone marrow (BM) to further characterize progenitor cells which respond to pre-B cell differentiation factors. In vitro culture with DNA synthesis inhibitors HU or 5-fluorodeoxyuridine in the presence of 2 different pre-B cell differentiation factors (CN-urinary activity or condition medium from BM stromal cell line S17) suggests that pre-B cell generation from 14.8- cells required DNA synthesis. Metabolic inhibition of RNA synthesis also abrogated pre-B cell generation. When BM was assayed 2 hrs post-in vivo HU treatment, little or no response to differentiation factors was observed even if BM cells were held in culture for up to 4 days. Taken together, these data suggest that B lineage progenitor cells, which preceed pre-B cells in development, are actively proliferating and require both DNA and RNA synthesis to differentiate to μ expression. Supported by grants AI-93250 and DK-39898 from the National Institutes of Health.

G 220 THE EFFECT OF LYMPHOKINES ON THE GROWTH OF HUMAN FETAL BONE MARROW CELLS. F.M. Hofman, M. Brock and B. Lyons, University of Southern California, Los Angeles, CA 90033.

Using a long-term human bone marrow culture system, developed in this laboratory, we analyzed the effects of different lymphokines on the proliferation of bone marrow-derived B cells. These B-cell cultures were characterized phenotypically with a panel of monoclonal antibodies identifying cells at different stages of development. The cells maintained in culture were shown to be early B cells based on the expression of cytoplasmic mu chain, BA-1 and B-1 antigenic determinants. These fetal bone marrow cells could be maintained in vitro for as long as four weeks with viability greater than 50%. In order to identify which factors regulate early B-cell proliferation, we exposed the B-cell cultures to a series of lymphokines. Our results show that cell line-derived supernatant containing IL-3 caused cell proliferation, while IL-1 and IL-4 did not have this effect.

G 221 **IMMUNOGLOBULIN μ AND δ HEAVY CHAINS EFFECT CELLULAR PROLIFERATION DIFFERENTLY.** Nobumichi Hozumi, Roland Tisch and Chaim Roifman*, Mt. Sinai Hospital Research Institute and *Hospital For Sick Children Toronto, Ontario, Canada M5G 1X5

We have recently shown that surface IgD (sIgD) and sIgM function similarly with regard to antigen binding capacity and antigen presentation efficiency using a B cell line transformed with IgD_{TNP} or IgM_{TNP} vectors carrying the Ig genes specific for the hapten, TNP (Tisch et al., Proc. Natl. Acad. Sci. USA 84:3831, 1987). To obtain a clue as to whether the two sIg receptors function differently, the response of B cell proliferation following crosslinking of sIgM and sIgD was examined. An immature B cell line, WEHI231, expressing sIgM was used as a recipient cell for gene transfer. Proliferation of WEHI231 cells is arrested by anti-μ antibody treatment. Proliferation of sIgD expressing transformants was evaluated following treatment with anti-Sp6 antibody which recognizes an idiotypic determinant associated with the V_H for TNP specificity and with anti-μ antibody. Anti-μ antibody treatment was able to inhibit proliferation of the transformants completely. In contrast, addition of anti-Sp6 antibody to the WEHI231 transformants induced an increase in ^{3}H-thymidine uptake. Thus, these results clearly demonstrate that μ and δ chains expressed on the immature B cell transformants effect cellular proliferation differently. Currently we are analyzing signal transduction in these transformants. The membrane exons of μ and δ chain genes are being shuffled to δ and μ domains respectively to map the regions responsible for the altered cellular proliferation. These results will be discussed.

Supported by MRC and NCIC.

G 222 THE B LYMPHOCYTE LINEAGE RELATED GENE mb-1: cDNA STRUCTURE AND RNA EXPRESSION IN DIFFERENT CELL LINEAGES, Shin-ichiro Kashiwamura, Nobuo Sakaguchi, Masao Kimoto, Philipp Thalmann, and Fritz Melchers, Saga Medical School, JAPAN and Basel Institute for Immunology, SWITZERLAND.

A gene, called mb-1, was isolated from a murine pre-B (70Z/3) minus T lymphocyte (T cell hybridoma, K62) subtracted cDNA library which is selectively expressed in cells of the B lymphocytes lineage. It is expressed at low to medium abundance in early progenitors of the B lineage, in pre B and in mature B lineage cell lines, in normal resting B lymphocytes, in polyclonally activated B cell blasts, but not in plasmacytomas. It is also not expressed at detectable levels in monocytes, T lymphocytes or fibroblasts, not in thymus, liver, kidney, heart, lung or brain.

And by the RNA in situ hybridization analysis, mb-1 message is expressed in 5 to 15 percent of cells in day 15 fetal liver, but not expressed in fetal thymus. Its preferential expression in early to mature B lymphocytes suggested it useful as the B cell differentiation marker.The mb-1 cDNA gene encodes a putative membrane glycoprotein, MB-1, with 220 amino acids which include a leader sequence, an extracellular domain with two potential N-glycosylation sites, a transmembrane portion with a charged amino acid in it, and an intracellular domain.

Furthermore, we have established the monoclonal antibodies against the MB-1 protein by immunizing chimeric protein purified from bacterial product and showed the presence of MB-1 protein on the surface of B lineage cells but not in other cells.

G 223 CHARACTERIZATION OF B CELL POPULATIONS BEARING Fcε RECEPTOR IN NORMAL AND CBA/N MICE, Marilyn R. Kehry and Susan A. Hudak, Department of Immunology, DNAX Research Institute, 901 California Ave., Palo Alto, CA 94304. The majority of splenic B lymphocytes express low affinity Fc receptors for IgE (FcεR). It has recently been suggested that FcεR may serve as a stage-specific marker in human B cell differentiation. To define the populations of mouse B cells expressing FcεR we have used two color staining and flow cytometry to analyze B lymphocytes for FcεR and several markers of B cell differentiation (IgD, IgM, and Ia). In normal adult BALB/c and CBA/J mice all B cells bearing FcεR expressed high levels of Ia and surface IgD and low levels of surface IgM. Thus, FcεR is expressed on a specific population of mature B cells, but not on pre-B or plasma cells. A B cell subpopulation that expresses high surface IgD and low surface IgM has been found to be absent in immune-defective *xid* mice, CBA/N. Examination of cells from the CBA/N strain showed that B lymphocytes expressing FcεR were present as a reduced percentage of the total B cells (4-fold lower frequency than B cells from normal mice). IL4 treatment of normal B cells induces increased expression of FcεR; IL4 treatment of B cells from CBA/N mice induced a higher proportion of FcεR-bearing cells which expressed high levels of both surface IgD and IgM. Thus, a B cell subset in CBA/N mice could be induced to express FcεR, but this population was altered in phenotype.

G 224 $V_H \rightarrow$ PARATOPE MAPS: ANALYSIS OF V_H EXPRESSION AMONG PC- AND HEL-SPECIFIC B CELL COLONIES. G. Kelsoe, R.M. Micell, J. Cerny and D.H. Schulze, Department of Microbiology, University of Texas Medical Branch, Galveston, TX 77550. The structure of the Igh-V locus suggests that V_H families arose by repeated duplications of an ancestral family followed by sequence divergence. The nature of this divergence and of the forces driving it are not understood. Do V_H families represent selected stores of particularly useful structural information (e.g., anti-pathogen) or does V_H divergence reflect potential diversity that is phenotypically neutral? If V_H families preferentially encode specific sets of structural information, the distribution of antibody (Ab) specificities among V_H families should be non-uniform. In contrast, should V_H families represent unselected (phenotypically neutral) reservoirs of potential diversity, Ab specificities should be distributed more or less equally among V_H families. We present $V_H \rightarrow$ paratope maps for some 20,000 LPS-induced B cell colonies screened for PC- or HEL-specificity and V_H expression. Splenocytes from adult C57BL/6 mice were plated onto filter paper discs and Ab-secreting colonies induced by culture with LPS. Each colony arises from a single founder B cell and is immobilized within the paper filter. Colonies secreting specific Ab were detected by immunoblotting discs on PC- or HEL-coated nitrocellulose; foci of bound Ab identifying specific colonies were detected via radioiodinated anti-kappa or -idiotope Abs. After blotting, discs were fixed and V_H expression (X-24, S107, Q52, J558) determined by *in situ* Northern hybridization. Thus, Ab specificity and V_H expression within single colonies could be determined. Analysis of 4,500 antigen-specific colonies demonstrate *no* preferential V_H:paratope associations.

G 225 PATTERNS OF IMMUNOGLOBULIN VARIABLE REGION EXPRESSION AMONG NEONATAL HA-RESPONSIVE B CELLS: EVIDENCE FOR PREFERENTIAL GENE EXPRESSION DURING LATER STAGES OF DEVELOPMENT. L. Kienker, J. Korostoff and M. Cancro, Dept. of Pathology, Univ. of Penna. Medical School, Philadelphia PA 19104-6082.

Earlier studies showed a regular emergence and turnover of B-cell clonotypes reactive to influenza hemagglutinin (HA). Preferential V_H gene rearrangements have been suggested to underlie this patterned emergence and turnover. To assess this possibility, hybridoma libraries were established whose specificities mirror the BALB/c HA-specific B-cell repertoire at one and two weeks of age. These were generated through chronic immunization regimes previously shown to induce oligoclonal responses dominated by clonotypes extant at the age of initial immunization. Cytoplasmic RNA from these hybridomas was analyzed by dot blot hybridization against 8 mouse V_H gene family probes. The results indicate that although genes from most known V_H families can generate HA-specific antibodies, the specificities prevalent during the first week of life disproportionately utilize V_H7183 gene segments. In contrast, hybridomas representative of the repertoire in two-week old individuals express V_H7183 minimally and instead preferentially use V_H36-60 and V_HX24 gene segments. These results are consistent with a preferential use of particular V_H gene families during early development. Specifically, they extend previous findings suggesting that the J-proximal V_H family, 7183, appears earliest within the B-cell pool. Further, the diminution of V_H7183 usage between 1 and 2 weeks of age coupled with the disproportionate representation of V_H36-60 and V_HX24, provides evidence that subsequent V_H gene rearrangements are also stochastically skewed toward particular families.

G 226 A LIPOPOLYSACCHARIDE (LPS) RECEPTOR ON 70Z/3 CELLS. T.N. Kirkland*, G.D. Virca**, R.J. Ulevitch**, and P.S. Tobias**. University of California, San Diego* and the Research Institute of Scripps Clinic, La Jolla**.

Research Institute of Scripps Clinic, La Jolla.

To identify an LPS receptor on 70Z/3 cells, we analyzed the cellular proteins labeled by a radioiodinated, photoactivatable derivative of *Salmonella minnesota* Re 595 LPS (^{125}I-ASD-Re) by SDS-PAGE and autoradiography. At least 10 distinct bands were labeled; our criterion for specific, saturable binding was inhibition of labeling of a band in the presence of excess *E. coli* J5 LPS. One band, in the 18 kD region, was prominently labeled and the labeling was consistently inhibited by an excess of J5 LPS. ^{125}I-ASD-Re binding to this band was not dependent on protein synthesis or cellular metabolism. The labeled 18 kD protein was found in a membrane-containing fraction of homogenized cells. Lipid A and its precursor IV_A (two biologically active fragments of Re LPS) inhibited the binding of the 18 kD to ^{125}I-ASD-Re. Therefore, the 18 kD protein binds to the lipid A region of LPS. In addition, polymyxin B, and a monoclonal anti-LPS antibody which blocks LPS-induced 70Z/3 cell activation inhibited the labeling of the 18 kD protein by LPS. Two anti-LPS mAbs which do not block LPS-induced cellular activation did not block 18 kD protein labeling. Thus, binding of LPS to the 18 kD protein is important for cellular activation. The 18 kD protein was labeled in B and T cells from C3H/FeJ and C3H/HeJ mice, but not in L929 cells or sheep red blood cells. Since the 18 kD protein binds to the lipid A region of LPS in a specific, saturable manner, and inhibitors of LPS activity block binding, we propose that the 18 kD protein is at least a part of the LPS receptor on 70Z/cells.

G 227 IL-4 CAUSES ISOTYPE SWITCHING TO IgG1 AND IgE IN CLONAL B CELL CULTURES. Deborah Lebman & Robert Coffman. DNAX Research Institute, Palo Alto, CA 94304.
IL-4 has been shown to increase both IgG1 and IgE secretion in cultures of LPS-stimulated B cells, but it has not been clearly established if IL-4 induces isotype switching to IgG1 or if it allows for the preferential growth of IgG1 committed precursor cells. IL-4 is one of the lymphokines produced by the murine T_H2 subset of helper T cells. Thus, in order to ascertain if IL-4 causes a switch to IgG1, limiting dilution analysis was performed using an excess of T_H2 cells specific for the $F(ab')_2$ fragment of rabbit IgG and limiting numbers of B cells expressing the appropriate haplotype. In the presence of rabbit anti-mouse IgM, 20%-30% of B cells gave rise to clones that could be detected by antibody secretion. The amounts of IgM, IgG1, and IgE were measured in culture wells that had a high probability of clonality as judged by Poisson analysis. The majority of IgM-secreting clones also produced IgG1, and in fact, clones producing IgG1 but no IgM occured frequently. In the presence of a blocking monoclonal antibody directed against IL-4, there was a substantial increase in the proportion of clones that secreted IgM without IgG1. Additional IL-4 appeared to cause some enhancement in both the amount of IgG1 secreted and the frequency of responding B cells. Although stimulation with a T_H2 clone could induce the formation of B cell clones that secreted IgE, the proportion of IgE secreting cultures was enhanced by additional IL-4. These results provide evidence that IL-4 can enhance the rate of isotype switching to IgG1 and IgE.

G 228 DIFFERENTIAL ATTACHMENT OF NORMAL AND TRANSFORMED PRE-B CELLS TO FIBRONECTIN, François M. Lemoine, Shoukat R. Dedhar, Connie J. Eaves, Terry Fox Laboratory, B.C. Cancer Research Centre, Vancouver, B.C., Canada, V5Z 1L3.
A key feature of murine long-term B lymphoid marrow cultures (LTBMC) is the presence of a layer of stromal cells that produce a growth factor essential for normal pre-B cell proliferation. Whether this control of pre-B cell proliferation by stromal cells involves additional mechanisms, for example involving cell-cell contact, is not known. To examine the possible role of fibronectin (FN) in facilitating stromal cell-mediated support of normal pre-B cell proliferation, we tested whether addition of a RGD synthetic peptide or an anti-fibronectin receptor antibody (FN-R Ab) could block the ability of pre-B cells from LTBMC to proliferate when cultured on a cloned line of marrow stromal cells. Both agents (but not anti-vitronectin receptor antibodies) were inhibitory in such co-cultures. To determine whether the ability of pre-B cells to bind to fibronectin might be altered in transformed pre-B cells, since these become less stromal cell dependent, normal pre-B cells and a number of spontaneous or Abelson virus transformed derivatives were compared with respect to their ability to adhere to various extracellular matrix proteins, i.e. FN, vitronectin, and collagen type I. Normal pre-B cells adhered strongly only to FN and this adhesion could be inhibited by either the RGD peptide or FN-R Ab. All transformed pre-B cells tested were unable to adhere to FN. These data suggest that mesenchymal stimulation of normal pre-B cell proliferation requires a specific interaction between the two cell types that is mediated by FN, and that malignant transformation of pre-B cells commonly induces quantitative and/or qualitative changes in FN-R expression such that the transformed pre-B cells can no longer participate in this interaction.

G 229 IN VIVO ANALYSIS OF SPLENIC PROGENITORS TO SECONDARY B CELLS, Phyllis-Jean Linton and Norman R. Klinman, Scripps Clinic and Research Foundation, La Jolla, CA 92037.
In vitro clonal analyses have demonstrated that splenic progenitors to secondary B cells can be separated from primary precursors to antibody forming cells (AFC) based on their low expression of the determinant recognized by the J11D monoclonal antibody. The vast majority of primary splenic B cells (90-95%) are "$J11D^{hi}$" and respond to primary but not secondary in vitro antigenic challenge. In contrast, at least half the "$J11D^{lo}$" splenic precursor cells require both primary and secondary in vitro antigenic stimulation to produce any antibody and the remaining "$J11D^{lo}$" B cells that produce antibody after primary in vitro stimulation produce more after secondary stimulation. To further verify that "$J11D^{hi}$" splenic B cells are progenitors to primary AFC whereas "$J11D^{lo}$" B cells are progenitors to secondary B cells, cell populations enriched for "$J11D^{hi}$" vs "$J11D^{lo}$" B cells were transferred into SCID mice. These mice were subsequently immunized and their immune responses analysed in vivo. After primary immunization of mice reconstituted with "$J11D^{hi}$" B cells antibody production peaked at three weeks and no increase in serum titer was observed upon rechallenge. Primary immunization of mice reconstituted with "$J11D^{lo}$" B cells resulted in low levels of specific antibody; however, boosting these mice was followed by the rapid generation of a very high titer antibody response which persisted for several weeks. Thus, the in vivo antibody responses generated by the "$J11D^{lo}$" vs "$J11D^{hi}$" B cells parallel the responses previously found in vitro for these cell subpopulations and support the conclusion that distinct splenic progenitors exists for primary AFC vs secondary B cells.

G 230 SELECTIVE INFLUENCES OF T-CELL SUBSETS IN THE SELECTION OF B-CELL LINEAGES, M.A.R. Marcos, A. Sánchez, C. Márquez, M.L. Toribio, A. de la Hera, J.M. Alonso and C. Martínez-A, Centro de Biología Molecular, C.S.I.C., Madrid.

Previous studies using both nude.xid and B-mice (ATxB) have concluded the T-cell dependence of B-cell development in xid mice. On the other hand, two B-cell lineages have been described and defined by the expression of Ly-1 (CD5) surface markers. We have shown a selective expansion of Ly-1$^+$ B cells in the absence of the conventional bone marrow-derived lineage in CBA/N upon the induction of a Cyclosporine A-mediated autoimmune syndrome, which also express Mac-1 antigens. Moreover, neonatal thymectomy (3rd day of extrauterine life) of CBA/N mice, although decreasing sIg$^+$ cells in absolute values, also induced a preferential expansion of sIg/Ly-1^{dull} B cells. Isotype analysis of naturally activated Ig-secreting splenic cells revealed low numbers of IgM, but near-normal T-cell dependent isotypes (IgG2, IgA). It appears that neonatal thymectomy in these mice acts on the process of acquisition of different TcR-bearing populations ($\alpha\beta$ vs $\gamma\delta$), being the created imbalances responsible for the Ly-1$^+$ B-cell expansion. Recent studies with CBA/N mice neonatally suppressed with anti-mouse T3 MonAb (145-2C11) are in course in order to further expand these questions. Thus, regulatory incidences in acquisition of T-cell repertoires appear to influence lineage relationships in B-cell compartments.

G 231 HETEROGENEITY IN THE CDR3 REGION OF MURINE MONOCLONAL ANTIBODIES THAT BIND 3-FUCOSYLLACTOSAMINE (3-FL). D. M. Marcus, H. Kimura and S. Buescher*, Baylor College of Medicine and The University of Texas Health Science Center*, Houston, Texas 77030

Our previous studies of anti-3-FL monoclonal antibodies (mAbs) PMN6, PMN29 and PM81 indicated a restricted use of gene segments. The heavy chains were encoded by V_H441, DQ52, J_H4, and the light chains by V_K24B and J_K1 gene segments. Since Abs against levan and galactan use the same V_H segment but differ from anti-3-FL Abs in the CDR3 region, we suggested that the fine specificity of these 3 families of Abs is determined by the CDR3 sequences and light chains. We are now studying 5 additional anti-3-FL mAbs. Southern filter hybridization analyses indicated that all 5 antibodies are encoded by the V_H441 gene segment. Partial sequences are summarized below and are compared to PM81.

Ab	D	N	J	
PM81	Q L G	X N	A M D Y	DQ52, J_H4
M5,72	Q L G	E	A M D Y	
M6,32	S D G	H	Y A M D Y	DSP2, J_H4
M3	I T T A	R	A Y	DFL16.2, J_H3

Four of 5 Abs use J_H4 and M3 uses the J_H3 gene segment. M5 and 72 use DQ52, but M6 and 32 use DSP2, and M3 uses DFL16.2. In summary, all 8 anti-3-FL Abs studied to date are encoded by V_H441, and 7/8 use J_H4, but there is appreciable diversity in the use of D segments and in the amino acid sequences of CDR3.

G 232 RECURSIVE SELECTION OF B- AND T-CELL REPERTOIRES, C. Martínez-A, A. Coutinho, M.L. Toribio, A. de la Hera, C. Márquez, M.A.R. Marcos and P. Pereira, Centro de Biología Molecular, C.S.I.C., Madrid and Institut Pasteur, Paris.

Normal BALB/c mice produce TNP-I-A^a specific T helper cells expressing a receptor heterpdimer which share with anti-TNP antibodies an idiotope defined by the F6(51) anti-idiotypic antibody. Expression of this T_H idiotype is controlled by immunoglobulin heavy chain allotype-linked genes and results from antibody-dependent selection of T-cell repertoires. We now present evidence for the recursive nature of T--B cell repertoire selection and suggest that the Ly-1$^+$ B cell lineage operates in the early phases of this process. Thus, the T_H idiotype is absent in BALB/c mice which are either suppressed for the first 4 weeks of life with anti-antibodies, or reconstituted with autologous bone marrow after lethal irradiation as adults, treatments which deplete Ly-1$^+$ B cells. Furthermore, supplementation of bone marrow reconstitution with syngeneic Thy-1$^-$, Ly-1$^+$ peritoneal B cells, selects T_H cell repertoires that are undistinguishable from normal mice as to expression of the F6(51) clonotype. Interestingly, large, in vivo "naturally" activated Ly-1$^-$ splenic B cells can also reconstitute T_H idiotype expression if they are isolated from normal, but not from athymic, nude donors. However, transfer of normal large splenic T cells to adult nude mice "educates" the splenic large B cell compartment in these animals such that they acquire the ability to recursively select, upon transfer to bone marrow reconstituted recipients, the T_H clonotype. Small resting lymphocytes in either the B or T cell lineage fail to exert these selective effects. These observations constitute formal evidence for recursive repertoire selection in the naturally activated lymphocyte compartment of normal animals.

G 233 THYMUS-DEPENDENT IN VIVO REGULATION OF IgE SYNTHESIS IN AN IgE-SECRETING MURINE HYBRIDOMA, A. Mathur, B.G. Van Ness & R.G. Lynch, Univ. of Iowa, Iowa City, IA 52242. In previous studies we demonstrated that BALB/c mice bearing ascitic tumors of the IgE-secreting hybridoma B53 (ε, k, anti-DNP) developed large numbers of Lyt 1^{+} 2^{+} FcεR(+) T lymphocytes in response to elevated serum IgE concentration, followed by a progressive decrease in the levels of serum IgE in spite of continued proliferation of the hybridoma cells. This sequence of events suggested that the IgE-secreting hybridoma triggered a suppressive immunoregulatory circuit of the host that inhibited IgE expression by the hybridoma cells. The present studies were undertaken to investigate the basis for the decline in serum IgE levels in mice with B53 tumors and to identify host factors that might be involved. We observed that ascitic B53 cells recovered at increasing time points from BALB/c mice exhibited a selective decline in steady state levels and rates of synthesis of ε heavy chain protein and mRNA. The expression of k light chain protein and mRNA appeared relatively unchanged. Decrease in ε heavy chain gene expression did not occur when B53 tumors were passaged in nu+/nu+ mice reconstituted with neonatal BALB/c thymus and in BALB/c mice depleted of L3T4+ cells (helper/inducer cell lineage). That FcεR(+) T lymphocytes were directly involved in the inhibition of IgE expression was supported by the earlier and more pronounced inhibition of B53 IgE in mice infused with FcεR(+) T lymphocytes. We conclude from these findings that: a) the decline in serum IgE levels that occurs towards the end of each generation of in vivo passage of the B53 hybridoma is due to decreased production of IgE by the hybridoma cells, b) the decreased production of IgE is due to 1)a selective loss of ε mRNA expression, and 2) the is dependent on the presence of Lyt 2+ cells, and c) FcεR(+) T lymphocytes participate in the mechanism by which IgE production is suppressed.

G 234 IL5 AND IL2 ARE REQUIRED FOR THE MAXIMAL INDUCTION OF J CHAIN-mRNA. Kiyoshi Matsui, Kenji Nakanishi, Tomohiro Yoshimoto, Keisai Hiroishi, Toshikazu Hada and Kazuya Higashino, 3rd Dept. of Internal Med. Hyogo College of Medicine, Nishinomiya, Hyogo 663 Japan
We have established BCL_1-CL-3 (CL-3)cells which can respond to IL5 and IL2. There seem to be two distinct differentiation pathways for CL-3 cells; IL2-independent, IL5-driven differentiation pathway without preceding upregulated IL2R expression and IL5 plus IL2-dependent augmented differentiation pathway with preceding upregulated IL2R expression. Here we studied how IL5 and IL2 could work in a synergistic manner for the development of CL-3 cells into ISC (IgM synthesizing cells). We measured the expression of mRNA for the secretory form IgM and J chain protein after stimulation with IL5 and/or IL2. Striking increase of μs-mRNA expression was obtained by both IL5 and IL5 plus IL2 stimulations. About the induction of J chain-mRNA expression, modest increase of this message was obtained by IL5 stimulation, and the maximal induction of J chain-mRNA expression required IL5 plus IL2 stimulation. Moreover this induction of J chain-mRNA expression clearly depended upon the concentration of IL2, although IL2 alone could not induce either μs-mRNA nor J chain-mRNA. Thus, maximal induction of μs-mRNA expression associated with modest increase of J chain-mRNA expression can explain partial induction of IgM synthesis by IL5 and maximal induction of both μs-mRNA and J chain-mRNA expression can explain maximal induction of ISC by IL5 plus IL2 stimulation.

G 235 ALTERATION IN THE EXPRESSED S. TYPHIMURIUM-SPECIFIC B-CELL REPERTOIRE AFTER STIMULATION WITH BACTERIAL STRAINS THAT DIFFER IN ONLY A SINGLE LPS EPITOPE, Eleanor S. Metcalf and Christy A. Ronald, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814.
Recently our laboratory has been studying the variables which regulate the expression of the adult murine B-cell repertoire. To this end, we have established the requisites for stimulation of B-cell responses to Salmonella typhimurium, an organism which cross-reacts with the normal environmental flora of the mouse. In those studies, an acetone killed and dried preparation of S. typhimurium strain TML (AKD-TML) was employed to stimulate salmonella-specific B cells, utilizing a modification of the B-cell limiting dilution splenic fragment assay. In the present study, the role of antigen form in the regulation of salmonella-specific B-cell repertoire expression has been evaluated using this system. The characteristics of secondary B-cell responses to AKD-TML have been compared with an AKD preparation of S. typhimurium strain SL1898, (AKD-SL1898) an antigen which differs only in the expression of a single O-antigen epitope on the LPS molecule of the bacterial cell membrane. The results show that the frequency of B cells stimulated with each of these antigens is significantly different, 1.29 vs. 0.77, respectively. Moreover, fine specificity analysis indicates that 65% of the AKD-TML B-cell clones were directed against the core polysaccharide or lipid A region of the LPS molecule, while greater than 90% of the SL1898-specific clones were directed against the O antigen of the LPS molecule. Furthermore, O-region antigen-specificity also differs after stimulation with these two antigens. Cross stimulation studies are currently underway to further characterize the responses to these antigens. These studies should help to elucidate some of the variables involved in the capacity to stimulate B-cell subsets which comprise a given antigenic B-cell repertoire, and also provide some understanding of the antigenic factors necessary to produce effective, protective vaccines. Supported by NIH grant AI-22436.

G 236 HIGH RATE IgM SECRETION BY LY-1 POSITIVE MURINE B LYMPHOCYTES, D. Robertson, R. Gulizia, and D.E. Mosier, Medical Biology Institute, 11077 North Torrey Pines Road, La Jolla, CA 92037.

Splenic or peritoneal B lymphocyte populations enriched for the Ly-1-positive subset have been adoptively transferred to irradiated syngeneic or scid (severe combined immunodeficient) recipients. The number of B cells, their phenotype, and the serum immunoglobulin profile was determined in such recipients. Three pertinent observations were made. First, large amounts of serum immunoglobulin M were detected despite the recovery of small numbers of B cells. Second, the B cells reconstituted recipients contained both Ly-1 negative and Ly-1 positive B cells as well as substantial numbers of plasma cells. Thirdly, we did not detect any overrepresentation of autoantibodies against DNA, thymocytes, IgG1 (rheumatoid factors), or bromelain-treated mouse RBC in the serum of such recipients. These results suggest that Ly-1-positive B cells either directly or indirectly contribute to high rate IgM synthesis, but that there is no necessary correlation between their numbers and auotantibody production.

G 237 PLURIPOTENT HEMATOPOIETIC STEM CELLS AND RESTRICTED PROGENITORS IN MOUSE BONE MARROW, C.E. Müller-Sieburg+, G.F. Tidmarsh#, I.L. Weissman#, G. Spangrude#, C.A. Whitlock#, and D. Rennick*, +Lilly Research Laboratories, La Jolla, CA 92037, #Dept. of Pathology, Stanford University School of Medicine, Stanford, CA 94305, and *DNAX Research Institute, Palo Alto, CA.

Due to heterogeneity of the hematopoietic organs, the early events of lympho-hematopoietic differentiation and their regulation have been difficult to study. We have succeeded in isolating and characterizing several novel hematopoietic progenitor stages. Pluripotent stem cells are highly enriched in a small population of bone marrow (0.2%) expressing low levels of the cell surface antigen Thy-1 but not markers associated with mature lymphocytes or myeloid cells. Sorted Thy-1^{lo} stem cells injected in low doses completely repopulate lethally irradiated mice and give rise to all hematopoietic cell lineages. Furthermore, our data indicate the existence of a myeloid-B lineage restricted progenitor. This bone marrow population lacks stem cells or T cell precursors, but gives rise both in vivo and in vitro to mature B cells, macrophages and granulocytes. A third population co-expressed the Thy-1 and B220 antigens and contains both B and T cell precursors, but not stem cells or myeloid-erythroid precursors and thus is a good candidate for a lymphoid restricted progenitor.

G 238 AN EBV-TRANSFORMED HUMAN LYMPHOID PROGENITOR-LIKE FETAL LIVER CELL LINE DIFFERENTIATES INTO $CD2^+$ SUBLINE WITH TCRγ CHAIN REARRANGEMENT.

Atsushi Muraguchi, Yasuhiro Horii, Sachiko Suematsu, and Tadamitsu Kishimoto, Osaka University, Osaka, Japan 565.

Characterization of human lymphoid progenitors as well as very early stage of lymphocyte differentiation pathway are poorly understood. In the present study, we have established lymphoid progenitor-like cell lines from fetal liver cells by Epstein Barr virus infection. These cell lines expressed antigenic markers characteristic of B lymphoid cells and retained germline Ig H gene. In these B progenitor-like cell lines, alternation of chromatin struture as well as appearance of sterile transcripts of Cμ gene was found, indicating that Cμ gene segment become active before D-J_H Ig gene rearrangement. In addition, CD2 molecule, the earliest surface antigen of T lineage cells, was induced in one of the cloned B-progenitor-like cell line, FL8.2.6, during propagation in culture. CD2 molecule on $CD2^+$ FL8.2.6 cells was functional since $(Ca)^{2+}$-inflex and growth promotion could be induced with anti-CD2 MoAb stimulation. In these $CD2^+$ FL8.2.6 cells, TCRγ gene rearrangement occured by the further cultivation, suggesting that this cloned cell line are capable of producing cells committed to early T or NK cell lineage. These cell lines provide a model system for understanding the regulation and commitment of lymphoid progenitors to matured lymphocytes in humans.

G 239 IL5 SELECTIVELY INDUCES HIGH AFFINITY IL2R ON A CLONED B LYMPHOMA LINE. Kenji Nakanishi, Kiyoshi Matsui, Tomohiro Yoshimoto, Keisai Hiroishi, Toshikazu Hada and Kazuya Higashino, 3rd Dept. of Internal Med. Hyogo College of Medicine, Nishinomiya, Hyogo 663 Japan

We have established BCL_1-CL-3 (CL-3) cells capable of responding to IL5 and IL2 by up-regulated IL2 receptor (IL2R) expression and IgM synthesis. CL-3 cell has high affinity and low affinity IL2R, but IL2 by itself cannot stimulate either proliferation or Ig secretion. However CL-3 cells stimulated with IL5 become competent to respond to IL2 stimulation by up-regulated IL2R expression followed by striking IgM synthesis. Here we show that IL5 stimulation has the capacity to increase the number of high affinity IL2R (3-fold) without increasing the total IL2R number and IL2R-mRNA expression and that IL2 acts on IL5 stimulated CL-3 cells to preferentially induce low affinity IL2R (9.5-fold). Kinetic study has revealed that high affinity IL2R expression begins to increase at 12h after stimulation with IL5 or IL5 plus IL2, whereas low affinity IL2R expression increases at 18h and becomes maximal at 24h after stimulation with IL5 and IL2. These results implicate that high level expression of high affinity IL2R is required for CL-3 cells effectively respond to IL2 stimulation. Actually, CL-3 cells stimulated with IL5 for 12h, having high level expression of high affinity IL2R and capable of internalizing bound IL2, can respond strikingly to IL2 by up-regulated IL2R expression and IgM synthesis.

G 240 B CELL REGULATION ON T AND B CELLS BY B CELL DERIVED BSF-2/IL-6 AND IL-1α, N. Nisimoto, K. Yoshizaki, L.T. Tseng, T. Kuritani, C. Kiyotaki, Y. Takahara, T. Hirano, and T. Kishimoto, DEPT. MEDICINE, OSAKA UNIV. MED. SCH., OSAKA, JAPAN.

The effect of B cell derived immunoregulatory molecules was studied against B and T cell stimulation. Partialy purified culture supernatant from B cell line (B-BCDF fraction) showed B cell differentiation activity as shown in our previous paper. In this study the activity for IL-2 receptor and IL-2 induction on T cell line was observed in B-BCDF fraction. It was proved BCDF activity was carried out by BSF-2 molecule secreted from B cell line, that is, cDNA for BSF-2 could hybridize with mRNA from BCDF producing B cell line, and polyclonal anti BSF-2 antibody neutralized the BCDF activity in B-BCDF fraction. Recombinant BSF-2 against T cells did not induce the IL-2 production from murine T cell line, LBRM-33-IA5 cells, however slight induction of IL-2 receptor on T cells was observed by rBSF-2 solution. rBSF-2 showed the augmenting effect on the induction of the IL-2 receptor expression with IL-2 molecule which was also detected in B-BCDF fraction. IL-1α production from B cell line was testified by the transcription of mRNA for IL-1α, and the neutralization of IL-2 inducing activity in B-BCDF fraction by anti IL-1α antibody. These results suggested that the immunoregulatory molecules, BSF-2 and IL-1α were produced from B cells, indicating that B cells may regulate the T-B and B-B interaction through BSF-2 and IL-1 molecules on the mechanism of antibody production.

G 241 THE RESPONSE OF BCL_1 CELLS TO A VARIETY OF LYMPHOKINES. A. O'Garra, D. Barbis, J. Wideman, M. Bond, F. Lee and M. Howard. Proliferation *in vitro* of the *in vivo* passaged murine B cell tumor line BCL_1 has been used as a standard assay for interleukin-5 (IL-5) [BCGf II] for a number of years. We demonstrate that this line will also respond to human IL-5. The response to murine IL-5 is abrogated by both gamma-interferon (IFN) and transforming growth factor-β (TGF-β), suggesting a regulatory role for these lymphokines in the proliferation of B cells induced by IL-5.

The lymphokines IL-1, IL-2, IL-3 and IL-6 had no effect on the growth of BCL_1. Both IL-4 and GM-CSF, however, stimulate growth of this cell. These effects could be inhibited by specific antibodies directed against the respective lymphokines. This raises the possibility that GM-CSF may be another factor which could be involved in B cell replication although it still remains possible that this is merely a property of a B cell tumor.

G 242 DEFINITION OF A LATE-STAGE B CELL DIFFERENTIATION ANTIGEN BY A NEW RAT MONOCLONAL ANTIBODY, Lynn Ogata, Margaret Gulley, John Thorson, Paul Naumann, John Kemp, University of Iowa College of Medicine, Iowa City, IA 52242.

We have developed a rat monoclonal antibody (S7) which will probably be a useful reagent in studies of the terminal phases of mouse B cell differentiation. S7 was produced by immunizing a (DA x Lou) F1 rat with the MOPC-315 plasmacytoma and fusing with Y3. FACS analysis shows that splenic B cells are negative or dull for the S7 antigen but antigen expression begins to increase rapidly after 48 hours of culture with LPS. After 5 days in culture, cells with plasma cell morphology are contained within the S7 bright fraction and can be readily sorted by FACS. S7 also reacts with all peripheral T cells, thymocytes, and all granulocytic cells in the bone marrow. By Western blot analysis, S7 recognizes a broad 85-95 Kd band. This reactivity pattern resembles that of the mouse anti-rat monoclonal antibody W3/13.

Although other antibodies with anti-plasma cell activity have been described, none seem to have been employed in FACS analysis and sorting so far. Since S7 stains late-stage maturing B cells and plasma cells brightly, it appears ideal for this purpose. The availability of a reagent like S7 may allow the identification of the stage at which a B cell commits to terminal differentiation and can no longer function as a memory cell.

Supported by NIH Grant CA37252.

G 243 INDUCTION AND THE FUNCTIONAL ROLE OF A NOVEL (p70/75) INTERLEUKIN-2 BINDING MOLECULE IN HUMAN B-CELLS, Osamu Saiki, Toshio Tanaka, Satoru Doi, and Susumu Kishimoto, Osaka University, Osaka, 553, Japan.

Induction of p70/75 interleukin-2(IL-2) binding molecules and their functional roles in immunoglobulin (Ig) secretion by IL-2 were examined in human B-cells. IL-2 at high concentrations, induced high levels of Ig secretion in SAC activated B-cells than at low concentrations. About 50% of SAC activated B-cells, lacking Tac antigen, were also responsive to IL-2, although the required dose of IL-2 was higher than that for Tac positive B-cells. Anti-Tac antibody did not inhibit the induction of Ig secretion by high concentrations of IL-2 in both Tac negative and positive B-cells, suggesting that IL-2 might induce Ig secretion through a receptor distinct from that defined by the anti-Tac monoclonal antibody. In contrast, IL-2 was ineffective in the absence of SAC stimulation even high concentrations.

Upon analysis of SDS-PAGE, p70/75 IL-2 binding molecules were detected on Tac-negative SAC activated B-cells. Similar IL-2 binding molecules distinct of Tac antigen(p55) were detected in both Tac positive B and T-cells. However, neither p55 nor p70/75 IL-2 binding molecules could be detected in the absence of SAC stimulation.

These observations suggest that p70/75 IL-2 binding molecules are induced in human B-cells in the presence or absence of Tac-antigen by SAC stimulation and the these determinants play an important function in the transduction of IL-2 associated signal for B-cell differentiation.

G 244 MONOCLONAL ANTIBODIES TO B CELL DIFFERENTIATION ANTIGENS, R. Michael E. Parkhouse, A. Rodriquez-Acosta, M.A. Campbell, A. Gonzales-Martinez, J. Delgado, G.C. Preece and L.J. Murray. Division of Immunology, National Institute for Medical Research, Mill Hill, London NW7 1AA.

Monoclonal antibodies have been raised against resting, activated and neoplastic murine B cells in an attempt to identify molecules which may function as receptors for B cell growth and differentiation factors. A number of monoclonal antibodies show differential reactivity with B cells at various stages of differentiation, such as resting versus activated. Of particular interest are two antibodies recognising molecules selectively expressed on the surface of LPS-activated B cells. These antibodies have been screened on lymphoid tissue sections and B cell cDNA libraries and in bio-assays for IL-4 and IL-5. Their variable surface representation during B-cell activation was also studied using the FACS.

G 245 CHARACTERIZATION OF LY-1$^+$ B LYMPHOCYTES IN THE PROXIMAL COLON, Greg A. Perry, Dept. of Anatomy, University of Nebraska Medical Center, Omaha NE 68105.
Recently we have described a lymphoid nodule in the proximal colon of rats and mice (Anat. Rec, in press). This proximal colonic lymphoid tissue is found 25% of the distance from the cecum to the rectum. It is composed almost entirely of 14.8$^+$ IgM$^+$ B cells, 10-20% of which are Ly-1$^+$ in normal Balb/c mice as determined by two color FACS analysis. This tissue is distinct from other secondary lymphoid tissues in its apparent lack of germinal centers and in that lymphocytes derived from this nodule are unresponsive to the mitogen LPS. In addition, this tissue is uniquely steroid sensitive among B cell populations, demomstrating an 80% reduction in tissue weight after hydrocortisone treatment, as compared to 5% for Peyer's patch. Finally, when fetal colon was grafted under the kidney capsules of adult syngeneic recipients, proximal colonic lymphoid tissue developed in the absence of luminal antigenic stimulation. On the basis of these experiments we speculate that proximal colonic lymphoid tissue may be a potential non-bone marrow source of B cells, including cells of the Ly-1$^+$ B cell subset in mammals. (Supported by NIH grant AI 25222).

G 246 SIGNAL TRANSDUCTION THROUGH THE EGF RECEPTOR TRANSFECTED INTO INTERLEUKIN-3-DEPENDENT HEMATOPOIETIC CELLS, Jacalyn H. Pierce, Marco Ruggiero, Timothy P. Fleming, Pier Paolo Di Fiore, Joel S. Greenberger[1], Joseph Schlessinger[2], Giovanni Rovera[3], and Stuart A. Aaronson, National Cancer Institute, Bethesda, MD 20892, [1]Univ. of Massachusetts, N. Worcester, MA, [2]Weizmann Institute of Science, Rehovot, Israel, [3]Wistar Institute, Philadelphia, PA.
An expression vector for the epidermal growth factor (EGF) receptor was introduced into the 32D myeloid cell line, which is devoid of EGF receptors and absolutely dependent on interleukin-3 (IL-3) for its proliferation and survival. Expression of the EGF receptor conferred the ability to utilize EGF for transduction of a mitogenic signal. When the transfected cells were propagated in EGF, they exhibited a more mature myeloid phenotype than was observed under conditions of IL-3 directed growth. Moreover, exposure to EGF led to a rapid stimulation of phosphoinositide metabolism as determined by the formation of phosphatidic acid and inositol phosphates. In contrast, IL-3 had no detectable effect on phosphoinositide turnover either in control or EGF receptor transfected 32D cells. Although the transfected cells exhibited high levels of functional EGF receptors, they remained nontumorigenic. In contrast, transfection of v-erbB, an amino-terminal truncated form of the EGF receptor with constitutive tyrosine kinase activity, not only abrogated the IL-3 growth factor requirement of 32D cells, but caused them to become tumorigenic in nude mice. These results demonstrate that a naive hematopoietic cell expresses all of the intracellular components of the EGF signaling pathway necessary to evoke a mitogenic response and sustain continuous proliferation.

G 247 CLONAL ANALYSIS OF THE REGULATORY ROLE OF LYMPHOKINES IN THE B CELL ACTIVATION PATHWAY, Beverley L. Pike, Mark R. Alderson, Michael S. Loughnan and G.J.V. Nossal, The Walter and Eliza Hall Institute of Medical Research, Melbourne, Victoria 3050, Australia.
The regulatory role of a variety of lymphokines and antigen in the B cell activation pathway has been assessed in assay systems in which a single, isolated, hapten specific B cell is the unequivocal target of action. We have arbitrarily divided into four overlapping stages for the purpose of these studies, namely, early activation (blastogenesis), cell division (proliferation) leading to clone formation, IgM secretion, and isotype switching. The bioactivity(s) of recombinant IL4, IL5, IL2, IL-6, IL1 and gamma IFN, when acting in vitro, either alone, or with an authentic "T independent" antigen, on murine B cells will be discussed. Data will be presented which clearly shows that a single factor is able to exert an influence at more that one stage of the B cell activation pathway, rather than being exclusively promoting activation, growth or antibody secretion. For example, IL4 is able to promote early activation and proliferation, but not IgM secretion. It also acts as an IgG1 switch factor. IL5 acts with antigen to promote growth and IgM secretion. IL-5 also induces IL2 receptors on B cells. IL6 acts late to enhance Ig secretion. The response of hapten specific B cells from tolerant donors will also be discussed.

G 248 INTERLEUKIN 5:AN IG A ENHANCING FACTOR, Simone Schoenbeck, Douglas T. Mc Kenzie*, Martin F. Kagnoff, Laboratory of Mucosal Immunology,Department of Medicine and *Department of Biology, University of California, San Diego, La Jolla, Ca 92093

Recent evidence indicates,that Interleukin 5 (IL5) selectively enhances IgA secretion in LPS stimulated murine B cells.This response is augmented by addition of Interleukin 4 (IL 4).In this study we have addressed the question of whether IL5 induces isotype switching from IgM to IgA or whether it acts on B cells already committed to IgA production. Surface IgM+(sIgM+) or surface IgA + (sIgA+) B cells from murine spleen or Peyer's Patch were purified by fluorescence cell sorting and incubated for 24 h with LPS.Then, IL 5, IL 4 or a combination of both were added and immunoglobulin secretion measured by ELISA after 1 week.In pure sIgM+ B cells IL 5 + IL 4 had only a minor effect on IgA secretion (LPS alone: 23.3 ng/ml, IL 5: 58.1 ng/ml) as compared to unsorted B cells (LPS alone: 77.2 ng/ml, IL 5: 734.4 ng/ml) and in limiting dilution assays there was no detectable IgA secretion in single sIgM+ cells.'IL 4 alone had no influence on IgA secretion. These results suggest that IL 5 acts primarily as an enhancing factor in B cells that already are committed to IgA production.Type 2 helper T cells which produce both IL 4 and IL 5 appear to play an important role in regulating IgA secretion.

1) Murray,P.D., D.T. McKenzie, S.L. Swain, M.F. Kagnoff. Interleukin 5 and Interleukin 4 produced by Peyer's Patch T cells selectively enhance immunoglobulin A expression. J. Immunology, 139, 2669/2674, 1987

G 249 CLONAL ANALYSIS OF B CELL RESPONSES TO SYNTHETIC PEPTIDE ANTIGENS OF PLASMODIUM FALCIPARUM

Alison J Venn, Beverley L Pike, Robin F Anders and Ken Shortman. The Walter and Eliza Hall Institute, Melbourne, 3050, Australia.

A series of peptides have been synthesized corresponding to the 3' and the 5' repeat sequences of the malarial RESA antigen. These have been shown to cross-react extensively with each other and with other defined malarial antigens. It has been suggested that these provide an immunological "smokescreen", by causing continued stimulation of a range of somatically generated variant antibody forming cells (AFC) producing non-protective antibody. We are using limit-dilution cultures of murine B cells to analyse responses to these antigens. A microculture system has been established using LPS as a stimulus and 3T3 fibroblasts as fillers, the supernatants being assayed for anti-RESA antibodies using an ELISA assay. A lov but definite frequency of anti-RESA-specific B cell AFC-precursors has been demonstrated. The characteristics of RESA-specific B cells and changes in their frequency after responses to malarial antigens is under investigation. A plaque assay for anti-RESA peptides is being developed, in order to screen clones developing form virgin and memory B cells for specificity variants, using panels of these closely related peptides.

G 250 B CELL RECONSTITUTION FOLLOWING HUMAN BONE MARROW TRANSPLANTATION.

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Following human bone marrow transplantation recovery of phenotypically identifiable circulating B lymphocytes occurs within the first 2 to 4 weeks and includes two major subpopulations: CD5 (Leu1 or T1) positive and CD5 negative B cells. Recovery of CD5+ B cells generally precedes that of CD5- cells and may represent up to 50% of circulating lymphocytes. The CD5+ B cells are of donor origin, are polyclonal, express approximately equivalent levels of surface IgM and IgD, and do not appear to express IL2 receptors. Recovery of these cells post transplant shows a strong negative correlation with graft versus host disease (both acute and chronic) and a positive correlation with serum Ig levels. They appear in the blood of patients undergoing both MHC matched and mismatched allogeneic transplants as well as in patients receiving syngeneic transplants. The time course of recovery is similar in patients who received donor marrow depleted of T cells with anti-Leu1 MoAb plus complement as it is in patients receiving no donor marrow T depletion or T depletion using MoAb directed against other T-cell associated surface antigens. In terms of proliferative response and in vitro Ig secretion in response to polyclonal activators, the CD5+ B cells are approximately equivalent to CD5- B cells.

G 251 MOLECULAR EVOLUTION OF THE ANTI-PC-KLH ANTIBODY RESPONSE ORIGINATES FROM DIVERSE ANTIGEN BINDING AND V GENE USAGE, Mary P. Stenzel-Poore and Marvin B. Rittenberg. Oregon Health Sciences Univ., Portland, Oregon 97201.

We have found that although the response to PC-KLH begins with a highly restricted binding phenotype, immune maturation results in two distinct populations that differ markedly in epitope recognition, idiotype and isotype composition and V region expression. In primary responses to PC-KLH the predominant antibodies (Group I) use VH1, are T15 id^+, express IgM, IgG and IgA, and bind the haptens PC and nitrophenyl PC (NPPC). The memory response exhibits, in addition to Group I antibodies, new antibodies (Group II) that bind NPPC but not PC dominate IgG1/G2, and express V genes not seen in the primary response as well as V elements used by Group I antibodies (VH1, Vk8 and Vk24). V gene analyses of Group II hybridomas reveal that Q52, S107 and J558 VH families contribute substantially to the Group II response and L chain usage derives predominantly from Vk1-3 and Vk24. Thus, Group II differs in epitope recognition and exhibits more V gene diversity than Group I.

We are analyzing the evolution of epitope recognition in serum anti-PC-KLH antibodies to determine the basis for antibody selection that allows Group II antibodies to develop in the face of overwhelming Group I dominance (99% of the primary response). We find that although Group II antibodies appear much later (day 14) they bind PC-protein 1000-fold better than do Group I antibodies. Interestingly, the Group II response does not appear to gain improved binding to PC-protein as the response matures. In contrast, Group I antibodies change markedly with time and evolve to bind PC-protein nearly as well as Group II antibodies. The superior intrinsic ability of Group II antibodies to bind PC-protein early provides a reasonable explanation for their selection from a minor population to represent >50% in the memory response . Thus although Group I antibodies dominate early they appear unable to compete as well for antigen until late in the response thereby allowing Group II antibodies to gain a foothold in the anti-PC-KLH response. Supported by NIH Grant AI 14985.

G 252 A HUMAN B CELL LINE THAT PROLIFERATES IN RESPONSE TO RECOMBINANT(R.) HUMAN IL-4, W. Tadmori, Xing Xia, Y.S. Choi, Ochsner Medical Foundation, New Orleans, LA 70121.

Unlike murine IL4, human IL4 show a weak or no B cell growth(BCGF) activity in the standard anti-IgM antibody costimulatory assay. Recently it has been found that the responsivness of activated B cells to IL4 is dependent on the nature of the preactivation signals. Epstine Barr Virus (EBV) transformed B cell lines represent an activation state of B cells. Recently we have selected an EBV transformed line (A_4) and developed a highly sensitive assay for BCGF activity (manuscript in preparation). Here we utilized this system to investigate the BCGF activity of R. human IL4. Our data show that R. human IL4, (but not IL1 or IL2), induces DNA synthesis of this cell line in a dose dependent fashion. The increase in [^{3}H] TdR uptake of A_4 cells in the presence of IL4 reflected cell division since cell counts in culture containing IL4 was markedly higher than cells cultured in the absence of IL4. The proliferative response to IL4 appears to be independent of serum and B cell growth factor(s) that mediate the autocrine growth of these cells. At a low level of autocrine growth of A_4, IL-4 induces synergistic proliferative response. However, as autocrine growth level reaches maximal level, responsivness to IL4 become minimal. These data suggest that IL4 could provide an alternative B cell growth factor for activated B cells. Currently the A_4 cells are being used to investigate the mechanism of action of IL4 as a BCGF and as target cells in a sensitive assay for IL4.

G 253 EXPRESSION OF NOVEL INTERLEUKIN 2 BINDING MOLECULES (P70/75) IN B CELL DIFFERENTIATION, Toshio Tanaka, O. Saiki, S. Doi, M. Suemura, S. Negoro and S. Kishimoto, The Third Department of Internal Medicine, Osaka University, Osaka, 553, Japan.

Expression and functional roles in the differentiation of B cells into immunoglobulin (Ig) secreting cells of novel interleukin 2 (IL-2) binding molecules (P70/75) were explored by using several human B cell lines and tonsillar B cells. Affinity-crosslinking studies revealed that five of nine B cell lines expressed P70/75 without Tac antigen (P55) expression and the expression was associated with B cell maturation. In tonsillar B cells, small high-density B cells did not express P70/75, whereas large low-density B cells, which were thought to be activated "in vivo", expressed them. Binding assays of radiolabeled IL-2 showed that the affinity of P70/75 was intermediate (Kd=I-3nM, 700-3,000sites/cell). Furthermore, high concentrations of IL-2 (above 100u/ml) induced Ig productions in large B cells and two of five cell lines, all of which were stimulated to produce more Ig by TPA or B cell stimulating factor 2 (kindly provided by T. Kishimoto and T. Hirano). In these five cell lines, IL-2 was rapidly internalized via P70/75 irrespective of responsiveness or unresponsiveness to IL-2. These results taken together suggest that B cells may express novel IL-2 binding molecules, associated with B cell maturation and differentiate into Ig secreting cells by IL-2 through novel IL-2 binding molecules without Tac antigen and we discuss about the mechanism of signal transduction by IL-2 through P70/75.

G 254 ISOLATION AND STRUCTURE OF A cDNA ENCODING THE B1 (CD20) CELL-SURFACE ANTIGEN OF HUMAN B LYMPHOCYTES, Thomas F. Tedder, Michel Streuli, Stuart F. Schlossman, and Haruo Saito, Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA 02115.
B1 is a Mr 33,000 phosphoprotein on the surface of human B lymphocytes that may regulate B cell proliferation and differentiation. In this report, a cDNA clone that encodes the B1 molecule was isolated and the amino acid sequence of B1 was deduced. B cell-specific cDNA clones were selected from a human tonsil cDNA library using differential hybridization with labeled cDNA derived from either size-fractionated B cell mRNA or T cell mRNA. Of the 261 cDNA clones isolated, three cross-hybridizing cDNA clones were chosen as potential candidates for encoding B1 based on their selective hybridization to RNA from B1+ cell lines. The longest clone, pB1-21, contained a 2.8 kb insert with an 891 base pair open reading frame that encodes a protein of 33 kDa. mRNA synthesized from the pB1-21 cDNA clone *in vitro* was translated into a protein of the same Mr as B1. Limited proteinase digestion of the pB1-21 translation product and B1 generated peptides of the same sizes demonstrating that the pB1-21 cDNA encodes the B1 molecule. Northern blot analysis indicated that pB1-21 hybridized with two mRNA species of 2.8 and 3.4 kb in only B1+ cell lines. The protein sequence deduced from the pB1-21 nucleotide sequence lacked an apparent signal sequence and contained three extensive hydrophobic regions. The deduced B1 amino acid sequence shared no significant homology with other known proteins although the overall structure of B1 is similar to that of two other phosphoproteins, rhodopsin and the B-adrenergic receptor. The structural and functional properties of B1 suggests that it may be a signal transducing protein central to the normal function of B lymphocytes.

G 255 MEMORY DEVELOPMENT TO A T-CELL DEPENDENT FORM OF α(1,3) DEXTRAN IS ACCOMPANIED BY THE APPEARANCE OF NEW λ-CLASS ANTIBODIES THAT DIFFER IN IDIOTYPE AND IN FINE SPECIFICITY. Thomas V. Tittle, McKay Brown and Marvin B. Rittenberg. Oregon Health Sciences University, Portland, Oregon 97201.
BALB/c mice were primed with nigerosyl-(N)-protein conjugates to induce λ-class memory to the α(1,3) diglucosyl epitope. Mice were boosted with either N-protein or with B1355 dextran; spleen cells of individual mice were fused. All cloned hybridomas described bind both B1355 and N-BSA. Only 2 of 12 hybridomas studied were positive for expression of the cross-reactive idiotype (IdX) of J558/M104E. N-protein priming led to a shift in idiotype expression in the memory B cell pool. Two binding phenotypes could be discerned based on fine specificity analyses. Phenotype 1 contains antibodies which have an I_{50} ratio for nigerose inhibition on B1355 relative to N-BSA of one or greater whereas in phenotype 2 the ratio is less than one. Phenotype 2 antibodies can be induced by either B1355 or N-KLH in the memory response. All hybridomas had binding site sizes larger than a disaccharide. VH gene analysis of 8 hybridomas and M104E showed that 7/8 shared the 4.4 kbp band which is the productive rearrangement of M104E. One phenotype 2 hybridoma did not share this band nor could its fragment size be accounted for by the use of a more 3' JH segment. All were positive by Northern analysis with a J558 coding region probe which identifies related members of the J558 gene family. Two phenotype 2 hybridomas, μλ-1 and γ_1λ-4, were produced in the same fusion and by Southern analysis share productive and non-productive JH rearrangements suggesting they may be clonally related. These two hybridomas have distinct fine specificity patterns suggestive of affinity maturation. Supported by ACS Grant IM172G.

G 256 LYMPHOKINE REGULATION OF IMMUNOGLOBULIN ISOTYPE PRODUCED BY B CELLS ISOLATED FROM MURINE SPLEENS AND PEYER'S PATCHES, Susan L. Tonkonogy, School of Veterinary Medicine, North Carolina State University, Raleigh, NC 27606.
The T cell-derived lymphokines interleukin-4 (IL4) and interleukin-5 (IL5) influence both the levels and the patterns of immunoglobulin isotype secreted by B cells isolated from the spleens and Peyer's patches of normal (C57Bl/6 x DBA/2)F1 mice. We observed marked synergy between IL4 and IL5 in stimulation of IgM, IgG3 and IgG1 production and additive effects for IgA production by both spleen and Peyer's patch B cells cultured in the absence of lipopolysaccharide (LPS). In the presence of LPS, however, IL4 drastically reduced the levels of IgM and IgG3, had little effect on IgA, and enhanced IgG1 produced by B cells from both lymphoid organs. IL5 can eliminate a portion of the IL4 mediated suppression of IgM but not of IgG3 production by LPS stimulated B cells. Synergistic effects of IL4 and IL5 were observed for IgA production by LPS stimulated Peyer's patch but not splenic B cells. While the patterns of isotype expression by spleen and Peyer's patch B cells appear very similar, Peyer's patch cells stimulated with lymphokines plus LPS secrete more IgA, less IgM, and similar amounts of IgG3 and IgG1 compared with spleen B cells.
These results indicate that IL4 and IL5 differentially regulate the quantities of immunoglobulin isotypes produced by B cells residing in systemic and mucosal lymphoid organs. Also, the effects of lymphokines on the isotypes produced by LPS stimulated B cells are markedly different from the effects of lymphokines on the isotypes produced by B cells which have not been stimulated with LPS.

G 257 INCREASED PRESENCE AND ALTERED ACTIVATION STATE OF LY-1 B CELLS FROM SM/J MICE, Raul M. Torres, L. David Engel, Peter S. Rabinovitch and Edward A. Clark, University of Washington, Seattle, WA 98195.
The $CD5^+$ (Ly-1/Leu-1) subpopulation of B lymphocytes is now known to play an important role in certain autoimmune diseases. This subpopulation is also the major B cell phenotype observed in fetal spleen, the initial B cell population arising in bone marrow transplant patients, and the primary chronic B cell in lymphocytic leukemias. The regulation and function of this subset, however, is not yet well understood. Utilizing murine splenic B cells from the SM/J mouse strain, we have sorted and analyzed both $Ly\text{-}1^+/IgM^+$ and $Ly\text{-}1^-/IgM^+$ subpopulations for cell cycle and proliferative response to anti-IgM and BCGF. We report here that $Ly\text{-}1^+$ B cells are enriched in the G_1 stage of the cell cycle, and are capable of directly responding to the progression signal delivered by BCGF. In contrast, sorted $Ly\text{-}1^-$ B cells cannot respond to BCGF alone, but rather require an initial signal administered by anti-IgM. Taken together these results suggest, in SM/J mice, Ly-1 B cells have an altered activation state enabling them to bypass initial competence signals. (This work supported in part by NIH training grant AG00057).

G 258 RESPONSIVENESS OF B CELLS FROM AUTOIMMUNE MICE TO INTERLEUKIN 5 (IL-5), Shelby P. Umland, Ning Fei Go, and Maureen Howard, DNAX Research Institute, Palo Alto, CA 94304.
Three murine strains (NZB/W F_1, BXSB and MRL/lpr) develop an autoimmune disease that is clinically and immunologically similar to human systemic lupus erythematosus (SLE). A characteristic of these mice is a polyclonal B cell hyperactivity. To explore whether this may be related to a hyper-responsiveness to B cell stimulatory factors, we have investigated the proliferative and secretory responses of B cells from these mice to semi-purified natural and recombinant IL-5. T-depleted spleen cells isolated from the interface of 50/60% Percoll density gradients, which contain those B cells activated in vivo, were used as target cells. This B cell population from NZB/W mice secreted IgM and incorporated ^3H-thymidine at significantly higher levels in response to IL-5 than did the comparable population from several normal murine strains. Small resting B cells from NZB/W and normal mice did not proliferate in the presence of IL-5. In contrast, no difference was observed in the magnitude of the proliferative or secretory responses between in vivo activated B cell populations from normal or autoimmune BXSB and MRL/lpr mice. Thus, this B cell hyper-responsiveness to IL-5 is not a feature common to these three models of autoimmunity.

G 259 **PARTICIPATION OF AUTO-ANTI-IDIOTYPE AND A MINOR IDIOTYPE IN THE SELECTIVE EXPANSION OF THE DOMINANT T15 IDIOTYPE,** *Meenal Vakil and John F. Kearney, University of Alabama at Birmingham, Birmingham, AL, 35294.*
Manipulation of newborn BALB/c mice wih small amounts of phosphorylcholine (PC) containing antigen as well as syngeneic monoclonal anti-PC (idiotypic) or anti-idiotypic antibodies leads to a modulation of the anti-PC repertoire which is reflected in the antibody response to the pneumococcal vaccine (R36a) as adults. R36a primes the total anti-PC response by preferentially expanding $T15^-$ B cells whereas, $T15^+$ antibody suppresses the expansion of $T15^+$ B cell clones. In contrast, $M167^+$ antibody enhances the total anti-PC response with the accompanying dominant expression of T15 idiotype. BD2, a neonatally derived monoclonal anti-T15 antibody expands both the $T15^+$ and $T15^-$ PC-specific B cells but the T15 idiotype dominates in the subsequent response to antigen. 4F-1, a monoclonal antibody directed toward the M167 idiotype has no modulatory effect on the T15 idiotype in adult mice, yet, in newborn mice, it abrogates the clonal expansion of $T15^+$ B cells. Since T15 and M167 are connected through BD2, it is inferred that suppression of T15 idiotype by 4F-1 is the result of functional inactivation of B cells expressing M167 idiotype during ontogeny. In summary, the striking dominance of T15 idiotype in the PC specific repertoire in BALB/c mice is not the result of antigen selection, rather, it reflects the selective expansion of $T15^+$ B cells by neonatal anti-idiotype and is aided by B cells expressing the minor idiotype. (Supported by NIH grants CA16673, CA13148, and AI14782.)

G 260 THE EFFECT OF ANTI-IGM AND LYMPHOKINES ON THE GROWTH OF BKS-2, A NEOPLASTIC ANALOGUE OF MATURE LY-1 B CELLS, V. Udhayakumar, and B. Subbarao, Sanders-Brown Center on Aging and Department of Microbiology and Immunology, University of Kentucky, Lexington, KY 40536.
BKS-2, a B lymphoma of CBA/ca origin, has been recently isolated in our laboratory and has been adapted to grow in CBA/N mice. This cell line expresses high level of membrane IgM (mIgM), low IgD, MHC class I, II as well as Ly-1 antigens and thereby making it analogous to mature Ly-1 B cells. Crosslinking of mIgM but not mIgD by anti-immunoglobulin antibodies leads to inhibition of growth in these cells. This inhibition does not seem to be mediated through Fc receptor, since F(ab)2 goat anti-mouse mu itself is able to mediate the same effect. Binding of antibodies to other surface molecules, such as MHC and Ly-1 molecules does not affect lymphoma growth. On the other hand, an antigen specific T cell supernatant fraction (TGG S), enriched for BSF-1 activity, enhances the growth of these cells. However, the TGG S is unable to rescue these cells from anti-mu mediated growth inhibition. Thus it appears that the negative signals generated by anti-mu antibody override the positive signals induced by the TGG S. Since these lymphoma cells express both IgM and IgD, it appears that the acquisition of mIgD, a characteristic of mature B cells, does not prevent B cells from receiving the tolerogenic signals generated by the crosslinking of mIgM. This lymphoma provides a unique system to study the biochemical basis of signalling mechanism(s) involved in the growth regulation of mature Ly-1 B cells at the clonal level. This work is supported in part by the NIH grants AI-21490 and AG-05731.

G 261 INTERLEUKIN 4 CAN MODULATE THE DEVELOPMENTAL EXPRESSION OF THE MURINE B CELL IgE Fc RECEPTOR, Thomas Waldschmidt, Daniel Conrad and Richard Lynch, The University of Iowa, Iowa City, IA 52242 and The Johns Hopkins University, Baltimore, MD 21205.
It has been recently established that all mature murine B cells display Fc receptors for IgE, and that the expression of this receptor can be significantly increased by IL-4. In order to gain more information regarding the possible function of the B cell IgE Fc receptor, we have examined the ontogenic appearance and lymphoid tissue distribution of this receptor with 2 color flow cytometry. In addition, a panel of B cell tumors representative of various stages of ontogeny and differentiation, were examined for the presence of the IgE Fc receptor. The results show that the B cell IgE Fc receptor is a very late appearing developmental marker, and is expressed predominantly on mature IgM, IgD bearing cells. The receptor was also shown to be absent on B cells which had switched to express another isotype. We further examined whether IL-4 can modulate the delayed expression of the IgE Fc receptor on maturing B cells. It was found that IL-4 can markedly accelerate the appearance of the IgE Fc receptor on immature B cells, indicating that the developmental expression of this receptor may not be inherently programmed, but may be influenced by IL-4 producing cells.

G 262 CD23 ($Fc_\varepsilon R_L$) EXPRESSION AND LMW-B CELL GROWTH FACTOR (BCGF) RESPONSIVENESS OF CLONAL POPULATIONS OF HUMAN B LYMPHOBLASTOID CELL LINES, Richard J. Warrington, University of Manitoba, Winnipeg, Manitoba, Canada, R3A 1M4.
Recent studies suggest a relationship between the low affinity IgE receptor ($Fc_\varepsilon R_L$) and the receptor for LMW-BCGF, since anti-CD23 antibodies can induce B cell proliferation and fragments of the CD23 antigen may possess autocrine growth factor activity. We have recently described an EBV transformed B lymphoblastoid cell line JR-2(82) that exhibits a preferential responsiveness to LMW-BCGF resulting in proliferation. JR-2(82) does not respond to IL-1, IL-2, γ-IFN (<1000 U/ml) or BSF-1 and proliferates poorly to HMW-BCGF (Namalwa). Clonal populations of JR-2 were generated and two clones, B10 and D3 were studied. D3 was generated and maintained on the LMW-BCGF preparations while B10 was not. Both clones responded with proliferation to LMW-BCGF but did not respond to IL-1, IL-2, or γ-IFN. But while D3 expressed high levels of the CD23 antigen by fluorescence activated cytography (99% positive), equivalent to the RPMI 8866 positive control, B10 was negative for CD23, using monoclonals to the CD23 antigen such as MHM6. B10 did not express CD23 antigen in the resting state or after incubation with recombinant BSF-1 or LMW-BCGF. However both D3 and B10 cells absorbed growth factor activity from LMW-BCGF preparations to an equivalent extent. In addition, both B10 and D3 cells release factor(s) possessing BCGF-like activity. This suggests that either two receptors for LMW-BCGF exist on B lymphoblastoid cells or the low affinity Fc_ε receptor is not the receptor for LMW-BCGF.

G 263 EFFECTS OF LEUKEMIC-DERIVED AND MESENCHYMAL GROWTH FACTORS ON STROMAL CELLS AND PRE-B CELL GROWTH IN LONG TERM CULTURE, Pamela Witte, Jerome Zack, William Simmons, R Graham Smith, and Brad Ozanne, Univ. Tex. Hlth. Sci. Cnt., Dallas, TX 75235. A human pre-B cell leukemia cell line SMS-SB secretes a novel transforming growth factor, TGF-LD. TGF-LD is active on a variety of rodent and human fibroblast lines. The activity is distinct from other known TGFs, interleukins, and mesenchymal growth factors. To ascertain if such factors affect cells of the lymphopoietic microenvironment, we studied the proliferation, CSA production, and lymphocyte support capacity of marrow stromal cells after treatment with TGF-LD and other growth factors. Stromal cells that maintain early B-lymphocyte production were isolated from Whitlock cultures by electronic cell sorting. TGF-LD, TNF, PDGF, and EGF stimulated proliferation of the isolated stromal cells. Negligible CSA was detected in the supernatants of unstimulated cells, as determined in a soft agar colony assay. CSA production, which appeared to be CSF1, was enhanced 25-100 fold within 24hr of treatment with TGF-LD, IL1, TNF or increased serum concentrations, and to a lesser extent with PDGF, EGF, and acidic FGF. Only TNF suppressed the maintenance of lymphocytes transferred to isolated stromal cells, while TGF-LD slightly augmented proliferation. However, in the presence of the heterogeneous, unsorted adherent cells from Whitlock cultures, TGF-LD suppressed the lymphocyte support function of these cells. These studies demonstrate that leukemic-derived factors can affect normal lymphopoiesis and myelopoiesis via stimulation or repression of the functions of microenvironmental cells. The outcome may involve a complex series of cellular interactions which ultimately alter a pivotal stromal cell type. By such means, leukemic cells may gain a growth advantage in the marrow-dependent stage of disease.

G 264 FUNCTIONAL ROLES OF GAMMA INTERFERON IN PROLIFERATION AND DIFFERENTIATION HUMAN B CELL SUBPOPULATIONS, Xing Xia and Y.S. Choi, Alton Ochsner Medical Foundation, New Orleans, LA. 70121

The multiple functional effects of interferon-gamma (IFN-r) on proliferation and differentiation of human B cell subpopulations were investigated. When B cell subpopulations were separated by Percoll gradient centrifugation and stimulated by Staphylococcus aureus Cowan I (SAC), these subpopulations responded differently to lymphokines. Small B cells (60/80% Percoll) were stimulated to proliferate by IFN-r alone. Large B cells (50/60% Percoll) did not respond to IFN-r but proliferated in response to B cell growth factor (BCGF) free of interleukin-2 (IL-2) and IFN-r.Although IFN-r alone could not induce the differentiation of SAC-activated B cells and did not support the growth of large B cells, it enhanced the proliferation and differentiation of both subpopulations in the presence of BCGF and IL-2. Pretreatment of B cells with IFN-r for 48 hr had a minor effect on the proliferation but significantly enhanced the differentiation in the presence of BCGF and IL-2. Therefore, IFN-r may act as a differentiation factor. However, in a later stage of culture, IFN-r inhibited B cell differentiation. Our experimental data suggest that IFN-r is a growth factor for a distinct subpopulation of SAC-activated human B cells and enhances the proliferation and differentiation of B cells, in the presence of other lymphokines.

G 265 PATHOGENIC ANALYSIS OF CASTLEMAN'S DESEASE — HYPER-PRODUCTION OF BSF-2/IL-6 FROM HYPERPLASTIC LYMPH NODE — , K. Yoshizaki, N. Nishimoto, T. Kuritani, T. Lee, H. Tagoh, T. Komori, S. Kishimoto, K. Aozasa, T. Nakahata, A. Muraguchi, T. Hirano and T. Kishimoto, DEPT. MEDICINE, OSAKA UNIV. MED. SCH., OSAKA, JAPAN.

Pathogenic analysis of Castleman's disease (C.D.) is first reported through the production of BSF-2/IL-6 which is known as a multi-functional cytokine, e.g. BCDF (BSF-2) activity, HPGF activity, T cell activation activity, hepatocyte stimulation activity. C.D. was characterized by the unique pathological feature of lymph node which showed hyperplasia of lymph follicules, enlarged germinal center, multiangiofollicular filtration, and massive plasma cell infiltration, accompanied with hyper-γ-globulinemia and increment of acute phase protein for many years. However the pathogenesis as well as ethiology of C.D. has been completely unknown. In this study we showed the hyper-production of BSF-2/IL-6 in hypertrophic lymph node on C.D. High BSF-2 activity and HPGF activity were observed in the culture supernatant of hypertrophic lymph node as well as in the serum, and were neutralized by anti BSF-2 antibody. BSF-2 production was confirmed by the transcription of BSF-2 mRNA from the lymph node. Immunohistochemical analysis showed production of BSF-2 was localized at germinal center by staining with anti-BSF-2 antibody. The striking evidence was that after the resection of solid hyperplastic lymph node, not only high BSF-2 level in the serum has been within normal range, but also the patients have gotten free from symptome and abnormal laboratory findings have come into normal range. These data indicated that chronic hyper-production of BSF-2/IL-6 molecule from hypertrophic lymph node meight be one of pathogenic cause of Castleman's disease.

G 266 RELATIVE TRANSCRIPTION OF μ AND δ GENES IN BONE MARROW PRE-B AND B CELLS. Dorothy Yuan and Pamela Witte. University of Texas Southwestern Medical Center, Dallas, Texas 75235.
Newly formed B cells first express IgM and subsequently display IgD on the cell surface. In the present studies we examined, by means of nascent RNA chain labeling, the relative levels of μ to δ gene transcription in bone marrow B cells, pre-B cells and earlier progenitors of B cells. Pre-B cells were obtained from Whitlock-type long term cultures of bone marrow cells from both normal and C.B 17 scid mice. Both populations were found to transcribe the δ gene at very low, but detectable levels. A similarly low level of δ transcription was found to occur in surface IgM positive cells from both cultured and freshly isolated bone marrow cells. In all populations analysed, termination of the majority of polymerases occurred within a discrete one-kb region located between the μ_M and Cδ1 exons. These results when compared with our previous findings, which showed that in both neonatal and adult splenic B cells δ gene transcription occurs at a relatively high level, suggest that activation of regulatory signals that allow polymerases to progress beyond the termination site 3' of the μ_m exons may occur when newly formed B cells migrate from marrow to the splenic environment.

Antigen Presentation, Autoimmunity, Immunodeficiency, Oncogenes, Activation, Transgenic Models - I

G 300 COMPARISON OF IMMUNODEFICIENCY IN PATIENTS WITH MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE OR MULTIPLE MYELOMA, E.Joy Andrews, Bernard A.Reuther, Michael J.Mant and Linda M.Pilarski, University of Alberta, Edmonton, Alberta, CANADA T6G 2H7.
This study included 36 patients with monoclonal gammopathy of undetermined significance (MGUS)-IgG or IgA type, and 23 patients with newly diagnosed multiple myeloma (MM). In both patient groups there were reduced numbers of circulating sIg+ B cells; in 59% of the MM cases and in 32% of the MGUS cases, the number of IgM+ B cells did not correlate with the serum IgM level . By measuring the reactivity of in vitro EBV-transformed B cell clones it was found that, in the majority of MM patients, an abnormally high proportion of clones produced antibody specific for tetanus toxoid (TT), conversely, the serum titer was severely reduced. Only a few MGUS patients showed a similar enrichment for anti-TT clones and most MGUS patients had a normal serum-antibody titer. Clones were also tested for reactivity with determinants on $F(ab')_2$ fragments of Ig. MM patients had a significant increase in auto-immune anti-Ig reactivity, but MGUS patients did not. It remains to determine, by temporal studies, whether this can be useful in the early diagnosis of progression to MM which occurs in some cases of MGUS.

G 301 CHARACTERIZATION OF A SHORT-RANGE HELPER T CELL-B CELL INTERACTION INVOLVED IN THE T CELL-DEPENDENT GROWTH OF RESTING B CELLS, Barbara J. Beaudoin and David C. Parker, University of Massachusetts Medical School, Worcester, MA 01605.
We have previously demonstrated that size-selected, resting mouse B cells efficiently present monovalent Fab' rabbit anti-mouse Ig antibodies to rabbit globulin-specific helper T cell lines for the elicitation of T cell help. The T cell-dependent growth of resting B cells in this in vitro polyclonal system requires an early, cell contact-dependent helper T cell-B cell interaction. B cells acquire the ability to proliferate in response to cell-free supernatants (SN's) from stimulated helper T cells as a consequence of the contact-dependent or short-range interaction, which is required for at least 8 hours following T cell recognition of antigen/Ia on B cells. Fixation of helper T cells with paraform-aldehyde abolishes their ability to deliver the short-range signal to B cells. We compared the ability of Th1 (IL-2 and IFN-γ producers) and Th2 (IL-4 producers) helper T cell lines to deliver the short-range inductive signal leading to B cell growth. A 12 hour interaction of monovalent antigen-pulsed B cells with either Th1 or Th2 rabbit globulin-specific T cells allows subsequent B cell proliferation in the presence of SN's from Con A stimulated helper T cells. In both instances, the B cell proliferative response is greater in the presence of Th2 SN's than in the presence of Th1 SN's. These results suggest that both Th1 and Th2 helper T cells are competent to deliver early, short-range activation signals which lead to B cell growth in the presence of appropriate late-acting lymphokines.
Supported by grants from the American Cancer Society (IM-327), the National Institutes of Health (RO1 AI24303) and the Cancer Research Institute.

G 302 VDJ REARRANGEMENTS IN B CELL LINES DERIVED FROM TRANSGENIC RABBITS. Robert S. Becker, Mark Suter, Helga Spieker-Polet and Katherine L. Knight, University of Illinois Medical Center, Chicago, IL 60612. We have established B cell lines from the peripheral blood and bone marrow of transgenic rabbits which developed leukemias. The rabbit c-myc gene was isolated and cloned downstream of the rabbit $E\mu$; this construct was used to produce transgenic rabbits which in turn developed B cell leukemias within 20 days of birth. We have cloned and analyzed the VDJ genes from these leukemia cells and B cell lines. Four of the six cloned VDJ rearrangements reside on 4.1 Kb Hind III fragments and the remaining two rearrangements on 4.8 Kb Hind III fragments. All of these VDJ genes have Hind III sites approximately 1.5 Kb upstream of their VH gene segments; the size difference of the Hind III fragments is the result of the VD gene segments utilizing two different JH gene segments. These conserved Hind III sites suggest that the utilized VH genes have a conserved 5' sequence; sequencing of these 5' regions has confirmed this prediction. Southern blot analysis of cosmids containing approximately 100 germline VH genes indicated that only a portion of germline VH genes have 5' sequences similar to the rearranged genes. Previous studies have demonstrated that the VH genes encoding VHa+ molecules only make up 30-40% of the germline VH gene pool but are utilized in 80-90% of the VDJ rearrangements. We suggest that the utilized VH genes have conserved 5' regions which are not present 5' of the unutilized VH genes.

G 303 IN VITRO GROWTH INHIBITION OF A HUMAN B LYMPHOMA LINE, Margaret Beckwith, Dan L. Longo,* Catherine D. O'Connell, Chantal M. Moratz and Walter J. Urba, Program Resources, Inc. and *Biological Response Modifiers Program, NCI-FCRF, Frederick, MD 21701-1013.

RL is an EBV-genome negative, surface IgM and IgD positive B lymphoma cell line that was isolated from the ascites of a patient whose nodular lymphoma had transformed into a diffuse undifferentiated lymphoma. We compared growth regulation of normal B cells to malignant cells (RL) after in vitro culture with anti-IgM or phorbol esters. In contrast to the increased proliferation observed with normal B cells, treatment of RL with rabbit anti-IgM-sepharose resulted in marked inhibition of ^{3}H-thymidine uptake and surface IgM levels. In addition, increased levels of CD20 (B1) and HLA-DR were seen within 24 hours of culture. Similarly, phorbol myristate acetate (PMA) increased proliferation of normal B cells, while causing growth inhibition of RL cells. A decrease in surface and secreted IgM, and increased expression of B1 and HLA-DR were also observed. No effect was seen when an inactive analog of PMA (Methyl-TPA) was used, suggesting that the inhibitory activity is mediated through a protein kinase C (PKC) pathway. The growth inhibition seen with both anti-IgM and PMA is not due to cell death, but appears to be irreversible. We plan to measure the effects of antibodies to other cell surface antigens, examine changes in oncogene expression, and further evaluate mechanisms by which the signals that stimulate the growth of normal B cells result in the growth arrest of malignant B cells.

G 304 TRANSCRIPTION OF THE C-MYB PROTO-ONCOGENE IS DIFFERENTIALLY REGULATED BY A BLOCK TO TRANSCRIPTION ELONGATION IN MURINE B-LYMPHOID TUMOR CELL LINES, Timothy P. Bender and W. Michael Kuehl, the University of Virginia, Charlottesville, VA 22908 and the National Cancer Institute, Bethesda, MD 20814

Expression of c-myb mRNA expression is down-regulated 10 to greater than 100 fold by events occurring at the pre-B cell/B cell junction. We have examined the mechanism regulating c-myb mRNA expression in a series of murine and human B lymphoid tumors which represent the pre-B cell, immature and mature B cell and plasma cell stages of B cell development. The down-regulation of c-myb mRNA expression is not due to differences in specific mRNA stability (c-myb mRNA has a half-life of 160-190 minutes in these cell lines) but is regulated at the transcriptional level. We have used nuclear run-on studies in conjunction with single stranded genomic DNA clones to show that sense transcription initiates at an equivalent rate at all stages of B cell maturation but that a block to transcription elongation (attenuation) appears to be the primary mechanism regulating c-myb mRNA levels. Using appropriate probes we have also found that anti-sense transcription occurs across exon one at all stages of B cell development. Assay of DNase I sensitivity has defined a site of major quantitative difference in DNase I hypersensitivity between cells which express high and low levels of c-myb mRNA suggesting the possibility that a DNA binding protein may be associated with attenuation. Regulation of c-myb mRNA levels by attenuation may provide a more rapid and sensitive response to external signals than initiation of transcription.

G 305 PRODUCTIVE PRESENTATION OF BEE VENOM PHOSPHOLIPASE A2 (PLA2) BY TETANUS TOXOID (TT) SPECIFIC B CELLS TO PLA2 SPECIFIC T CELLS RESULTS IN THE PRODUCTION OF ANTI-TT ANTIBODIES. C.D. Benjamin, M.A. Harvey (1), Debra A. Young (2) and A.C. Lua (3). Biogen Research Corporation, Cambridge, MA 02142.

Our laboratory has established an antigen dependent, T cell-B cell cooperative response to the small protein PLA2 from honeybee venom. PLA2 is the predominant allergen in bee venom and we have investigated regulation of the isotype response using PLA2 as a model in mice. The response to PLA2 is not under genetic control; all mouse strains tested (10) made a T cell proliferation and an antibody response to as little as 1 ug of PLA2 when emulsified in complete Freund's adjuvant. We established an in vitro system using PLA2 specific T cell lines from A/J strain mice to investigate isotype regulation in the response to PLA2. The cooperative response to PLA2 is absolutely PLA2 and T cell dependent. However, using A/J strain mice hyperimmunized with the unrelated antigen tetanus toxoid (ostensibly our negative control), we found that productive presentation of PLA2 by these B cells to our PLA2 specific T cell line provided sufficient stimulus to drive them to anti-TT antibody secretion. There was no spontaneous or background secretion of antibody in the absence of PLA2 or in the presence of tetanus toxoid. The anti-TT response could not be replaced by PLA2 stimulated, antigen depleted T line supernatants; nor could polyclonal T cell activators drive the anti-TT response. Our results indicate that an antigen-dependent, H-2 restricted T cell-B cell cooperative response which results in B cell activation and antibody secretion can occur even when the B cell carries immunoglobulin receptors specific for an unrelated antigen.

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G 306 TOLERANCE, MHC RESTRICTION, ANTIGEN AND PRESENTATION ANALYSES IN $E\alpha$ MUTANT TRANSGENIC MICE. C. BENOIST[1], Y. RON[2], W. VAN EWIJK[3], J. MONACO[4], J. FEHLING[1] and D. MATHIS[1]. 1 : Laboratoire d'Immunologie, I.G.M.E., 11, rue Humann, 67085 Strasbourg cedex, FRANCE. 2 : Research Institute of Scripps Clinic, 10666 North Torrey Pines Road, La Jolla, California, U.S.A. 3 : Erasmus University of Rotterdam, Dept. of Cell Biology, Postbus 1738, 3000 DR Rotterdam, PAYS-BAS 4 : Virginia Commonwealth University, Dept. of Microbiology and Immunology Box 678, MCV Station, Richmond, Virginia, U.S.A.

We have previously demonstrated the feasibility of constructing mouse lines that carry an E^k_β transgene. In these lines, $E\alpha$ is expressed efficiently, accurately, and with tissue and cell-type specificity. Most importantly, the transgene confers new immunological capabilities on the B6 x SJL recipient : it becomes tolerant to I-E and capable of responding to E-restricted antigens.

The fidelity of w.t. E^k_β expression and function in transgenic mice has allowed us to dissect promoter elements important for expression in different tissues. By making various deletions in the promoter regions of the injected clones, we have created lines that have restricted $E\alpha$ expression; consequently, surface E_α:E_β occurs only in certain compartments of the immune system:

- In several lines, expression is correct in essentially all cell types (thymus cortex and medulla, macrophages, dendritic cells) but is absent in the vast majority of B cells.
- Another line exhibits a very selective defect, since the I-E complex is present essentially everywhere but not on the thymic cortical epithelium.
- In another line, the opposite pattern is found in the thymus, where I-E expression is strong in the cortex but virtually absent in the medulla.

Those lines have been analyzed for various I-E directed immune phenomena. The results obtained address the issues of B cell function in antigen presentation, and of the role of thymic regions in the acquisition of an MHC selected T cell repertoire.

G 307 ACCUMULATION OF SOMATIC MUTATIONS WITH TIME. Claudia Berek, Institute fur Genetik, Koln, FRG.

The antibody diversity at different stages of the immune response to the hapten 2-phenyl-oxazolone has been analysed. The increase in the number of somatic mutations between day 7 and day 14 of the primary response suggests an antigen dependent induction of a hypermutation mechanism in B cells. However the analysis of antibodies of the memory response suggests that the somatic diversification of the immunoglobuln genes is not restricted to the early stages of the immune response. The accumulation of somatic mutations from the primary, to the secondary, to the tertiary response antibodies indicates that antigen can reactivate the hypermutation mechanism in long-lived memory B-cell clones.

G 308 DIFFERENCES IN ACTIVATION REQUIREMENTS OF B LYMPHOCYTES FROM LYMPH NODE AND SPLEEN, Bondada Subbarao and S.N. Goud, Sanders-Brown Center on Aging and Department of Microbiology and Immunology, University of Kentucky, Lexington, KY 40536.

Bondada Subbarao and S.N. Goud, Sanders-Brown Center on Aging and Department of Microbiology and Immunology, University of Kentucky, Lexington, KY 40536.

B cells from peripheral lymph nodes (inguinal, axial, popliteal, brachial and tracheobronchial areas) failed to respond to TNP-Ficoll *in vitro* as well as *in vivo*. B cells from mesentric lymph nodes gave modest responses while splenic B cells responded extremely well to TNP-Ficoll. However, B cells from all these lymphoid organs responded equally well to stimulation with the TI-1 antigen TNP-*Brucella Abortus* (BA). The *in vitro* lymph node response to TNP-Ficoll could be reconstituted with irradiated spleen cells and the critical cell in the irradiated spleen population was a sephadex G-10 adherent accessory cell but not a T cell. In addition, the peripheral lymph node B cells responded to TNP-Ficoll, if the cultures were supplemented with partially purified or recombinant derived interleukin (IL)-1 confirming the accessory cell deficiency in the lymph node. Recombinant IL-4 and IL-2 were not effective in restoring the lymph node response to TNP-Ficoll. *In vivo*, subcutaneous challenge with TNP-Ficoll induced a response in the spleen but not in the peripheral lymph nodes. Injection of unconjugated BA along with TNP-Ficoll in the foot pad elicited a PFC response in the draining lymph node as well as in the spleen. The action of BA appeared to be due to recruitment of a responding B cell or an accessory cell from the spleen since splenectomy severly decreased this lymph node response to stimulation with TNP-Ficoll + BA. This work is supported in part by the NIH grants A1-21490, AG05731 and an award from the Tobacco and Health Research Institute of University of Kentucky.

G 309 DETECTION OF *REL*-RELATED RNA AND PROTEIN IN HUMAN LYMPHOID CELLS, E. Brownell[1], F.W. Ruscetti[2], R.G. Smith[3], and N.R. Rice[1]. [1]NCI-Frederick Cancer Research Facility, Frederick, MD 21701, [2]National Cancer Institute, Frederick, MD 21701, [3]University of Texas Health Science Center, Dallas, TX 75235.

We used a portion of the human c-*rel* protooncogene to study c-*rel*'s pattern of expression in human leukemic cell lines and normal lymphocyte populations. Unlike most other protooncogenes, c-*rel* is apparently expressed most abundantly in differentiated lymphoid cell types, and the major transcript displays a significant species-specific variation in length. Both peripheral B-cells from normal donors and Burkitt lymphoma-derived cell lines exhibit the highest levels of c-*rel* transcripts; peripheral T-cells and late-stage T lymphoblast cell lines express about 50% less c-*rel* RNA. In addition, we used antisera made to portions of the v-*rel* transforming protein to detect, for the first time, an 82,000 MW human *rel*-related protein in Daudi cells, which display high levels of c-*rel* RNA. Research sponsored by the National Cancer Institute, DHHS, under contract NO. NO1-CO-74101 with Bionetics Research, Inc. The contents of this publication do not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

G 310 SOMATIC MUTATION IN TRANSGENIC MICE. Patricia Busto, Theresa O'Keefe, and Thereza Imanishi-Kari, Tufts University, Boston, MA 02111.

Somatic point mutations have been proposed as a mechanism to generate antibody diversity during the immune response. Although it has been demonstrated that somatic mutation does occur in rearranged immunoglobulin genes, it has been more difficult to conclude that these point mutations indeed create new antigen specificity. Transgenic mice constructed by introducing on the germ line of C57/B16 mice a rearranged VH gene 17.2.25 linked to a Cu constant region gene of Balb/C origin were used in these studies. The transgene binds the hapten NP when it associates with lambda 1 light chain. Anti-DNP hybridomas were produced from transgenic mice immunized with DNP-RNase. Analysis of hybridoma supernatant specific for DNP revealed that a reasonable frequency of those, expressed the transgene in association with lambda light chain. These antibodies bound with different affinities to the original NP hapten. Affinity chromatography indicates that DNP binding antibodies are the same antibodies that bind NP hapten. These results indicate that probably a new antigen specificity was created by somatic mutations of the transgene, the lambda gene or both. Molecular analysis of the expressed VH and VL genes are in progress in order to determine what region of the gene have been affected and how much mutation actually occurred.

G 311 INAPPROPRIATE EXPRESSION OF THY-1 IN TRANSGENIC MICE CAUSES A LYMPHOPROLIFERATIVE ABNORMALITY, Shizhong Chen, Carlisle P. Landel, Florence Botteri, Herman van der Putten and Glen A. Evans, The Salk Institute, P.O. Box 85800, San Diego, CA 92138.

Thy-1 is an abundant cell-surface glycoprotein of unknown function found on mouse T lymphocytes, neurons and hematopoietic stem cells. In order to investigate its function, we re-directed the expression of Thy-1 to the B cell lineage in transgenic mice with a hybrid Thy-1 transgene containing the transcriptional enhancer of the mouse immunoglobulin heavy chain. These mice express the Thy-1.2 transgene on mature B cells and their progenitors and develop a heritable lymphoid hyperplasia characterized by the appearance of several cell populations expressing large amounts of Thy-1 in the bone marrow and lymph nodes. These abnormal cells include a population of large cells expressing both Thy-1 and the pre-B cell antigen B220, and a population of rapidly dividing cells expressing large amounts of Thy-1 but lacking most other cell-surface markers characteristic of the hematopoietic lineage. These mice suffer no grossly detectable immune defects, although we are searching for more subtle effects. We are investigating the function and the developmental potential of these abnormal cells *in vitro* and by transplantation to lethally irradiated mice.

G 312 DOWNREGULATION OF HUMAN B CELL ACTIVATION BY TRANSFORMING GROWTH FACTOR B1 AND B2, Edward A. Clark, Geraldine Shu and Larry Ellingsworth, University of Washington and Collagen Corporation, Seattle, WA 98195

Two distinct but closely related factors, transforming growth factor (TGF)-B1 and TGF-B2 were purified to homogeneity from bovine demineralized bone (Seyedin et al., J Biol Chem 262:1946-49, 1987) and then tested for their effect on B cell activation and proliferation. Both TGF-B1 and TGF-B2 inhibited the proliferation of B cells stimulated with mAb to surface IgM or to the B cell differentiation antigens CD20 or CDw40. The inhibitory effects of these factors resembled the activity of cyclic AMP inducers forskolin and dBcAMP, yet were effective at 10^5 to 10^6 lower molar concentration than either forskolin or dBcAMP. However, neither factor actually induced cAMP or inhibited the induction of cAMP by other agents. Both TGFs inhibited activated B cells from entering the G1 and S phases of the cell cycle. However, they had no effect on early activation events, including anti-IgM-induced $Ca2^+$ mobilization, activation of cAMP, or induction of increased levels of Class II MHC antigens on B cells. Thus, TGF-B1 and TGF-B2 do not block the initial activation of B cells, but rather act to inhibit recently activated B cells from traversing the cell cycle. (Supported by Collagen Corporation, who provided TGF-B1 and TGF-B2, and by NIH grants DE08229 and GM37905.)

G 313 EXPRESSION OF HLA-B27 GENE IN TRANSGENIC MICE. C.S. David, S. Prakash, S. Savarirayah, S. Banerjee, T. Haqqi and R. Little. Dept. Immunol., Mayo Clinic, Rochester, MN 55905.

HLA-B27 gene from a patient with ankylosing spondylitis (courtesy of H. Coppin, France) was injected into (B6 x SJL)F_1 mouse embryos. Of the 150 embryos injected, 70 were transferred to pseudopregnant females which delivered 27 pups. Of the 27 pups, 2 mice expressed the HLA-B27 gene on the basis of Southern blot analysis of tail DNA. Dot blot analysis indicated a high copy number. RNA analysis indicated expression of the gene in most tissues where class I genes are expressed. FACS analysis using peripheral blood lymphocytes with an anti-HLA-B27 monoclonal antibody indicated expression of the B27 antigen on the cell surface in association with the mouse β_2-microglobulin. The transgenic mice were backcrossed to B10 strain to produce the progeny. Approximately 50% of the backcross generation showed expression of the B27 antigen on the cell surface. Skin graft analysis between these mice showed that grafts from B27 positive animals were rejected by the B27 negative full sibs in a time period typical of most class I antigens. Thus, our studies so far have indicated that the HLA-B27 gene expressed in these transgenic mice is found in most tissues, the molecule is expressed on the cell surface in association with mouse β_2-microglobulin and can function as a class I transplantation antigen. Further breeding is in progress to produce a congenic mouse expressing the B27 gene. Other structure/function studies are underway using the transgenic mice.
Supported by NIH grants CA-24473 and AI-14764.

G 314 RELATIONSHIPS BETWEEN LY-1 B CELL AND MYELOID DIFFERENTIATION, Wendy F. Davidson, Jacalyn H. Pierce, Stuart Rudikoff and Herbert C. Morse, III, NIH, Bethesda, MD 20892. A cell line, HAFTL-1, derived by in vitro transformation of fetal liver cells with Ha-v-ras, was found to have the molecular and phenotyic characteristics of pro-B cells recently committed to the Ly-1$^+$ B cell pathway. Stimulation of these cells with LPS resulted in their differentiation within either the B lymphocyte or myelomonocytic lineages. Thus, lines derived from LPS-stimulated HAFTL-1 cells exhibited characteristics of pre-B cells (expression of ThB and Ia, continuing DJ rearrangement of the IgH locus) or mature macrophages (expression of Mac-1 and Mac-2, loss of the B lineage markers Lyb-2, Lyb-8 and Ly-5 (B220), production of lysozyme and nonspecific esterase and phagocytosis) while maintaining their Ly-1$^+$ phenotype. Both the pre-B cell lines and macrophage lines also produced GM-CSF. The macrophage lines, in contrast to the pro-B cell and pre-B cell lines, expressed high levels of fos, fms and bcl-2 and low levels of myb mRNA transcripts. These results suggest that events resulting in irrevocable committment to a single lineage are carefully orchestrated and can occur late in differentiation, at least within the pathway yielding Ly-1$^+$ B cells and a proposed population of Ly-1$^+$ macrophages.

G 315 INDUCTION OF TNP-SPECIFIC ANTIBODY IN PRIMED AND UNPRIMED B CELLS BY Th1 AND Th2 T CELL CLONES. R.H. DeKruyff, D.T. Umetsu. Stanford University, Stanford, CA 94305.

We examined the induction of anti-TNP responses in primed and unprimed B cells using a panel of nominal antigen specific (KLH or GL$^\beta$) Th1 (IL2 producing) and Th2 (IL4 or BSF-1 producing) inducer T cell clones. With primed B cells, 8 of 10 Th1 clones induced little or no antibody synthesis. However, two Th1 clones (D3 and E10) were extremely efficient at inducing IgG anti-TNP PFC in primed B cells. Clone D3, a KLH specific clone, induced 22,000 IgG anti-TNP PFC/culture in the presence of 0.1 ng/ml of antigen. Cognate B-T cell interaction was required, since only B cells syngeneic to the clone were activated, and supernatants of clone D3 did not induce antibody production. The 5 Th2 T cell clones examined were heterogeneous with regard to the capacity to induce TNP specific antibody synthesis in primed B cells. While 3 Th2 clones induced TNP antibody synthesis, one clone (B5) failed to induce any anti-TNP PFC and another clone (AK1) induced only IgM and not IgG antibody. All Th2 clones were high producers of IL4 and could respond to antigen presented by B cells.

With unprimed B cells, all Th1 clones induced minimal levels of antibody. All Th2 clones except B5 were efficient in inducing primary anti-TNP responses. These studies indicate that TNP-specific antibody can be induced in memory B cells by both Th1 and Th2 T cell clones.

G 316 REGULATORY ROLE OF CD19 MOLECULES IN B-CELL ACTIVATION AND DIFFERENTIATION, Menno A. de Rie, Ton N.M. Schumacher, Gijs M.W. van Schijndel, René A.W. van Lier and Frank Miedema, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, incorporating the Laboratory of Experimental and Clinical Immunology of the University of Amsterdam, Amsterdam, The Netherlands.

The functional role of CD19 molecules in B-cell activation and differentiation was studied. CD19 antigens represent B-lymphocyte-specific molecules, expressed on virtually all human cells of the B-lymphocyte lineage except plasma cells. We produced a new anti-CD19 Mab, designated CLB-CD19, that immunoprecipitated a 52 kD glycoprotein from ^{35}S-methionine-labeled Daudi cell lysates. Binding of CLB-CD19 Mab did not inhibit the Staphylococcus aureus (Sac)-induced proliferation of normal and leukemic B cells. However, both intact and F(ab')$_2$ fragments of the CLB-CD19 Mab inhibited the pokeweed mitogen (PWM) and IL-2-induced immunoglobulin (Ig) synthesis as well as the accessory-cell-independent differentiation of normal B cells. PWM- and Sac-induced Ig synthesis of leukemic B cells was inhibited equally well by the CLB-CD19 Mab. Delayed addition studies in the PWM system showed that once the differentiation of B cells has been induced, CD19 molecules have lost their regulatory function. Measurements of intracellular calcium in Indo-1-labeled cells showed that anti-CD19 Mab did mobilize free intracellular calcium in Daudi cells, whereas anti-CD19 Mab did not interfere with the anti-IgM-induced rise in free intracellular calcium in neoplastic and normal lymph-node B cells. Taken together, these data indicate that CD19 molecules play a regulatory role during T-cell-dependent terminal differentiation of normal and leukemic B cells.

G 317 ANALYSIS OF DEFECTIVE SIGNAL TRANSDUCTION IN B-CLL, Hans G. Drexler, Malcolm K. Brenner, Suzanne M. Gignac, Elaine Coustan-Smith and A. Victor Hoffbrand, The Royal Free Hospital, London NW3 2QG, U.K.

B-CLL cells are arrested at immature stages of differentiation, but can be induced to differentiate *in-vitro*. The cells from 27 patients with B-CLL were treated with phorbol ester TPA, calcium ionophore A23187, B-cell differentiation factors BCDF (from mitogen-activated normal B-cells) and T24 (supernatant from the bladder carcinoma cell line T24), and recombinant IL-2 singly and in combinations for 6 days in culture. Changes in morphology, RNA synthesis (measured by 3H-uridine uptake), immunoglobulin production (tested by ELISA), activation of c-fos and c-myc genes (Northern blotting analysis) and expression of tartrate-resistant acid phosphatase (detected by isoelectric focusing) were used as indicators of positive signal transduction. TPA alone and TPA plus A23187 synergistically induced the cells to differentiate morphologically and functionally to plasmacytoid cells. A23187 alone and BCDF, T24 and IL-2 alone or in combination were not effective in inducing differentiation. Initial events of signal transduction are bypassed by TPA and A23187 as these reagents directly stimulate second messengers (TPA activates protein kinase C; A23187 increases intracellular Ca^{2+}). Signal transduction downstream of protein kinase C appears to be intact in B-CLL. The physiological extracellular stimuli BCDF, T24 and IL-2 effective in normal B-cells did not trigger the B-CLL cells to differentiate. These results suggest that the defect in signal transduction in B-CLL might be found upstream of protein kinase C.

G 318 ISOTYPE SWITCHING BY A MICROINJECTED REARRANGED μ IMMUNOGLOBULIN HEAVY CHAIN GENE IN TRANSGENIC MICE, Jeannine Durdik, Rachel M. Gerstein, Paul F. Robbins, Alfred Nisonoff and Erik Selsing; Rosenstiel Basic Medical Sciences Research Center, Brandeis University, Waltham, MA 02254.

C57B1/6 mice transgenic for a μ heavy chain gene containing the BALB/c Cμ exons linked to a recombined VDJ region isolated from R16.7 (an arsonate specific hybridoma) expressed high levels of the transgene. Even without immunization, significant levels of immunoglobulins expressed determinants peculiar to the transgene. Also observed in unimmunized animals were low levels of p-azophenyl-arsonate (ARS)-binding antibody and an idiotype, CRI_A. ARS binding and CRI_A are most likely dependent upon expression of a specific light chain with the injected heavy chain.

Following immunization with ARS, the levels of ARS specific, idiotype positive antibodies increased. However, these ARS specific, idiotype positive antibodies were predominantly of heavy chain classes other than μ. Several of serological criteria strongly suggest that the injected transgene VDJ region is being utilized in the isotype switching. Sequence from an ARS specific IgG producing hybridoma is also consistent with switching of the transgene. Because the microinjected μ plasmid lacks γ constant regions, the IgG must be derived in part from endogenous loci.

G 319 THE KINETICS OF CONJUGATE FORMATION AND DISSOCIATION BETWEEN HELPER T CELLS AND ANTIGEN PRESENTING B CELLS. Douglas Rowe, Susan L. Swain and Richard W. Dutton, University of California, San Diego, La Jolla, CA 92093-0063.

Cloned B cell and T cell lines have been separately internally labelled with fluorescent dyes so that the formation and dissociation of antigen specific conjugate between helper T cells and antigen presenting B cells could be studied in a model system. The rate of association was rapid and firm conjugates formed only if the B cells were pre-incubated with specific antigen. The rate of association was dependent on temperature, the cell density, and shaking of the cultures but was not affected by the presence of a large excess of irrelevant B cells. In contrast, dissociation of specific conjugates was slow with half lives in excess of several hours. Dissociation rate also did not appear to be increased in the presence of irrelevant competing B cells. These studies suggest that stable long term conjugates form when T cells recognize Ag presenting by B cells. The significance of these observations will be discussed.

G 320 CONTROL OF TISSUE SPECIFIC REARRANGEMENT EVENTS DURING LYMPHOCYTE DIFFERENTIATION

Pierre Ferrier, Andrew Furley, Bernie Krippl, Frank Costantini, Wendy Cook, and Frederick W. Alt, The Howard Hughes Medical Institute and Departments of Biochemistry, Microbiology and Human Genetics, College of Physicians and Surgeons of Columbia University, New York, New York, 10032

We have suggested that tissue- and stage-specific regulation of Ig and TCR variable region gene assembly is controlled by modulating accessibility of substrate gene segments to a common VDJ recombinase; accessibility could be correlated with transcription of the substrate gene segments. To elucidate controlling mechanisms, we introduced into transgenic mice a recombination substrate that consisted of a TCR β chain V, D, and J segments separated from a Cμ gene by a DNA segment which either did or did not contain the Ig heavy chain enhancer. Analyses of normal lymphocytes in multiple transgenic strains demonstrated that the heavy chain enhancer was necessary and sufficient to obtain DJβ rearrangements in normal B and T cells, but that Vβ to DJβ occurred only in T cells. This has been confirmed preliminarily by analyses of A-MuLV transformed B and T cell lines from the transgenic strains. The specific regions and activities of the Vβ gene which confer tissue-specific rearrangement are under investigation. In accord with implications of our previous cell culture studies, the enhancer may be necessary to achieve general access to the locus, but elements associated with the Vβ gene, perhaps the promoter, may provide tissue specificity of variable region gene assembly.

G 321 MOLECULAR CHARACTERIZATION OF THE LYMPHOCYTE ACTIVATION MARKER BLAST-1, Donald E. Staunton, Robert C. Fisher, and David A. Thorley-Lawson, Tufts University School of Medicine, Boston, MA 02111.

BLAST-1 is an early activation-associated glycoprotein expressed on the surface of human lymphocytes. In order to study the structure of BLAST-1 and its function in cellular activation, a full-length 1.2kb cDNA encoding BLAST-1 was isolated and characterized. The translated sequence contains 5 putative N-linked glycosylation sites and a short hydrophobic carboxyl terminus. Subsequent analysis indicated that BLAST-1 is anchored to the plasma membrane via a phosphatidylinositol-containing lipid. In addition, the translated sequence reveals that BLAST-1 is related to members of the immunoglobulin superfamily, especially to CD4 and MHC classII. The homology to these proteins is greatest in their amino termini where they demonstrate 30-32% identity. Following anti-IgM stimulation of B cells, BLAST-1 mRNA is detected within 30 minutes indicating a possible role for BLAST-1 in early B cell activation. Preliminary analysis suggest the BLAST-1 gene is approximately 10-15kb.

G 322 T1 (CD5) ANTIGEN DEFINES A DISTINCT SUBSET OF ACTIVATED HUMAN B CELLS, Arnold Freedman, Gordon Freeman, and Lee M. Nadler, Dana-Farber Cancer Institute, Boston, MA 02115.

Anti-Ig, EBV, anti-CD20 monoclonal antibody, and IL-4 all activate human B cells via unique receptors, each inducing distinct activation events. Following activation of highly purified splenic B (B1+) cells with the phorbol ester TPA, the T1 (CD5) antigen is induced. Prior to stimulation and at 24 hr approximately 7% of B cells co-expressed T1. At 48 hr, 40% expressed T1 and at 72 and 96 hr T1 expression decreases to about 20%. Northern blot analysis of total cellular RNA demonstrated induction of T1 mRNA by 8 hr with increasing amounts up to 48 hr. Whereas TPA induced T1 expression, other B cell stimuli including anti-Ig, EBV, anti-CD20 (1F5), rIL-1, rIL-2, rIL-4, rIFN-q, and BCGF all failed to induce T1. Although rIL-4 did not induce T1, of the above stimuli it was the only one which when co-cultured with TPA inhibited T1 expression. Dual fluorochrome analysis of these T1+ B cells demonstrated co-expression of the B cell activation antigens B5, IL-2 receptor, and CD23. TPA activated splenic B cells were then sorted into the B1+T1- and B1+T1+ populations and examined for response to anti-Ig, rIL-2, and BCGF and combinations of anti-Ig and rIL-2 or BCGF. At 48 and 96 hours, the B1+T1- cells demonstrated significantly greater (2-3 fold) ^{3}H-TdR incorporation and IgG secretion than the B1+T1+ cells. These studies suggest that T1 identifies a distinct subpopulation of activated B cells. Moreover, the inhibition of T1 expression by rIL-4 suggests a role of T cells in the regulation of this pathway of B cell activation.

G 323 THE XLR PROTEIN IS LOCALIZED IN THE NUCLEUS OF SECRETORY B-CELLS AND INDUCES PHENOTYPIC CHANGES IN TRANSFECTED NON-SECRETORY B-CELLS, H-J. Garchon, G. Jedd, A. Stahl and M.M. Davis, Departments of Medical Microbiology and Genetics, Stanford University, Stanford, CA 94305.

The XLR (X-linked, Lymphocyte Regulated) gene family is located on the X-chromosome and is linked to the xid mutation of CBA/N mice. The major transcript of this locus, which is expressed in secretory B-cells, contains an open reading frame of 208 a.a. We have expressed large quantities of this protein using a bacterial expression vector and raised antibodies against the purified material. A doublet of apparent m.w. 30-32 kDa was found by immunoprecipitation only in the most mature, Ig-secreting B-cells but not in non-secretory B-cells or in pre B-cells. Immunofluorescence staining of fixed cells indicated that the XLR protein is confined to the nucleus. The developmental regulation of XLR expression and its nuclear location suggested that it might be a regulatory gene. We have tested this hypothesis directly by transfecting an expression vector containing the XLR cDNA into BAL17 lymphoma cells which normally do not express it. Four stable transfectant cell lines were established and were shown to synthesize the XLR protein. They were then analyzed with a panel of nine surface antigens using the FACS. Comparison with parental BAL17 cells reveals in all four lines an increase in IgD expression (up to 10-fold) whereas the Bla-2/14G8 antigen was turned off (40 to 100-fold less expression). The relevance of this phenotypic change to the process of B-cell differentiation and to the xid mutation will be discussed.

G 324 PROGRESSION OF bcl-2 REARRANGED FOLLICULAR LYMPHOMA RESULTS IN PRE-B-CELL LEUKEMIA WITH A t(8;14)(14;18) TRANSLOCATION. Charlotte E. Gauwerky, Yoshihide Tsujimoto, Frank G. Haluska, Peter C. Nowell*, Carlo M. Croce. The Wistar Institute, Philadelphia, PA 19104 and *University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

In 85% of all cases with follicular lymphoma, the malignant cells carry the t(14;18) translocation involving the bcl-2 gene. In Burkitt's lymphoma with the t(8;14) translocation the c-myc oncogene translocates into the immunoglobulin heavy chain locus of chromosome 14. We studied two cases with acute B-cell leukemia and two translocations, t(8;14) and t(14;18). One patient had a five-year history of follicular lymphoma prior to developing pre-B-cell leukemia. Southern analysis revealed rearrangements of bcl-2, c-myc and J_H in both cases. $c\mu$ and $c\kappa$ were deleted; $c\lambda$ and $c\gamma$ were rearranged. Genomic libraries were constructed. Genes for t(14;18), t(8;14) and the reciprocal bcl-2 gene on chromosome $18q^-$ were cloned. The breakpoint t(14;18) occurs in the bcr of the bcl-2 gene, which was joined to the J_4 segment of the immunoglobulin heavy chain gene. An N region is identified, and heptamer and nonamer signals are seen on both sides of the breakpoint. Analysis of the t(8;14) translocation revealed the breakpoint 543 bp 5' of the first exon c-myc joining into $c\gamma_2$ on chromosome 14. The reciprocal of bcl-2 on chromosome $18q^-$ was found to join into the D_2 repeat unit. Our data support our hypothesis on the evolution of B-cell malignancies: During V-D-J joining a t(14;18) translocation occurs in a pre-B cell that develops into a follicular lymphoma clone with an activated bcl-2 gene. Within this clone a t(8;14) translocation occurs during heavy chain isotype switching, resulting in the deregulation of c-myc involved in the translocation.

G 325 IDIOTYPE VACCINATION AGAINST B-CELL LYMPHOMA GENERATES VARIANT SURFACE-Ig NEGATIVE TUMOURS, WITH ALTERED TISSUE DISTRIBUTION, DIVISION RATE AND RESPONSE TO GROWTH FACTORS, Andrew J.T. George, Shelley, G. Folkard and Freda K. Stevenson, Lymphoma Research Unit, Tenovus Research Laboratory, Southampton General Hospital, Southampton SO9 4XY, U.K.

Tumours of B lymphocytes tend to have a predominant localization in a particular lymphoid tissue. Thus the mouse B-cell lymphoma, BCL_1, like its human counterpart prolymphocytic leukaemia, is a spleen-seeking tumour and tumour cells are found in blood and other lymphoid tissue only late in the disease.

By immunising syngeneic mice with idiotypic IgM derived from the BCL_1 cells, we have immunoselected two variants of the BCL_1 tumour which appear to differ from the parent only in that they fail to express surface Ig. Concomitant with this there is a change from splenic to hepatic localization and division rate, as measured by S-phase analysis, in the spleen is reduced. The variants also show a more benign course in vivo.

Parental BCL_1 cells respond well to LPS by both DNA synthesis and IgM secretion but this response is ablated in the variants, suggesting a requirement for surface Ig in such a response. The BCL_1 tumour is also sensitive to growth factors such as BCGF II (IL-5) and comparative responses of the variant tumours to the available growth factors will be reported.

The approach of immunising mice or eventually patients with idiotypic IgM derived from their tumour might lead to generation of such variants but it appears possible that a more benign disease might result.

G 326 MOLECULAR CHARACTERIZATION OF REARRANGED MU TRANSGENE EXPRESSION IN LYMPHOID CELLS. R. Gerstein, J. Durdik and E. Selsing, Brandeis University, Waltham, MA 02254.

A gene containing the variable region from the CRI_A-bearing hybridoma R16.7 linked to the μ constant region was injected into fertilized eggs of C57Bl/6 mice. Analysis of RNA from tissues of transgenic mice indicates expression is lymphoid specific; splenic and thymocyte RNA both contain transgene mRNA. Membrane μ to secreted μ ratios in transgenic spleen are similar to those of normal spleen, while transgenic thymocytes make more membrane μ than spleen cells, suggesting that the transgene is subject to differential splicing. RNase protections indicate that much of the secreted μ message is likely to be encoded by the transgene.

Preliminary analysis of RNA from hybridomas suggests that within some individual hybrids there is both μ and γ transgene message, as hybridizations of Northerns with a V region probe displays mRNA of both sizes and constant region probes verify the identity of this message. Since isotype switching is observed following immunization in these transgenic mice, further analysis of these hybridomas may help elucidate the mechanism of this phenomenon. Southern blots will assess the status of endogenous Ig genes in these hybrids.

G 327 RESISTANCE TO LYMPHOID ENGRAFTMENT IN LPR RECIPIENTS OF NORMAL STEM CELLS, Roseann M. Glaser, David L. Perkins and Ann Marshak-Rothstein, Boston University School of Medicine, Boston, MA 02118. It has previously been shown that lethally irradiated MRL/lpr mice reconstituted with bone marrow from a non-autoimmune strain, A.Thy, developed a state of permanent split chimerism with respect to their hematopoietic system; all circulating erythrocytes were derived from the normal donor, while peripheral T and B cells were derived from the lpr host. In contrast, MRL/+ recipients were fully reconstituted by donor stem cells. We have extended these studies to investigate repopulation of additional hematopoietic cell populations. Bone marrow cells and macrophages in these mice predominantly express the donor phenotype. However, cells in the thymus, lymph node and spleen are derived from the lpr host, although they do not express any of the unusual cell surface markers associated with the lpr phenotype. The lack of mature donor lymphoid cells in these mice, in spite of the presence of normal stem cells, could be due to their selective down-regulation in the lpr host or to a strong growth advantage of both lpr T and B cells. To distinguish between these possibilities, protocols for overcoming the resistance effect were investigated. Partial donor engraftment for T cells, but not for B cells, has been achieved by pretreatment of lpr recipients with anti-asialo GM1 antibody or by irradiating recipients with a split dose, methods known to eliminate the NK-like populations responsible for Hh resistance.

G 328 HIGH DOSE ANTIGEN ARRESTS GROWTH AND DIFFERENTIATION OF A TNP-SPECIFIC HUMAN B CELL LINE, Basil Golding, Sanford R. Katz, Thomas Hoffman, George C. Tsokos and Stanley Pillemer. FDA and NIDDK, NIH, Bethesda MD 20892.

A human monoclonal B cell line was developed by primary in-vitro immunization with TNP-Brucella abortus, followed by EBV transformation and cloning by limiting dilution. The cells cycle rapidly, and during log phase growth a small percentage of the cells differentiate into anti-TNP secreting cells. The differentiated cells bind TNP and can be enriched by rosetting with TNP-SRBC. They were found to reside mainly in the G1 phase of the cell cycle. To determine if the EBV transformed cells retained responsiveness to external stimuli, TNP on different carriers was added. Addition of TNP-SRBC in increasing doses had a marked inhibitory effect on proliferation and anti-TNP production. The inhibition was more pronounced as the degree of haptenation of the TNP-SRBC was increased, and did not occur in the presence of soluble antigen, indicating that crosslinking was important. Cell cycle analysis revealed that cells treated with TNP-SRBC were arrested in G2M with a concommitant decrease in G1, corresponding with the decreased IgM secretion. These studies demonstrate that the EBV transformed B cell line remains sensitive to external signals. The inhibition observed with high dose antigen may represent the downslope of the antibody response normally observed in primary in-vivo responses.

G 329 SURVIVAL OF MEMORY B CELLS IS DEPENDENT ON THE PERSISTENCE OF ANTIGEN, David Gray, Basel Institute for Immunology, CH-4005 Basel, Switzerland.
Immunisation of chimaeras that have received κ allotype-marked memory cells results in the production of high serum levels of donor antibody that is maintained for many months. Antigen is known to persist within lymphoid tissues for periods in excess of 1 year in the form of immune complex on the surface of follicular dendritic cells. These data suggest that cells within memory clones undergo repeated stimulation over a long period of time. The corollary of this seems to be that the survival of memory B cell clones is dependent on the continued presence of antigen. This has been demonstrated by transfering κ allotype-marked thoracic duct lymphocytes from rats primed with DNP-haemocyanin into irradiated recipients. These chimaeras were then immunised at various periods after transfer (1, 3, 6, 11 weeks). The half-life of memory B cells in the absence of antigen is short (< 2 weeks) and indeed 11 weeks after transfer no memory response can be elicited with soluble antigen. Evidence is also provided that, in vivo, soluble antigen only elicits T cell help if it is processed/presented by "activated" B cells.

G 330 INVOLVEMENT OF SOME GTP BINDING PROTEIN (G PROTEIN) IN RECEPTOR IMMUNOGLOBULIN-MEDIATED SIGNAL TRANSDUCTION IN B LYMPHOCYTES, Subrata Haldar and John G. Monroe, Department of Pathology & Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia PA 19104.
Crosslinking of membrane associated immunoglobulin (mIg) on the surface of B lymphocytes prompts hydrolysis of inositol phospholipids (PI) by a PI specific phospholipase C. The molecular mechanism by which mIg is linked to phospholipase C is unknown. Based upon studies in other receptor systems involving PI hydrolysis, we have investigated involvement of GTP-binding proteins (G-proteins) in the transmembrane machinery linking mIg to inositol phospholipid hydrolysis. We have observed 2-3 fold increases in GTP binding to plasma membranes from normal as well as transformed murine B cells following mIg crosslinking by anti-Ig antibodies. The binding was specific for GTP and could be abrogated by pretreatment of the membranes with pertusis toxin. ADP-ribosylation studies using ^{32}P-NAD has identified a 41 kd protein as the primary pertusis toxin substrate. Finally, incubation of B cell membranes with anti-Ig antibodies in our in vitro system causes hydrolysis of inositol phospholipids as measured by generation of inositol phosphates, and GTP analogues have an augmenting effect on the accumulation of inositol phosphates in the presence or absence of anti-Ig antibody. Importantly, ligand-induced generation of inositol phosphates was inhibited by pretreatment of the membranes with pertusis toxin. These results are consistent with G-protein involvement in transmembrane signalling through mIg leading to inositol phospholipid hydrolysis.

G 331 Functional characterization of an TNP-specific B cell clone established by somatic hybridization. T. Hamano, T. Iwasaki, T. Yamasaki, Y. Murata and K. Nagai
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Splenic B cells of A/J mice primed with TNP-LPS were fused with 2.52M, a HAT-sensitive mutant of a B cell line, in the presence of PEG and DMSO. TP67.21, a subclone of a resulting hybrid, expresses IA^k, IE^k, IgM, B220, FcR, C3R and IL 2R on the cell membrane by FMF analysis. It also expresses receptor molecules for TNP on the cell surface, derived from TNP-reactive B cells of A/J mice immunized with TNP-LPS used for cell fusion, by a rosette forming assay with TNP-SRBC. In contrast, parental 2.52M lacks IA^k and IE^k on the cell membrane and does not bind to TNP-SRBC under the same condition. Thus, it is likely that TP67.21 is an antigen specific B cell clone directed against TNP. The antigen binding of the cells was markedly inhibited by the specific free hapten or anti-IgM antibodies. Interestingly, TP67.21 was induced to generate a significant amount of anti-TNP antibody when treated with TNP-conjugates such as TNP-LPS, TNP-KLH, TNP-BSA and TNP-OVA without T cell help; this was followed by a marked decrease in the expression of B cell surface markers on the cell surface. The present study suggests that the crosslinkage of receptor molecules on TP67.21 by antigen may directly provide a differentiation signal for maturation to a lineage of B cells, and consequently result in the generation of antigen-specific antibodies without T cell involvement.

G 332 IMMUNOGLOBULIN GENE ACTIVATION BY IL5 AND ANTIGEN, Wendy K. Hancock, Susan L. Swain and Richard W. Dutton, University of California, San Diego, La Jolla, CA 92093-0063.

The in vivo passaged CH12 lymphoma differentiates in vitro into antibody secreting cells when two synergizing signals-antigen (SRBC) and IL5 (BCGFII)-are present. 10 to 50 percent of the CH12 cells secrete IgM antibody after 72 hours under these conditions. No differentiation occurs when only one signal is present. cDNA probes for J chain and mu chain were used to investigate the induction kinetics of mRNA in CH12 cells. When both SRBC and IL5 were present, J chain mRNA was detectable at 24 hours and increased to a maximum only after 72 hours. In contrast, a marked increase of mu-secretory mRNA was seen at 24 hours. No J chain message was induced when cells were cultured with either SRBC or IL5 alone. However, moderate levels of mRNA for mu-membrane and mu-secretory were ininduced with IL5 but not SRBC alone. The significance of these and other observations will be discussed.

G 333 INDUCTION OF TWO INDEPENDENT MOLECULES ON THE B CELL SURFACE IMPORTANT FOR T CELL STIMULATION. Catherine M. Hawrylowicz and Emil R. Unanue. Dept. of Pathology, Washington Univ. School of Medicine, St. Louis, MO 63110.

Various recombinant derived cytokines induce a membrane form of interleukin 1 (IL-1) or an activity critical for T cell stimulation in the mixed lymphocyte response (MLR) on the B cell surface. B cells cultured with anti-immunoglobulin antibodies (anti-Ig) plus IL-2 for 2 days express mIL-1. Tumour necrosis factor α (TNFα) or IL-4 plus anti-Ig fail to directly stimulate the B cell mIL-1 expression, but enhance the mIL-1 activity induced by IL-2. Furthermore, interferon gamma (IFNγ) partially inhibits mIL-1 expression induced with IL-2. In contrast, the accessory activity required for stimulatory function in an MLR is induced on the B cell surface following overnight culture with anti-Ig plus IL-2 or IL-4. Unlike most other documented effects of IFNγ on murine B cells, IFNγ synergizes with IL-4 or TNFα to induce this activity and under certain circumstances culture with anti-Ig plus IFNγ alone causes expression of MLR accessory activity. These studies therefore demonstrate that IL-1 is not the sole cofactor required for T cell activation by B cells, and suggest that the nature of lymphokines produced during an immune response may bias which accessory molecule will be expressed by the B cell.

G 334 STUDY OF THE IMMUNODEFICIENCY OF RIIIS/J MICE, Jacques R. Hiernaux, Edmond A. Goidl* and Phillip J. Baker, NIAID, Bethesda, MD 20892 and* University of Maryland, Baltimore, MD 21201.

RIIIS/J mice lack an autosomal dominant gene(s) influencing the magnitude of the IgM antibody response to several representative helper T cell-independent antigens (Type III pneumoccocal polysaccharide (SSS-III), E. Coli 0113 and E. Coli 055 LPS, $\alpha(1\rightarrow3)$ Dextran, Bacterial Levan and phosphorylcholine (PC) present on the outer membrane of R36A bacteria. However, they give a good IgM antibody response to TNP-Ficoll and TNP-Dextran as well as to SRBC, a T-helper T cell-dependent antigen. By contrast, the PC-specific immune response to PC-HRBC is very low. Low responsivenes is not X-linked. In the case of SSS-III, low responsiveness is not due to an overexpression of suppresor T cell activity, or to a lack of amplifier T cells. Most strain of mice develop an oscillatory immune response after a single injection of LPS of E. Coli 0113 and they can be primed for memory by injection of small doses of LPS of E. Coli 0113; RIIIS/J mice do not elicit an oscillatory response and do not express immune memory to LPS of E. Coli 0113. RIIIS/J B cells give a normal proliferative response to the LPS of E. Coli 0113 in vitro. Interestingly, RIIIS/J mice do not produce an IgM antibody response to bromelain treated mouse red cells. They also have a low level of circulating IgM and IgG_3. This suggests that they lack the Lyl^+ subset of B lymphocytes. Nevertheless, they produce an antibody response to mouse transferrin and they develop an auto-antiiodiotypic response in the course of an anti-TNP immune response. Thus RIIIS/J mice, like CBA/N mice, might provide another example of an experimental model that can be used to study antigen-induced B cell activation.

G 335 EFFECTS OF MURINE BSF-2 ON THE PROLIFERATION OF B AND T LYMPHOCYTES.

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The recent cloning of murine BSF-2 (Lee, et al., manuscript in preparation) has allowed an examination of the effects of this factor on T and B lymphocyte growth. Preliminary results suggest that BSF-2 has little effect on proliferation of either small resting B cells or in vivo activated large B cells. Recombinant murine BSF-2 does enhance proliferation of plasmacytomas.

We have also found that recombinant murine BSF-2 acts on thymocytes by increasing their proliferative response to IL-4/PMA by 2-3 fold. This proliferation enhancement is restricted to the L3T4 single positive thymocyte populations. The mechanism of enhancement is being investigated, however it is clear from preliminary experiments that BSF-2 does not act on its own as a growth factor for IL-4/PMA activated thymocytes.

G 336 V-REL EXPRESSION INDUCES A VARIETY OF GENOTYPICALLY DISTINCT IgM POSITIVE B LYMPHOMAS.

Carolyn F. Barth and Eric H. Humphries, Southwestern Medical School, Dallas, Texas 75235

Infection of chicks with reticuloendotheliosis virus-strain T (REV-T) induces metastatic tumors that remain unclassified. It has been suggested that they may be early lymphoid tumors. We have found that the helper virus, REV-A, with which REV-T replicates, induces significant bursal atrophy that is associated with a loss of bursal function. Based upon this observation, we infected chicks with REV-T and a non-immunosuppressive helper virus. These chicks develop rapidly metastatic B-cell lymphomas capable of proliferation both in multiple microenvironments as well as in vitro. In situ analysis of these tumors indicates that they express IgM as well as 3 distinct cell surface markers associated with the avian lymphoid lineage. Analysis of both heavy and light chain immunoglobulin loci demonstrates that tumor-derived cell lines possess a variety of distinct genotypes. Some cell lines appear to have been blocked in differentiation prior to gene conversion. This finding suggests that v-rel and c-myc, another oncogene involved the development of avian B-cell lymphomas, use different mechanisms to block lymphocyte differentiation.

G 337 ACTIVATING MUTATIONS OF THE C-ABL PROTOONCOGENE.

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Studies of the two major c-abl protooncogene messages (types I and IV) suggest that retroviral overexpression of the genes coding for these two messages is not sufficient for transformation of murine fibroblasts. The type IV c-abl gene has a consensus sequence for N-terminal myristylation and has an N-terminus homologous to the c-src protooncogene. The lack of transformation by c-abl type IV is reminiscent of the differences between c-src and v-src. We have characterized 3 classes of mutations that activate c-abl IV, expressed in a retroviral vector, to transform murine fibroblasts: 1) addition of gag sequences; 2) N-terminal small deletions and linker insertions; 3) C-terminal point mutations. Lymphoid transformation by these viruses is under examination. These results will be considered in light of a model of regulation of c-abl and by way of comparison to analogous mutations in c-src.

G 338 THE ROLE OF THE B CELL IN THE ACTIVATION OF SUBSETS OF CD4-POSITIVE T CELLS HAVING DISTINCT FUNCTIONAL ACTIVITIES, Charles A. Janeway, Jr., Joseph Murray, Michael E. Katz, Barbara Broughton and Kim Bottomly, Department of Pathology and Section of Immunobiology, Howard Hughes Medical Institute at Yale University School of Medicine, New Haven, CT. 06510.
The activation of CD4-positive T cells is a critical event in the induction of an immune response. Two subsets of CD4 T cells have been defined, which we term helper and inflammatory T cells. These cells differ in their dominant functional activities, with the activation of resting, antigen-specific B cells being predominantly or exclusively a function of helper T cells. These CD4-bearing T cells are also distinguished by the lymphokines they secrete: Helper T cells secrete IL-4 as their typical lymphokine, while inflammatory T cells produce IL-2, interferony (IFN) and lymphotoxin (LT). We have observed that immunization of B cell depleted mice leads to the selective activation of CD4 T cells of the helper type, and no apparent activation of inflammatory T cells, as assayed by functional activity. In the present study, this is confirmed by analysis of the lymphokines produced by cells immunized with protein antigens in B cell deprived mice. We interpret our studies to mean that B cells are the initiating antigen presenting cell for inflammatory T cells responding to protein antigens, while helper T cells are immunized by another type of antigen presenting cell. We propose that the helper cell is primed by a macrophage, since helper T cells require IL-1 for autocrine growth, and since IL-1 is produced predominantly by macrophages. Finally, we propose that B cells are required for the activation of inflammatory T cells by protein antigens because of the ability of B cells to generate a high density of antigen:Ia ligand. Physiologically, macrophages infected with intracellular bacteria are probably the major inducing antigen presenting cell for inflammatory T cells. Supported by NIH grants AI-14579, CA-29606, and the Howard Hughes Medical Institute.

G 339 IMMUNOREGULATORY ROLE OF INTERLEUKIN 4 ON HUMAN B CELL RESPONSIVENESS. DF Jelinek and PE Lipsky. Southwestern Medical School, Dallas, TX 75235.
The role of interleukin 4 (IL-4) in human peripheral blood B cell activation, proliferation, and differentiation was examined. Highly purified B cells stimulated with Cowan I Staphylococcus aureus (SA) proliferated minimally and generated no immunoglobulin secreting cells (ISC). Recombinant interleukin 2 (rIL-2), but not recombinant IL-4 (rIL-4), alone was able to promote maximum proliferation and generation of ISC in cultures of SA-stimulated B cells. Addition of rIL-4 to rIL-2 supported cultures of SA-stimulated B cell cultures dramatically suppressed both B cell proliferation and differentiation. rIL-4 had to be present during the first two days of a five day incubation to cause inhibition of responsiveness. When a two-stage culture system was utilized, the presence of rIL-4 during the initial 48 hr activation of B cells with SA and rIL-2 resulted in a profound inhibition of the ability of the activated B cells to respond subsequently to rIL-2 or crude T cell factors. A similar 48 hour incubation with rIL-4 alone without SA had no effect on subsequent B cell responsiveness. rIL-4 partially inhibited the expression of the gp55 component of the IL-2 receptor by the activated B cells, as well as two additional activation antigens, the 4F2 molecule and the transferrin receptor. The presence of recombinant interferon (IFN)-gamma during B cell activation decreased the inhibitory effect of IL-4. Other cytokines including IFN-alpha, interleukin 1 and B cell growth factor, but not tumor necrosis factor alpha also diminished the inhibitory effect of IL-4. These results indicate that IL-4 inhibits the capacity of human peripheral blood B cells to be activated maximally by SA and rIL-2 and therefore suggest a new immunomodulatory role for this cytokine.

G 340 GENERATION OF SPECIFIC IgG SECRETING PFCs FROM MURINE IN VITRO IMMUNIZATION, L.M. Karavodin, J.L. Phelps and B.S. Wilson. Hybritech, Incorporated, San Diego, Ca. 92126
Requirements for murine primary and secondary antigen-specific in vitro immunization have been examined. We investigated parameters generating specific Ag binding IgG plaque forming cells (PFC) and hybridomas. With soluble HSA and OVA as model systems, Ag concentration and addition of specific T helper cells were found to be critical for optimizing the primary IgM response (as measured by Elisa-plaque assay and analysis of hybridomas). Although a wide range of Ag concentrations gave specific PFCs, the range capable of giving specific IgM producing hybridomas was narrow. OVA specific helper T cells added in the first five days of culture gave a several-fold increase in the number of PFC and % specific hybrids. For the secondary response, dose and kinetic dependant lymphokine addition was essential for generating specific IgG PFC. Mixed thymocyte culture media during the first four days of culture was necessary to generate both IgM and IgG Ag specific PFC. Long term culture could be supported with high concentrations of rIL-2 and induced to differentiate to Ag-specific IgG secreting PFC with IL-4. A large portion of the early Ab response is polyspecific. These data suggest that T cell help and lymphokine conditions are critical factors in isotype switch from in vitro immunization cultures.

G 341 MODULATION OF MURINE B LYMPHOCYTE ACTIVATION BY THREE RAT MONOCLONAL ANTIBODIES AGAINST THE TRANSFERRIN RECEPTOR, John Kemp, Paul Naumann, and John Thorson, University of Iowa College of Medicine, Iowa City, IA 52242.

Although the growth of B cells is known to be dependent upon the uptake of extracellular iron via the transferrin receptor, there is very little information available about whether lymphocyte subsets might vary with respect to their dependency upon normal transferrin receptor function. We have previously shown that an ATRA which we developed (C2F2) exhibits highly selective inhibitory effect on lymphocyte activation.[1] Although C2F2 reacts with both T and B cell blasts, and is as powerful as other ATRAs in the inhibition of T lymphocyte activation, it is remarkable for its inability to inhibit B lymphocyte activation. In order to learn more about these unexpected results, we have performed a side-by-side comparison of three ATRAs in T and B cell activation assays using several strains of mice, and have also carried out experiments aimed at understanding the mechanism of action of ATRAs. The results indicate that although T cell activation is more sensitive to ATRA mediated inhibition than is B cell activation, the strain being tested is a significant variable, just as is the monoclonal antibody being employed. These studies raise new questions about the roles of iron, transferrin and the transferrin receptor in lymphocyte activation in general, and add new intricacies to our understanding of the process of B lymphocyte activation in particular.

[1] J Immmunol 138(8):2422-2426, 1987. Supported by NIH Grant CA37252.

G 342 ALTERATION OF THE B CELL SURFACE PHENOTYPE, IMMUNE RESPONSE TO PC-KLH, AND THE B CELL REPERTOIRE IN M167 MU PLUS KAPPA TRANSGENIC MICE, James J. Kenny*, Fred Finkelman#, and Dan L. Longo$, *Program Resources Inc., NCI-FCRF, Frederick, MD 21701; #Dept. Medicine, Uniformed Services Univ. of the Health Sciences, Bethesda, MD 20814; $Biological Response Modifiers Program, NCI-FCRF, Frederick, MD 21701.

M167, mu plus kappa, transgenic mice have been analyzed for the expression of the transgene product as a cell surface, antigen-specific receptor and for their ability to respond to antigen. The vast majority of B cells in these H+L transgenics (97-99%) express large amounts of the transgene product on their surface and are capable of binding phosphocholine. Five to 15% of the B cells also express endogenous IgM and IgD heavy chain products. Following immunization with phosphocholine conjugated keyhole limpet hemocyanin (PC-KLH), greater than 1000 ug/ml of anti-PC antibody bearing the transgene IgM-A allotype marker are produced. Surprisingly, significant amounts of endogenous, IgM-B allotype, anti-PC, antibodies are also produced; however, these antibodies lack the T15-idiotype which dominates the anti-PC response in their non-transgenic littermate controls. The B cells producing these endogenous anti-PC antibodies also fail to switch to IgG synthesis, whereas, B cells producing anti-KLH antibodies readily undergo class switching. These findings may have important implications regarding repertoire development in transgenic mice, and such mice should prove to be a valuable source of essentially monoclonal, normal, resting B cells for biochemical studies involving antigen-induced B cell activation, proliferation and differentiation.

G 343 IMMORTALIZATION AND TRANSFORMATION OF MURINE PRE-PRE B CELLS IN VITRO. J.R. Kettman, B. Ozanne and G.J. Palumbo*, Department of Microbiology, University of Texas Health Science Center, Dallas, Texas 75235 and *Lab of Viral Disease, NIAID, National Institutes of Health, Bethesda, Maryland 20892.

We examined the tumorigenic potential of pre-pre B cells which represented different stages of Abelson murine leukemia virus (A-MuLV) induced transformation based on their growth potential *in vitro*. Cell lines with high growth potential were factor-independent in liquid culture, they were able to form colonies in semi-solid medium, and they formed tumors when inoculated into syngeneic mice. In contrast, cell lines with low growth potential in liquid culture were found to be dependent for growth in a dose dependent manner on conditioned medium obtained from Whitlock-Witte bone marrow cultures, they also had low colony forming ability in semi-solid medium, and they were not tumorigenic when inoculated into syngeneic mice. Both the factor-dependent cells and factor-independent cells contained Abelson provirus. A selection pressure which exists with culture of the factor-dependent cells *in vitro* leads to the emergence of factor-independent variants and the eventual progression of the factor-dependent population to factor-independence. Progression is due to the overgrowth of factor-dependent cells by factor-independent cells. With the emergence of factor-independent cells the cell line concomitantly becomes tumorigenic. Our results suggest that A-MuLV is sufficient to initiate transformation in the infected cell, but that an additional alteration is needed to confer a tumorigenic phenotype.

G 344 GENE REARRANGEMENTS AS TUMOR SPECIFIC MARKERS, Ilan R. Kirsch and Nita L. Seibel, NCI-Navy Medical Oncology Branch, National Institutes of Health, Naval Hopsital, Bethesda, Maryland 20814

We are trying to develop a procedurally simple rapid turnaround technique for utilizing tumor specific gene alterations as tumor specific markers. We have been able to determine the nature of a class of tumor specific gene rearrangements by direct sequencing of selected mRNAs extracted from tumor cells. In a pilot experiment we were able to determine the sequence of an immunoglobulin kappa variable region expressed by a certain Burkitt's lymphoma cell line by directly sequencing total RNA extracted from the tumor using a reverse transcriptase dideoxy nucleotide method with a 5' kappa constant region complementary oligonucleotide as a primer. A radiolabelled synthetic oligonucleotide complementary to the CDR3 segment of this K_V region uniquely recognized the Burkitt's cells when a mixture of lymphocytes was analyzed using the technique of tissue in situ hybridization. The technique is rapid and sensitive and when fully refined holds potential for increased sensitivity of diagnosis, classification, staging, and possibly treatment of a range of malignancies.

G 345 CO-EXPRESSION OF SURFACE IgM AND PRE-B CELL MARKERS IN MURINE CELL LINES, W. Michael Kuehl and Timothy P. Bender, National Cancer Institute, Bethesda, MD 20814 University of Virginia, Charlottesville, VA 22908.

The 18.81 Abelson virus transformed pre-B cell line expresses cytoplasmic mu heavy (H) chain. We have isolated several clones which spontaneously have formed a kappa light (L) chain gene by DNA rearrangement. Two of these clones express surface IgM but continue to express high levels of four markers which distinguish pre-B from B cells, i.e. c-myb, N-myc, and $\lambda 5$ mRNAs plus BP1 surface antigen (none of these markers are expressed in the WEHI 231 or Bal 17 immature B cell lines). Somatic cell hybrids formed by fusion of the 70Z/3B pre-B cell line (μ^+, L^-, La^-, J chain$^-$) with the A20/2J mature B cell line ($\gamma 2a^+$, L^+, La^+, J chain$^+$) co-express the H and L chain genes of each parent. Yet these hybrids have a phenotype which is otherwise identical to the 70Z/3B pre-B parent, including expression of high levels of c-myb and $\lambda 5$ mRNAs. These results indicate that expression of surface Ig is not a reliable marker to distinguish pre-B from B cells.

G 346 ANALYSIS OF THE MURINE COMPLEMENT RECEPTOR GENE FAMILY. C.B. Kurtz, M. Shaw, M.D. Miller, J.H. Weis, Department of Medicine, Harvard Medical School, Boston, MA 02115.

The human and murine complement receptors 1 and 2 are two related proteins which serve as ligands for specific proteolytic products of C3, and CR2 is the receptor for Epstein-Barr virus on the surface of human B lymphocytes. Using the human CR1 and CR2 cDNA's as probes, we have initiated experiments to define the genetics of the murine counterparts and to understand their biological roles. This analysis has been complicated by the fact that the murine homologues to the human proteins are not tightly conserved between the two species, that is, the human CR1 gene which encodes a protein of 200,000mr is most closely related to a series of murine genes which encode membrane proteins of 50,000mr. However, the murine and human CR2 genes are very tightly conserved in both sequence, size of the mRNA, chromosomal location and their tissue specific expression (B lymphocytes). Chromosomal walking experiments have linked the murine CR1/CR2 gene(s) to the second set of murine genes which are the human CR1 homologues. Comparison of this linkage and sequence analysis of these genes allows us to propose an evolutionary hierarchy of this related gene family.

G 347 IMMUNOMAGNETIC PURGING OF B-LYMPHOMA CELLS, Gunnar Kvalheim, Øystein Fodstad, Steinar Funderud, Bernt Dörken, Otto Sørensen, Kjell Nustad, John Ugelstad, and Alexander Pihl. The Norwegian Radium Hospital, 0310 Oslo 3, Norway (1), University of Heidelberg, West Germany (2), and SINTEF, Trondheim, Norway (3).
Previously we have reported the use of super-paramagnetic polymer particles (Dynabeads M-450) coated with high affinity monoclonal antibodies of the IgM isotype to remove B-lymphoma cells from human bone marrow. We have now developed an indirect method in which we incubate first with a panel of 3 different IgG1 pan-B-cell MoAbs, HD37 (CD19), HD6 (CD22), and HHI (CD37), and then with immunobeads (IBs) charged with antibodies directed against the Fc-portion of the primary MoAbs. Rael cells were admixed to mononuclear bone marrow cells in the ratio 1/9. With a ratio of IBs/total antibody-binding B-cells of 50/1 and two different cycles, a tumor cell depletion of more than 5 logs was achieved, as judged by a clonogenic assay. The concomitant reduction of CFU-GM and CFU-GEMM was about 10%. MoAbs recognizing the CD 19,22 and 37 antigens were tested with complement and as immunotoxins. The immunomagnetic purging was by far the most efficient method and has been scaled up to clinical use employing a closed transfusion bag system. Some preliminary data from patients will be presented.

G 348 IMMUNE DEFICIENCIES IN A TRANSGENIC MOUSE LINE, Marinus Lamers°, Rita Carsetti°, Jean Langhorne°, John Kearney* and Georges Köhler°, °Max-Planck-Institut für Immunbiologie, Stübeweg 51, 7800 Freiburg, FRG and *University of Alabama in Birmingham, 224 Tumor Institute, Birmingham, AL 35294, USA.
We have analyzed the immune response of a mouse transgenic for a mu heavy and kappa light chain gene with specificity for TNP. In this mouse line (Sp6 μ,k) the B-cell pool is reduced by 30%. The functional B-cell pool is reduced by 75%, because only 30% of the B-cells express endogenous genes while all B-cells express the transgenes. This is reflected in a severely impaired primary antibody response to T-cell dependent antigens, in particular $\delta^{}_{3}$, $\delta^{}_{2b}$ and $\delta^{}_{2a}$ responses are low (5%-10% of normal). Secondary responses, however, are only moderately effected with the exception of $\delta^{}_{3}$ antibodies which are virtually absent. We postulate that these - low - responses can be explained by the low number of functional B-cells.
In contrast the response to certain T-cell independent, bacterial antigens is totally absent, e.g. the response to α1,3 dextran, levan and phosphoryl choline (T15 positive). Precursors for α1,3 dextran however are present, albeit at a lower frequency (3-10x lower). At present we are testing mouse lines transgenic for the same variable region (derived from Sp6), but with a different constant region. Possible explanations for the failure to respond to T-independent antigens are: 1) ineffective activation of B-cells 2) inactivation due to idiotypic network interactions 3) lack of T-help.

Antigen Presentation, Autoimmunity, Immunodeficiency, Oncogenes, Activation, Transgenic Models - II

G 400 HYBRIDOMAS EXPRESSING A REPRESSOR OF IMMUNOGLOBULIN GENE ACTIVATION, Alexander R. Lawton and Vernon S. Summerlin, Vanderbilt University School of Medicine, Nashville, TN 37232
Differentiation of murine B lymphocytes induced by bacterial lipopolysaccharide (LPS) is inhibited by high concentrations of anti-μ antibodies, while proliferation is enhanced. Treatment with anti-μ greatly reduces concentrations of mRNA's for μ, κ, and J chains, but not for class I or class II histocompatibility antigens. This specific inhibition of transcriptional activation requires RNA and protein synthesis, suggesting mediation by a trans-acting repressor (J. Exp. Med. 166:864, 1987). We predicted that hybridomas derived from anti-μ treated B lymphocytes might constitutively express this putative repressor. A series of fusions of spleen cells treated with LPS + anti-μ or only LPS with Ag8.653 produced negative results. Anti-μ treatment reduced the frequency of hybridomas, but the fraction secreting IgM was the same as in controls. Fusions with the B lymphoma line M12.4.5 generated similar numbers of hybridomas from anti-μ treated and control cells, but fewer of the former secreted IgM (63% vs 86% at 24 hrs, 20% vs 86% at 48 hrs, 30% vs 88% at 72 hrs). To test for the repressor phenotype (RP) we determined the secretor status of secondary hybridomas. Non-secretor hybridomas chosen at random were recloned and grown in 8-azaguanine to select HAT-sensitive revertants. Six hybridomas derived from anti-μ treated cells (putative RP hybridomas) and 2 control hybridomas were fused to LPS-stimulated B cells. Control fusion partners yielded 35% and 45% IgM secreting hybridomas. Four fusions with RP hybridomas yielded secondary hybrids of which 0-9% synthesized IgM. The other 2 yielded intermediate frequencies of secretors (25% and 17%). These hybridomas may be a source of a unique DNA-binding regulatory protein.

G 401 A 53-KD PROTEIN COMPLEXED WITH P210$^{BCR-ABL}$ IN CELL LINES DERIVED FROM PHILADELPHIA CHROMOSOME (PH')-POSITIVE CHRONIC MYELOGENOUS LEUKEMIA (CML) PATIENTS. Wanjun Li[1], William S. Kloetzer[2] and Ralph B. Arlinghaus[1], [1]Univ. of Texas System Cancer System, M. D. Anderson Hosp. & Tumor Inst., Houston, TX 77030, [2]J & J Biotech Center, La Jolla, CA 92122. Leukemic cells from patients with Ph'+ CML contain a 210 kd protein (P210$^{bcr-abl}$) with a protein tyrosine kinase activity. We have prepared two monoclonal antipeptide antibodies, one from each gene, and have affinity purified each. Incubation of anti-abl (c-abl 51-64) immunoprecipitates of K562 cells with [γ^{32}P ATP] in protein kinase assays resulted in the labeling of P210$^{bcr-abl}$ and a 53 kd (P53) protein. Increasing concentrations of antibody detected similar ratios of P210$^{bcr-abl}$/P53 suggesting the presence of a complex between the two proteins. Of three different anti-bcr antibodies tested, only one detected the complex. SDS without 2-mercaptoethanol eluted P210$^{bcr-abl}$ and P53 from the monoclonal antibody in the form of complexes which migrated on a 6% SDS gel with a mol. wt. of 275 kd and more than 500 kd. Both complexes yielded P53 and P210$^{bcr-abl}$ upon treatment with SDS-mercaptoethanol. Further studies have indicated that P53 is not a fragment of P210; it is not the phosphorylated H chain of IgG; and it is not a cross-reactive abl-related protein. Previous studies have detected this protein in glycerol gradients as a 300 kd P210 complex which was precipitated with anti-abl (v-abl 389-403) sera (Maxwell et al., Cancer Res. 47:1731, 1987). P53 is phosphorylated on tyrosine and serine, and it is unrelated to P210 by Cleveland gel analysis. Studies are underway to determine the significance of the P210:P53 complex and the relationship of P53 to other known proteins such as the P53 protein complexed to the large T antigen of simian virus 40.

G 402 "NATURAL" IMMUNE ACTIVITIES VERSUS IMMUNE RESPONSES.
Inger Lundkvist, Denis Portnoi, Dan Holmberg and Antonio Coutinho, Department of Applied Cell and Molecular Biology, Umeå University, S-901 87 Umeå, Sweden and Department of Immunobiology, Institut Pasteur, F-757 24 Paris, France.

We have analysed the regulatory mechanisms governing the immune response to Dextran α,1-6 (Dex), and recently reported on the inverse correlation between the utilization of an anti-Dex Id (17-9) in specific immune responses versus its representation in pre-immune, natural antibodies. Thus, the small resting cells - which are not included in the expressed, actual repertoire - can be triggered by external antigen and give rise to "classical" immune responses. The large, internally acativated cells, on the other hand - the precursors for the natural antibody-producing cells found in the serum of normal, non-manipulated mice - are, most likely, involved in network-like interactions and have been shown to be biased for self-reactivity. We have now extended our studies on this issue and analysed the influence of the state of activation of Dex-specific and Id-positive clonal B cell precursors on the secondary response to thymus-independent antigens. Our results, suggesting that Id-specific regulation contributes to specific unresponsiveness in primed mice, will be discussed.

G 403 THE SUPEROXIDE GENERATING SYSTEM OF EBV-TRANSFORMED B CELL LINES (EBV-BLCL): STRUCTURAL HOMOLOGY WITH THE PHAGOCYTIC OXIDASE AND TRIGGERING VIA SURFACE IMMUNOGLOBULIN. #F.E. Maly, *A.R. Cross, *O.T.G. Jones, #C. Walker, #C.A. Dahinden and #A.L. De Weck, #Institute of Clinical Immunology, Inselspital, Bern, Switzerland and *Dept. of Biochemistry, University of Briston, UK.

We analysed EBV-BLCL generating superoxide in response to PMA (1). Phagocyte-typic low potential cytochrome b_{-245} was found in EBV-BLCL and also a 45 kDa Diphenylene-Iodonium -(DPI)-binding peptide, which we believe to be part of the phagocytic NAD(P)H-oxidase.Also,DPI inhibited O_2^- -production by PMA-stimulated EBV-BLCL and PMN.Further,EBV-BLCL showed O_2^- - production after addition of protein A - bearing staphylococci, but not of staphylococci devoid of protein A. Such response could also be triggered by a monoclonal antibody directed against human IgG.In conclusion, superoxide production by EBV-transformed B lymphocyte lines appears related to expression of an electron transport chain structurally homologous, if not identical, to the "phagocytic" NAD(P)H -oxidase. Possibly also non-transformed B cells may, at a certain differentiation stage, express a superoxide generating chain, which might have an important role, e.g. in inflammation or autoimmunity.
(1) Volkman, D.J., Buescher, E.S., Gallin, J.I. and Fauci, A.S. (1984) J. Immunol. 133:3006-3009.

G 404 HUMAN RETROVIRUS MAY PLAY AN INDIRECT ROLE IN B CELL NEOPLASIA, Dean L. Mann, Mikulas Popovic, George Mark, and William Blattner, NCI, Bethesda, MD 20892. B cell chronic lymphocytic leukemia (CLL) has been shown to occur more frequently in HTLV-I seropositive individuals than would be expected based on serologic screening in a population where this retrovirus is endemic. In addition, an increased incidence of B cell lymphomas has been shown to occur in HIV-1 infected individuals. We tested the hypothesis that these tumors may be an antigen committed B cell that had undergone malignant transformation in the following experiments: HTLV-I negative CLL cells from 2 HTLV-I seropositive patients (IC, LL) and 1 seronegative patient (HP) were fused with a HAT sensitive B lymphoblastoid cell line. The resulting hybridomas were cloned and the culture fluids tested for antibody activity to HTLV-I protein. Igm was produced by all the hybridomas from these fusions and reacted as follows: IC with HTLV-I p-24 gag protein, LL with HTLV-I large envelope, and no reaction to viral proteins with HP. HIV-1 negative B cell lymphomas were explanted from brain from 2 patients with AIDS and placed into culture. The cultured cells were cloned and culture fluids tested for reactivity with HIV-I. The IgG from one tumor reacted with the large envelope protein from HIV-1 on Western blots. The IgG from the 2nd tumor reacts with HIV-1 in ELISA assays and is being further characterized. These results suggest a possible indirect causative role for human retrovirus in B cell neoplasia.

G 405 **IDIOTYPE AS A BIOMARKER FOR THE IMMUNE RESPONSE OF THE AGING MOUSE** Susan J. Martin McEvoy, George K. Lewis and Edmond A. Goidl. Dept. of Microbiology and Immunology, School of Medicine, University of Maryland at Baltimore, Baltimore, Maryland 21201.

We have analyzed the anti-2,4,6-trinitrophenyl (TNP) antibody response of aged C57Bl/6NNIA mice (23-24 mo) at the monoclonal antibody (MAb) level. This response has been compared to MAbs obtained from young (2-3mo.) mice. MAbs were screened by plaque-forming cell assay (PFC). They were then tested against Bromelain autologous erythrocytes (Br-MRBC) and mouse transferrin (mTransf) as self-antigens in PFC assays. Ten of eleven MAbs from aged mice reacted to one or more self-antigens. Eight out of eleven were triple reactors (TNP, Br-MRBC and mTransf). Four of these Mabs also carried the AD8 idiotype which is a major cross-reactive antibody system. Two doubly-reactive MAbs were $AD8^+$ and one MAb was reactive only towards TNP was also $AD8^+$. Four MAbs out of eleven were $AD8^-$. Little anti-self cross-reactivity was seen among MAbs obtained from young mice and all were $AD8^-$. The AD8 idiotype may be a biomarker of the imunoglobulin idiotype repertoire expressed in the immune response of the aging mouse. (Supported by USPHS grant AG-04042)

G 406 Effects of Interleukin-4 Upon Protein Kinase Activities Associated with Murine B-Lymphocyte Plasma Membranes.
Gail McL. McGarvie, Valerie, A. Beattie and William Cushley, Department of Biochemistry, University of Glasgow, GLASGOW G12 9QQ, Scotland, U.K.
Interleukin-4 exerts specific immunological effects upon B lymphocytes including increased expression of MHC class II molecules and F_c receptors for IgE. IL-4 also acts as a competence factor for B cells rendering them responsive to signals received via the cell membrane receptor for antigen (i.e. membrane Ig). The signal transduction systems activated by IL-4 have not been identified, but recent data have suggested that hydrolysis ofinositol lipids is not involved.
The effects of addition of IL-4 to plasma membranes isolated from small, resting cells and from low density blast cells following 24 or 48 hr LPS stimulation upon protein kinase activites have been evaluated. IL-4 treatment caused a small increase in incorporation of ^{32}P (from $-^{32}P$-ATP) into total, acid-insoluble membrane protein in cell membrane preparations. Analyses of phosphoamino acid content suggested that the majority of ^{32}P was incorporated into serine phosphate; only a very small increase in tyrosine phosphate content was noted. Analysis of SDS-PAGE profiles of total phosphoproteins revealed no pronounced changes following IL-4 treatment, although a 45-47kD band was reproducibly phosphorylated. The specific phosphorylation of peptides of defined sequence will also be described.

G 407 REGULATION OF B CELLS BY NK CELLS. A. Michael, J. Hackett,Jr. V.Kumar, M. Bennett, D. Yuan. University of Texas Southwestern Medical Center. Dallas, Texas, 75235

In addition to their ability to lyse tumor cells recent evidence suggests that NK cells can regulate hematopoesis and other lymphoid subpopulations. We have utilized purified, IL-2 propagated NK cells obtained from mice with severe combined immunodeficiency to investigate possible regulatory effects of NK cells on B cells. Splenic B cells were fractionated by Percoll gradient centrifugation into small resting and large activated subpopulations. NK cells were able to decrease the proliferative response to LPS of the large activated B cells but had no effect on the response of small resting B cells. Experiments aimed at elucidating the mechanism of NK-mediated inhibition of activated B cells have shown that NK cells release gamma interferon upon interaction with activated B cells. The anti-proliferative effect of NK cells on large B cells can be reversed by antibodies to gamma interferon. NK cells were unable to lyse either population of B cells; however, in a cold target competition assay large activated B cells but not resting B cells reduce the killing of YAC-1 cells. Therefore NK cells recognize B cells at a certain stage of maturation, and regulate their activity via gamma interferon secretion.

G 408 EFFECTS OF N-RAS ONCOGENE EXPRESSION ON GROWTH AND DIFFERENTIATION FACTOR REQUIREMENTS OF MURINE B-CELLS. *Carol A Midgley, Ian A McKay, David J Chiswell, Amersham International, Amersham, England HP7 9LL.*

While a close association has been demonstrated between mutations in the human N-ras gene and various forms of human leukaemia (McKay I A et al Anticancer Res. 6, 483, 1986), little work has been done on expression of exogenous N-ras in cells of the haemopoietic lineage. We have constructed a murine retrovirus which expresses the human N-ras proto-oncogene with an ^{12}ASP mutation which renders it oncogenic. This virus, which transforms murine fibroblasts, has been used to infect murine cells of the B-cell lineage in order to study the effects of N-ras expression on the growth and differentiation of such cells. Infection of the myeloma cell line X63-Ag8.653 results in a dramatic alteration of cell phenotype. In infected cultures very large cells appear which, despite their capacity for continued division, require the support of uninfected 653 cells for cloning. Maintenance of cloned N-653 cells in culture for prolonged periods results in the production of increasing numbers of very large cells. These two facts, taken together, suggest that N-ras expression has altered the requirements of 653 cells for growth and for differentiation factors. Results will be presented on the responses of these cells to a number of B-cell growth and differentiation factors including IL-4, LPS, and TPA.

G 409 ROLE OF B CELLS IN ACTIVATION OF CONTRASUPPRESSOR T CELLS (Tcs) BY TYPE 3 PNEUMOCOCCAL POLYSACCHARIDE (S3). Helen Braley-Mullen, University of Missouri, Columbia, MO 65212.

Previous studies suggested that B cells might play a role in Tcs activation by S3 since Tcs could be activated only under conditions that also resulted in induction of anti-S3 antibody. Moreover, Tcs were restricted to interacting with target cells from mice compatible at the immunoglobulin heavy chain variable region with the Tcs donors and Tcs could not be induced in mice depleted of B cells by treatment from birth with anti-IgM. CBA/N x Balb male (xid) mice which are unable to produce antibody to S3 do not develop Tcs after immunization with S3. This is not due to an absence of S3-specific Tcs in xid mice but to the absence of a B cell population required for Tcs activation. If xid mice receive splenic B cells from normal Balb x CBA/N males prior to immunization with S3, active Tcs are obtained; B cell-depleted spleen cells are ineffective. The B cells given to the xid mice are not the source of Tcs since irradiated xid donors given B cells and S3 do not develop Tcs. Irradiation (1500R) of the B cells does not prevent their ability to induce Tcs in xid mice; thus cell division or production of anti-S3 is not required. That the B cells are functioning to present S3 to Tcs is suggested by the observation Tcs can be activated by S3 coupled to spleen cells (S3-SC) only if the SC contain B cells; S3-SC prepared from B cell depleted normal SC or from xid SC do not induce Tcs. Moreover, Balb x CBA/N B cells are unable to induce Tcs in xid mice when B cell donors are pre-immunized with S3 under conditions which induce tolerance in B cells. (NIH Grant CA25054.)

G 410 ANTIGEN PROCESSING BY ANTIGEN-SPECIFIC MURINE B LYMPHOCYTES, Christopher D. Myers and Ellen S. Vitetta, University of Texas Southwestern Medical Center at Dallas, Dallas, Texas 75235.

Much knowledge has recently been accumulated about antigen presentation. Cells, receptors and accessory molecules have been identified and regions of many antigens which can be presented to T cells have been defined. However, antigen processing, the mechanism by which antigen-presenting cells modify native antigen and express it on the cell surface such that it can be recognized by T cells, remains very poorly understood. In order to begin analysis of the biochemical events underlying antigen processing, we have utilized a highly purified population of trinitrophenyl-binding murine B lymphocytes (TNP-ABC). This allows us to compare processing of TNP-haptenated antigen and anti-Ig, a commonly used antigen analog. Antigen bound to TNP-ABC is degraded to form acid soluble fragments in the supernatant over a period of 4-6 hours. Appearance of these fragments in the supernatant parallel the kinetics with which antigen-pulsed B cells are able to present antigen to T cells as measured by T/B conjugation. Anti-Ig is also degraded to acid soluble peptides, levels of which plateau after 12 hours of incubation. Degradation of anti-Ia or anti-H-2 occurs much more slowly, but fragments continue to appear in the supernatant at a constant rate for greater than 24 hours. More recent studies have compared antigen processing by memory and virgin TNP-ABC. Memory cells are able to bind more antigen than virgin cells, especially at low antigen concentration. They also digest slightly more of the antigen bound. However, the kinetics of digestion were not significantly different between memory and virgin cells.

G 411 ALLELIC EXCLUSION IN TRANSGENIC MICE THAT CARRY HUMAN IgM HEAVY CHAIN GENES. M.C. Nussenzweig, A.Shaw, E. Sinn, K. Holmes, H. Morse, and P. Leder, Harvard Medical School, Boston, MA 02115.

In order to study heavy chain allelic exclusion we have created strains of transgenic mice which carry modified human u chain genes capable of directing the synthesis of either secreted or membrane bound forms of the heavy chain. Having the human gene in the mouse has made the analysis of protein and mRNA expression in primary lymphocytes straight foward. We find that the B cells of transgenic mice that produce the membrane bound form of the u chain do not make mouse u. In animals that carry and express the secreted form of the same gene individual B cells produce both the secreted form of the human IgM transgene and mouse IgM. Thus membrane u is sufficient to mediate allelic exclusion whereas secreted IgM is not. Our current studies focus on the regulation of the expression of the modified human u genes in these strains of mice, and their effects on B cell development.

G 412 ESTABLISHMENT AND CHARACTERIZATION OF TWO HUMAN LARGE CELL LYMPHOMA CELL LINES, [1]C.D. O'Connell, [1]W.J. Urba, [1]S.R. Levy, [2]E. Brownell, [1]J.J. Kasper, [3]J.M. Tuscano, [4]W.H. Wilson and [3]D.L. Longo, [1]PRI, [2]BRI, [3]BRMP, NCI-FCRF, Frederick, MD 21701; [4]MB, COP, NCI, Bethesda, MD 20892.

We have established cell lines from the ascites of 2 patients with diffuse large cell lymphoma in standard media without the aid of feeder layers. They are EBV negative, and have been maintained in culture for over a year. DB expresses B cell surface antigens CALLA, DR, B1 and Leu 12, contains rearranged Ig heavy chain genes and secretes IgG. SR appears to be an early lymphoid cell line, expressing Tac, Leu 10 and DR, but has no demonstrable Ig gene rearrangement and does not produce antibody. Differential levels of c-rel mRNA expression by the 2 cell lines also indicates that DB and SR are from different stages of lymphocyte development. The karyotype of both cell lines has been determined. DB is near octaploid, with many chromosomal alterations, including deletions on chromosomes 2,4,10 and 18, and translocations (4;13), (5;19) and (10;14). SR is near diploid, with 3 characteristic chromosomal alterations: 2 deletions on chromosome 1 and a pericentric inversion on chromosome 2. These cell lines should be useful in defining the genetic and biochemical basis for lymphoid cell transformation and for developing strategies to control lymphoma cell proliferation.

G 413 PRIMARY MURINE B LYMPHOCYTES AS TARGETS FOR GENE TRANSFER AND TRANSFORMATION BY ONCOGENE-CONTAINING RETROVIRAL VECTORS. Robert W. Overell, Karen Weisser, David Cosman, Bruce Hess and Kenneth H. Grabstein. Immunex Corporation, 51 University Street, Seattle, Washington 98101 USA.

Retroviral vectors were constructed containing a variety of oncogenes and selectable drug resistance genes. The biological activity of these vectors was analyzed on LPS-activated murine B cells. Primary resting B lymphocytes, purified from murine spleen, were activated for 24 hours with LPS and then infected by overnight co-cultivation with virus-producing ψ2 cells. Expression of the transferred genes was measured in two ways. B cells were infected with retroviral vectors containing the hph or neo genes, which confer resistance to hygromycin B and G418 respectively on mammalian cells. Expression was demonstrated by the formation of drug resistant antibody-secreting cells in the infected but not the control cultures. This analysis indicated that approximately 5% of the B cells were expressing the transferred drug resistance marker. Expression of transferred oncogenes was evaluated by Northern analysis. Infection of LPS-activated B lymphocytes with a retroviral vector containing two oncogenes, v-abl^{p160} and v-myc^{MC29}, resulted in the establishment of immortal B cell lines. However, these cell lines arose at a very low frequency indicating that secondary events were required for the induction of the immortal phenotype. The cell surface phenotype of these cells indicated that they were mature B cells, which were not of the Lyl subclass. Data on oncogene expression in the immortal B cell lines will be presented.

G 314 ROLES OF THE PHOSPHOINOSITIDE-DERIVED SECOND MESSENGERS IN THE GROWTH ARREST OF A B LYMPHOMA, Dawne M. Page and Anthony L. DeFranco, Univ. of California, San Francisco, CA 94143. Stimulation of the antigen receptor of the murine B lymphoma WEHI-231 with anti-IgM results in irreversible growth arrest and inositol phospholipid hydrolysis — producing diacylglycerol (DG), which activates protein kinase C, inositol 1,4,5-trisphosphate, which induces the release of calcium from intracellular storage sites, and other inositol polyphosphates. We wished to determine whether the second messengers generated from phosphoinositide hydrolysis mediate the growth arrest caused by anti-IgM in these cells. Accordingly, the roles of two of the possible second messengers, intracellular free calcium (Ca^{++}_i) and DG, were assessed by increasing $[Ca^{++}]_i$ with ionomycin and by activating protein kinase C with phorbol 12,13-dibutyrate (PdBu). The combination of 175-500 nM ionomycin and 4-7 nM PdBu caused growth arrest and cell volume decrease responses in WEHI-231 cells which were similar to those caused by anti-IgM, although the responses to the mimicking agents were clearly slower. The combination of 250 nM ionomycin and 4-7 nM PdBu was most similar to anti-IgM, as both treatments induced growth arrest of WEHI-231 cells in the G_1 phase of the cell cycle, and in both cases, this growth arrest was prevented by the addition of bacterial lipopolysaccharide. Also, 250 nM ionomycin plus 4-7 nM PdBu did not inhibit the growth of two other murine B lymphoma cell lines, each of which did exhibit phosphoinositide breakdown but not growth arrest upon addition of anti-Ig. Overall, the combination of 250 nM ionomycin and 4-7 nM PdBu mimicked the action of anti-IgM on WEHI-231 cells quite well, although not completely. Thus, the phosphoinositide-derived second messengers Ca^{++}_i and DG probably play important roles in mediating the action of anti-IgM on WEHI-231 B lymphoma cells.

G 415 THE EFFECT OF INTERLEUKIN-4 ON FcγR RECEPTOR-MEDIATED INHIBITION OF c-*MYC* mRNA LEVELS IN MOUSE B LYMPHOCYTES. **Nancy E. Phillips, Kathy A. Gravel, Kathleen Tumas, and David C. Parker. Department of Molecular Genetics and Microbiology, University of Massachusetts Medical Center, 55 Lake Ave North, Worcester, MA 01605.**

Mouse B cells are stimulated to proliferate by Fab'₂ fragments of rabbit anti-mouse immunoglobulin (Ig) antibodies but inhibited from proliferation by IgG anti-mouse Ig. We have previously shown that this inhibition is mediated by binding of the IgG anti-Ig to receptors for the Fc domain of IgG (FcγR) on B cells and the crosslinkage of these receptor to mIg. Mouse B cells do proliferate, however, to IgG anti-Ig in the presence of IL-4 [Proust *et al.* J. Immunol. 135:3056(86); O'Garra *et al.* PNAS 84:6254(87); and our, as yet, unpublished observations]. We have recently investigated what effect Il-4 has on mIg-induced increase in levels of c-*myc* mRNA, which we have shown is inhibited by FcγR-mIg crosslinking (Phillips and Parker, Molec. Immunol. In Press). Surprisingly, doses of IgG or Fab'₂ anti-Ig and Il-4 which induce comparable levels of ^{3}H-thymidine incorporation do not induce comparable levels of c-*myc* mRNA.

G 416 ADMINISTRATION OF GAMMA-INTERFERON TO RHEUMATOID ARTHRITIS PATIENTS DECREASES CIRCULATING B-CELL LEVELS, Seth H. Pincus and Grant Cannon, NIH Rocky Mountain Laboratory, Hamilton, MT 59840, and University of Utah, Salt Lake City, UT 84132.

Increased B-cell reactivity associated with rheumatoid arthritis (RA) is reflected in the production of rheumatoid factor (anti-immunoglobulin) and a polyclonal increase in serum immunoglobulin concentrations. To study immunologic function in RA, we monitored RA patients who were being treated with gamma-interferon. While there is no theoretical rationale for the administration of this agent in RA, empirical data suggest it reduces disease symptoms. Study patients received 100 mg gamma-interferon intramuscularly five times per week for three months. Immunologic monitoring consisted of analysis of cell surface antigens on peripheral blood leukocytes using monoclonal antibodies and flow cytometry. Twenty eight markers on B-cells, T-cells, NK-cells, monocytes and neutrophils were studied. Circulating cells bearing the CD20 (B1) marker fell from 197±36 per cubic mm prior to therapy to 94±16 after treatment ($p<.01$). Cells expressing CD21 (B2) also fell from 48±31 to 9±3 ($p<.05$) with treatment. The number of lymphocytes expressing HLA-DR (primarily B-cells) also fell, from 241±43 to 163±19 (NS). We are currently studying serologic parameters on these patients to see if the changes in circulating B-cell levels are reflected in changes in serum immunoglobulin and rheumatoid factor levels. Other significant changes seen in patients treated with gamma-interferon include an increase in monocytes expressing HLA-DR and a decrease in the total number of neutrophils. Gamma-interferon has specific effects upon circulating leukocytes, most profoundly upon the B-cell population. Whether these effects cause the anti-arthritic actions of gamma-interferon remains to be established.

G 417 B AND T CELL POPULATIONS IN MICE TRANSGENIC FOR AN IMMUNOGLOBULIN MU HEAVY CHAIN GENE, Evelyn M. Rabin, Thereza Imanishi-Kari and Henry H. Wortis, Tufts University School of Medicine, Boston, MA 02111.

Transgenic C57Bl/6 mice were made with a construct of the completely rearranged BALB/c μ heavy chain bearing the 17.2.25 NP^a idiotype. Sera and cell populations from two transgenic lines were analyzed for μ allotype and idiotype expression. The 17.2.25 idiotype is cross-reactive with a minor NP^b idiotype of C57Bl/6 mice, and introduction of the transgene results in the dramatic amplification of the minor endogenous NP^b 17.2.25-like idiotype in host mice. Transgenic mice were crossed to xid mutant mice which are characterized by several B cell abnormalities including the absence of the Ly 1 B cell subpopulation. Sera from xid/Y trans/+ offspring contained the increased levels of 17.2.25 idiotype associated with μ^a and μ^b characteristic of both xid/+ trans/+ littermates and +/+ transgenic mice. The subpopulations of B and T cells present in these xid transgenic mice were also analyzed and compared to those found in +/+ mice and non-transgenic xid mice.

G 418 PERITONEAL B CELLS RESTORE TI-2 RESPONSIVENESS IN XID MICE, James E. Riggs and Robert T. Woodland, Univ. Mass. Med. Ctr., Worcester, MA.

Unresponsiveness to thymus-independent type 2(TI-2) antigens is a functional phenotype of mice bearing the X-chromosome linked immune-deficiency xid. Adoptive transfer of T-cell depleted splenocytes, bone marrow, or fetal liver from normal donors restores TI-2 responsiveness in unirradiated XID mice. Recent studies from our laboratory suggest an association between sIgM>>sIgD B cells and this functional phenotype. Because representation of sIgM>>sIgD B cells is enhanced in the peritoneum, we conducted adoptive transfers to determine if peritoneal cells(PC) from normal donors can restore TI-2 responsiveness in syngeneic XID recipients. Since XID mice fail to produce IgG_3 antibodies, donor B cell antibody production was monitored by an RIA specific for IgG_3 anti-hapten antibody. When compared by titration, PC were 5-fold more effective than splenocytes at restoring TI-2 responsiveness. Complement-mediated depletion of Thy 1.2, Ly-1, Mac-1, B.23, or Ia bearing peritoneal cells does not abrogate responsiveness, whereas J11d or B220 treatment does. Confirmation that donor B cells mediated this response was provided by RIA and immunohistochemical analysis of allotype expression in adoptive recipients of cells from allotype congenic donors(C.BC-682[$H\text{-}2^d$,IgH^b]--->BALB.xid[$H\text{-}2^d$,IgH^a]). This experimental approach may provide insight as to the functional capabilities of these cells. (supported by NIH AI 20210, 21858,and CA 45940).

G 419 EXPRESSION OF B-CELL DIFFERENTIATION ANTIGENS B1, B2, C3B, AND IA DURING FOLLICULAR TRANSFORMATION, Jonathan W. Said, Cedars Sinai Medical Center, Los Angeles, CA 90048.
Immunoultrastructural techniques were used to localize surface antigens on follicular B-lymphocytes at various stages of transformation. In both cryostat sections and cytocentrifuge preparations of tonsillar B-lymphocytes B1 and B2 were localized to cell membranes including microvillous surface processes. B1, Ia, and C3b revealed uninterrupted linear surface membrane staining, while B2 appeared as localized aggregates at sites of antigen expression. In addition B2 was present on complex processes of dendritic histiocytes which interdigitated with those of lymphoid cells. Morphometric studies using computerized planimetry revealed that cleaved cells expressing B1 and B2 were present at all stages of follicular transformation and suggest that centrocytes (small cleaved follicular B-lymphocytes) and noncleaved cells transform to centroblasts along parallel lines without following the sequential differentiation schema proposed by Lukes and Collins.

G 420 CLONAL ANALYSIS OF THE ENHANCING ACTIVITIES OF INTERLEUKIN-4 ON THE C_E GENE EXPRESSION IN LPS ACTIVATED MURINE B CELLS
H.F.J. Savelkoul, R.L. Coffman#, and R. Benner;Department of Immunology, Erasmus University, Rotterdam, The Netherlands, and # DNAX Research Institute, Palo Alto, CA, USA.

Interleukin-4 (IL-4) is able to preferentially enhance murine IgE levels in the supernatant of LPS-stimulated T-cell depleted spleen cell cultures. Clonal and quantitative analysis of this response revealed that the enhanced IgE production is due partly to an increased IgE precursor frequency and partly to an increase in clonal burst size of IgE-secreting cells. IL-4 increased the precursor frequency and burst size of IgM-secreting cells not more than two-fold. Both the IgM and IgE response in LPS-stimulated B cells was completely inhibited by the addition of monoclonal anti-IgM antibodies (M41) to the cultures, indicating that the IgE-secreting clones developed as subclones from precursors commited to IgM-synthesis. These cells lacked expression of membrane-bound IgE upto day 5 of the culture. IL-4 thus selectively induced a class switch recombination from IgM to IgE. Application of feeder cells in these cultures resulted in an increased precursor frequency of especially IgM-secreting cells and an increased switch frequency to IgE that is due , partially, to IL-4 leaking out of the (irradiated) feeder cells. So far, testing cultures of intact spleen cells from various responder type mouse strains (BALB/c, AKR, SJL) suggested a correlation between the responder type and the IgE precursor frequency. It was shown, however, that the undetectable precursor frequency in SJL mice is not due to an intrinsic B cell defect but is caused by T cells defective in the production of IL-4 (T_H2 type cells).

G 421 MINIMUM ACTIVATION SIGNALS NECESSARY TO RENDER B CELLS SUSCEPTIBLE TO INFECTION BY VIRUS, Madelyn R. Schmidt and Robert T. Woodland, University of Massachusetts Medical Center, Worcester, Ma. 01605.
We have examined the ability of B lymphocytes from both normal mice and mice expressing the X-linked immune defect (XID) to support viral protein processing and virus production during activation from the resting state. Small (resting) B cells, prepared by treatment of spleen cells with anti-Thy 1, anti-Ly 2, and complement, followed by counter-current cell elutriation will not support Newcastle Disease Virus (NDV, a paramyxovirus) synthesis, as assessed by immunoprecipitation of S^{35} labelled proteins, infectious center assays, or virus production. Activation of these cells for 24 hours with B cell mitogens, such as LPS or anti-Ig, results in NDV viral protein synthesis and virus production following infection. Resting cells maintained in culture with no stimulus are still uninfectible after 24 hours. To determine the minimum requirement for transition of resting B lymphocytes to the susceptible state, factors from macrophage, T_{H1}, T_{H2}, or Conconavalin A activated spleen cells, which do not activate B cells to secrete antibody, were examined and found to be sufficient to support NDV viral protein synthesis. Therefore, the response of resting B cells to a variety of B cell growth and differentiation factors can cause significant biochemical changes in the cell sufficient to render them susceptible to infection or capable of supporting viral protein synthesis. (supported by NIH AI 20210, AI 21858, and CA 45940)

G 422 CORRECTION OF THE FAILURE OF IG H CHAIN VARIABLE REGION GENE REARRANGEMENT BY CELL FUSION, Jerrold Schwaber, Children's Hosp. & Harvard Med. School, Boston, MA 02115

X-linked agammaglobulinemia is a congenital, sex-linked antibody deficiency disease resulting from failure of B lymphoid development at either the stage of pre-B cells (major form) or immature B cells (minor form). The failure of B lymphoid development is associated with failure of variable region gene rearrangement. We have found that B lymphocytes and cell lines from an XLA (minor form) patient are limited to expression of truncated μ and δ H chains, with limited, delayed expression of L chain. The truncated H chains are composed of D-J_H-C(μ/δ), resulting from failure to rearrange V_H. A 9-12-7 consensus sequence for rearrangement of V_H is encoded upstream of the D region, preceded by a Leader Sequence which serves for translation of the shortened message. The structure of the consensus sequence appears normal. Further, X chromosome linkage of this disease indicates that the failure of rearrangement does not result from defective structural genes. The failure could then result from premature termination of rearrangement, or from abortive rearrangement. To determine whether there was premature termination, we fused a clone from the XLA B cell line with mouse myeloma cells. Resulting hybrid cells underwent class switch from δ to gamma. Gamma H chains and mRNA were of apparent full size. Sequence determination showed that the gamma chains were encoded by VDJCgamma, the product of complete variable region rearrangement. Thus, the failure of B lymphoid development in this XLA is associated with, and may result from, premature termination of rearrangement. XLA may result from an inherited defect in an X chromosome linked enzyme which regulates variable region rearrangement.

G 423 REVERSAL OF NEGATIVE SIGNALLING IN B CELL LYMPHOMAS. **David W. Scott, Garvin Warner, Nina Birnbaum and Daniella Livnat, University of Rochester Cancer Center, Rochester, NY 14642**

WEHI-231 and CH31 are B cell lymphomas which are inhibited in their growth by crosslinking of surface immunoglobulin (IgM) receptors during early G_1. This "negative signalling" process can be prevented or reversed if these cells are exposed in G_1 to either phorbol esters or the supernatant from a T cell line specific for ovalbumin (T_{OVA}). In this presentation, we use a novel cell synchronization procedure to demonstrate 1) that the prevention of negative signalling is only accomplished by active phorbol esters capable of activating protein kinase C; 2) that ionomycin ($\pm$ phorbol esters) fails to deliver a negative signal under conditions in which anti-Ig can significantly prevent cell cycle progression into S; and 3) that prolonged exposure to phorbol esters desensitizes these cells for phorbol-mediated reversal but has no effect on lymphokine-driven protection from negative signalling. The importance of receptor internalization and the insufficient role of calcium flux in the negative signal will be discussed (Supported by CTR grant #1840).

G 424 ALTERNATIVE PROMOTERS, SOMATIC MUTATION, AND TRANSCRIPTIONAL DEREGULATION OF THE Bcl-2-IMMUNOGLOBULIN FUSION GENE IN LYMPHOMA, Masao Seto, Ulrich Jaeger, Richard D. Hockett, Stanley J. Korsmeyer, Howard Hughes Medical Institute, Washington University School of Medicine, St. Louis, Missouri 63110

The most common translocation in human lymphoma, the t(14;18)(q32;q21), generates heterogeneous 4.2-7.2 Kb Bcl-2-immunoglobulin (Ig) chimeric mRNAs resulting from alternative Bcl-2 5' exons and varied Ig 3' untranslated regions (UT). The normal human Bcl-2 gene has a 3 exon structure with an untranslated first exon, a facultatively spliced 220 bp Intron I, but a huge 370 Kb Intron II defined by pulse field gel electrophoresis. S1 protection and primer extension analysis revealed two promoters. An upstream promoter in Exon I displayed multiple initiation sites in a GC rich region with 7 Sp1 binding motifs. Promoter 2 Exon II possesses classic CCAAT (-106) and TATAA (-88) elements and a decanucleotide (ATGCAAAGCA) (-119) homologous with Ig V region enhancers. Most t(14;18) breakpoints cluster within the 3' UT of Bcl-2 implicating that event in gene deregulation. The Bcl-2 gene introduced into the C_γ locus of SU-DHL-6 displayed somatic mutation. While Bcl-2-Ig mRNAs demonstrated an unaltered 2.5 hr half-life, the Bcl-2-Ig gene revealed an inappropriately high rate of transcription for a mature B cell. This indicates the translocated Bcl-2 allele has escaped normal control mechanisms.

G 425 INDUCTION OF ANTI-ds DNA ANTIBODIES IN BALB/c MICE. Rachel Shefner, Scott Snapper, Karin Zupko and Betty Diamond, Albert Einstein College of Medicine, Bronx, NY 10461.
The observation by Diamond and Scharff that a myeloma cell line producing a phosphorylcholine binding antibody bearing the T15 idiotype (S107) can undergo somatic mutation of a single nucleotide pair resulting in a single amino acid change and acquire specificity for dsDNA led us to pursue the question of whether such antibodies may also occur in vivo. We have identified a protocol leading to the production leading to the production of T15 positive anti-DNA antibodies in BALB/c mice. Previous studies have shown that anti-idiotype to anti-I-J^d interferes functionally with suppressor T cell activity in vitro. When mice are immunized with anti-I-J^d in vivo, they generate anti-idiotype to anti-idiotype to anti-I-J^d and high titers of T15+ anti-dsDNA antibodies. We are currently generating monoclonal T15+ anti-dsDNA antibodies from these animals. We have isolated hybridomas making T15+, IgG or IgM antibodies that bind dsDNA and have obtained both V_H and V_L sequences by RNA sequencing. RNA sequence analysis should reveal the relationship of these antibodies to antibodies of the T15 idiotype that bind to foreign antigens and will help elucidate the molecular genetic origins of antibodies with specificity for dsDNA.

G 426 CTL SPECIFIC FOR SOLUBLE ANTIGENS DEMONSTRATED BY KILLING OF B CELLS REACTIVE WITH THE SAME MOLECULE.
Nobukata Shinohara, NCI, NIH, Bethesda, MD 20892
Although CTL are generally thought to be restricted by class I MHC antigens, CTL responses of considerable magnitude can also be induced to allogeneic class II antigens. Since class II antigens are thought to be responsible for restriction of responses to soluble antigens, we have speculated that CTL specific for soluble antigens presented in the context of class II MHC antigen may exist and play a regulatory role in immune responses. Such CTL would be capable of preferentially killing antigen-specific B cells which have trapped antigen via immunoglobulin receptors. This killing would result in antigen-specific, MHC restricted suppression of humoral antibody responses. In an attempt to induce such CTL, lymph node cells from KLH or OVA immunized mice were restimulated with antigen in vitro and were then tested for killing activity in the presence of TNP-conjugated antigens on a B cell line transfected with the structural genes of an anti-TNP antibody. Both Lyt-2^+ and L3T4$^+$ CTL with the requisite specificity were induced. These CTL specifically lysed the B cells in the presence of, or after preincubation with, the relevant antigens. The presence of TNP groups on the antigen molecules greatly facilitated the process of rendering B cells susceptible to such killing, suggesting the major contribution of the immunoglobulin receptor.

G 427 BIOCHEMICAL AND MOLECULAR ANALYSIS OF CLONALLY RELATED HUMAN EPSTEIN-BARR VIRUS TRANSFORMED B-CELL LINES SECRETING PATHOLOGIC α PR_2 COLD AGGLUTININS.
Leslie E. Silberstein and Condie E. Carmack. University of Pennsylvania, Department of Pathology and Laboratory Medicine and Institute for Cancer Research, Philadelphia, PA
To study the biology of cold hemagglutin disease, we have characterized B-cell clones isolated from a patient with splenic lymphoma and hemolytic anemia due to a monoclonal IgMκ, α-PR_2 cold agglutinin (CA). Cytogenetic studies of splenic lymphocytes demonstrated an abnormal karyotype, (51XX,+3,+9,+12,+13,+18). After EBV transformation of splenic lymphocytes, eight clones secreting IgMκ, α-PR_2 CA were isolated. Each clone had the same abnormal karyotype as above. DNA isolated from the clones and spleen was analyzed by Southern blot hybridization with Cμ, J_H and Cκ probes; identical Ig gene rearrangements were seen in each case. α-PR_2 antibodies isolated from culture supernatants and serum were compared by IEF and demonstrated similar banding patterns. Distinctive banding patterns, however, were observed in two out of the seven clones, suggesting structural differences. Absorption studies with human RBCs showed that the observed IEF banding patterns were solely due to α-PR_2 CA. With TLC, the biochemical determinants recognized by the cold autoantibodies were defined as glycolipids containing Neu Acα2-3 Galβ1-4 Glc (NAC).
To further investigate the intraclonal structural relationship of the α-PR_2 antibodies, we have cloned the variable regions using specific oligonucleotide primers. Sequence analysis of these chains showed; 1) their clonal relationship; 2) heavy chains from the V_H1 subgroup, light chains from the Vκ3,a subgroup; and 3) limited somatic mutation. The relationship between somatic mutations, idiotype, and autoantigen binding is discussed.

G 428 THE ROLE OF STRUCTURAL IMMUNOGLOBULIN GENE DELETIONS IN HUMAN IMMUNOGLOBULIN GENE DEFICIENCIES, C.I. Edvard Smith, Jan Inge Henter, Lennart Hammarström and Annika Linde, Dept. of Clinical Immunology and Center for Biotechnology, Karolinska Institute at Huddinge Hospital, S-141 86 Huddinge and Dept. of Immunology, Stockholm University, S-106 91 Stockholm, Sweden.

Human immunoglobulin deficiencies can be subdivided according to the presence or absence of the structural genes encoding immunoglobulins. We have analysed 118 individuals with IgA deficiency or selective IgG subclass deficiencies and in only one case could we find evidence for a structural gene deletion. This form of IgG1 deficiency seems to be due to a new type of homozygous deletion of the structural gene for $C\gamma 1$. The Southern blot analysis indicated that the maternal haplotype contains a deletion encompassing $C\gamma 1$, $C\psi\varepsilon 1$, $C\alpha 1$ and $C\psi\gamma$, whereas the deletion on the paternal haplotype may be confined to the $C\gamma 1$ gene.

Antibodies against protein antigens are largely restricted to the IgG1 subclass with a varying contribution of IgG3 and IgG4 in man, whereas anti-carbohydrate antibodies in the adult are confined to the IgG2 subclass. The identification of individuals heterozygous or homozygous for IgG1 gene deletions has enabled us to study the influence of this defect on isotype restriction. We have analysed the immune response to a number of different protein and polysaccharide antigens in the patient and in her parents. The effect of IgG1 gene deletions on the antibody repertoire will be presented.

G 429 NEUTRAL PROTEASES REGULATE THE APPEARANCE OF MEMBRANE-ASSOCIATED PROTEINS PHOSPHORYLATED BY PROTEIN KINASE C, E. Charles Snow, K.E. Pollok and T. Burke, University of Kentucky Medical Center, Lexington, KY 40536.

Membranes purified from normal B cells stimulated with PMA and exposed to a short [γ-^{32}P]ATP reaction mixture, express at least ten uniquely or hyperphosphorylated proteins when compared to membranes prepared from control cells. The appearance of these phosphoproteins is sensitive to H-7, a specific inhibitor of PKC. However, membranes purified from B cells stimultaed with PMA and ionomycin do not possess PKC activity unless the cells were stimulated in the presence of leupeptin, an inhibitor of Ca^{2+}-dependent, neutral proteases. Such proteases are thought to cleave only membrane localized PKC into a cytosolic Ca^{2+}/phospholipid-independent enzyme called protein kinase M. The neutral proteases apparently represent a Ca^{2+}-dependent regulatory system operative in controlling membrane localized PKC activity and might be involved in mediated some aspects of the signal transduction mechanism.

G 430 ß-GALACTOSIDASE: A SURROGATE MINOR HISTOCOMPATIBILITY ANTIGEN, Ulrich Theopold and Hans-Georg Rammensee*, Max-Planck-Institut für Immunbiologie, Stübeweg 51, 7800 Freiburg, FRG and *Basel Institut for Immunology, Grenzacherstr. 487, CH-4005 Basel.

Based on work on cytotoxic T lymphocyte (CTL) recognition of viral proteins it was suggested that minor histocompatibility (H) antigens might be peptides derived from polymorphic intracellular proteins, presented to CTL in context of class I on the cell surface. To test the hypothesis that nonviral intracellular proteins, or fragments thereof, could serve as target structures for class I restricted CTL, BALB/c mice were immunized with syngeneic tumor cells transfected with the E. coli ß-galactosidase gene. CTL specific for this surrogate minor H antigen were derived; they lyse cells transfected with different constructs engineered to result in intracellular, membrane inserted or secreted product. The data suggest that real minor H antigens might also be derived from proteins expressed in those three ways.

G 431 IgG SYNTHESIS BY POKEWEED MITOGEN- AND EPSTEIN BARR VIRUS-STIMULATED HUMAN B CELLS IS RESTRICTED TO THE ECTO-5'-NUCLEOTIDASE POSITIVE SUBSET, Linda F. Thompson and Julie M. Ruedi, Scripps Clinic and Res. Fndn., La Jolla, CA 92037.
Ecto-5'-nucleotidase (ecto-5'-NT) is a differentiation antigen expressed on a subpopulation of human B cells. Ecto-5'-NT activity is often reduced on B cells of hypogammaglobulinemic patients and is 5- to 6-fold higher on normal adult peripheral B cells than on cord blood B cells, suggesting that the enzyme is acquired during B cell maturation. To confirm this hypothesis, we prepared highly-purified ecto-5'-NT positive and negative B cells using anti-5'-NT antibodies and the FACS and examined their ability to secrete IgM and IgG after stimulation with pokeweed mitogen (PWM) or Epstein Barr virus (EBV). For the PWM-stimulated cultures, ecto-5'-NT^+ B cells synthesized two-fold more IgM and 20-fold more IgG than the ecto-5'-NT^- B cells. For the EBV-stimulated cultures, ecto-5'-NT^+ B cells synthesized 1.5-fold more IgM and 4.5-fold more IgG than the ecto-5'-NT^- B cells. These results show that although both ecto-5'-NT positive and negative B cells can synthesize IgM in response to PWM or EBV, the synthesis of IgG is largely restricted to the ecto-5'-NT^+ subpopulation. Thus, ecto-5'-NT is different from other human B cell surface antigens such as IgD, Leu 8, HB-4, and the mouse erythrocyte rosette receptor, which are lost as B cells mature and become responsive to PWM. In preliminary experiments, the addition of anti-5'-NT antibodies to PWM-stimulated (but not EBV-stimulated) lymphocytes increased the concentrations of IgM and IgG secreted by 2- to 5-fold. Experiments are in progress to determine whether this phenomenon is mediated by T, B, and/or accessory cells and to further define the role of ecto-5'-NT in B cell differentiation.

G 432 CHARACTERIZATION OF AN ABERRANT REARRANGEMENT OF RAT IMMUNOGLOBULIN EPSILON HEAVY CHAIN AND C-MYC GENES. Shin-Shay Tian and Charles Faust, Texas Tech Univ. Health Sciences Center, Lubbock, TX 79430.
B-cells productively rearrange their immunoglobulin (Ig) light and heavy (H) chain genes to produce one functional Ig per cell, with the other alleles frequently being non-productively rearranged. Peripheral B-cells produce functional IgM and IgD, expressed as surface Ig, and on maturation, they may undergo H-chain class switching to express another H-chain Ig isotype. In this case, the other non-productively rearranged H-chain allele also frequently undergoes translocations through some aberrant form of H-chain class switching. On occasion this aberrant event with the non-productively rearranged H-chain involves the c-myc proto-oncogene located on another chromosome, and this chromosomal translocation correlates well with B-cell neoplasia. We have identified, cloned and characterized such a translocation from the IgE-secreting immunocytoma, IR162, involving the non-productive epsilon H-chain allele aberrantly rearranged with the c-myc proto-oncogene. The translocational junction has been characterized by DNA sequence analysis to contain the epsilon switch region and the 5'-end of the c-myc gene. All structural coding information of the rat c-myc gene, as well as some transcriptionally associated 5'-cis DNA elements, were transferred to the epsilon H-chain switch region in the translocation. However, the putative silencer region, containing a potential negative regulatory DNA sequence, was severed from the aberrantly joined c-myc gene and not transferred to the epsilon switch region. The c-myc mRNA is expressed in IR162, and RNAase mapping has confirmed promoter usage of the aberrantly translocated c-myc gene.

G 433 ALTERED PHOSPHORYLATION OF THE B CELL SURFACE PROTEIN CD20 IS A RESPONSE OF IMMUNE ACTIVATION, Mary A. Valentine, Edward A. Clark, David Einfeld, Joseph Brown and Jeffrey A. Ledbetter, University of Washington and Oncogen Corporation, Seattle, WA 98195.
Only a limited number of B cell surface proteins involved in activation are known to be phosphorylated following interaction with their ligands or specific antibodies. The major triggering molecule on mature B lymphocytes, surface IgM, itself has no known kinase activity or cytoplasmic phosphorylation sites, but its perturbation can activate membrane-bound protein kinase C (PK-C). The ability of resting B cells to respond to the IgM signal is augmented by the concurrent presence of several other agents, including a low molecular weight B cell growth factor (BCGF), phorbol myristate acetate (PMA), and an epitope-specific monoclonal antibody which recognizes the surface phosphoprotein CD20. As these molecules can interact functionally, we asked how anti-μ and BCGF would affect CD20 phosphorylation. We found that 1) both anti-μ and BCGF could alter CD20 phosphorylation, implying that this response is a physiologic reaction to immune activation, 2) long term exposure to either agent rendered B cells less responsive to PMA-induced changes and 3) responses of dense and buoyant tonsillar B cells to BCGF differed markedly, suggesting that functional modulation of a surface molecule may occur according to the activation state of the cell. In order to determine the structural basis of CD20 action, a cDNA for human CD20 was isolated and sequenced (Einfeld et al., submitted). The CD20 gene encodes for a protein with 291 amino acids with no strong homologies with proteins with available sequences. The CD20 protein spans the membrane four times, and both its N-terminus and C-terminus are located within the cytoplasm. The 85 residues of the highly charged cytoplasmic C-terminus has no tyr residues, but does contain 9 ser and 6 thr residues that are potential sites for phosphorylation after B cell activation. (Supported by NIH grant GM37905, ACS grant IM-422 and by Oncogen Corporation.)

G 434 THE EFFECT OF EXOGENOUS ANTIGEN ON IMMUNOGLOBULIN V-REGION GENE SELECTION IN AUTOIMMUNE MICE, Donald L. Very, Jr.[1], Drew Weissman[1], Lawrence J. Wysocki[2] and Ann Marshak-Rothstein[1],[1]Boston University School of Medicine, Boston, MA 02118 and [2]National Center for Immunology and Respiratory Medicine, Denver, CO 80220.
Systemic autoimmune diseases such as systemic lupus erythematosus are characterized by the presence of antibodies reactive with a wide variety of normal self-antigens. It has been hypothesized that autoreactivity in these disease states may result from the polyclonal activation of germline immunoglobulin variable region (Ig V-region) genes which can cross-react with self-antigens. In support of this hypothesis, it has been demonstrated that antibodies utilizing the germline heavy chain V gene associated with the strain A anti-p-azophenylarsonate (Ars) cross-reactive idiotype (CRI), can bind DNA. However, the relationship between these germline sequences and the antibodies involved in the pathogenesis of autoimmune disease is unclear. To address this issue, we have developed a strain of autoimmune mice designated (MxA)1pr.Id which express the autosomal recessive mutation 1pr and produce high levels of autoantibodies. These mice also contain the appropriate A strain Ig V-region genes enabling them to respond to Ars immunization by the production of the dominant CRI. Preliminary data indicate that (MxA)1pr.Id mice immunized with Ars-KLH produce a CRI positive response that is comparable in kinetics and titer to that obtained by immunization of age-matched A/J mice. Using this model, the effect of Ars immunization on the subsequent development of the autoantibody repertoire is examined.

G 435 INDUCTION OF NON-RESPONSIVENESS IN NORMAL B CELLS BY ANTI-Ig: MODULATION OF NEGATIVE SIGNALLING EVENTS, **Garvin Warner and David W. Scott, University of Rochester Cancer Center, Rochester, NY 14642**
We have examined the effects of anti-Ig pretreatment on antigen specific B cell stimulation. Non-responsiveness (in terms of the generation of fluorescein specific AFC's) to subsequent LPS stimulation could be induced by crosslinking sIg for 18-24 hrs with either intact or F(ab')$_2$ fragments of rabbit anti-Ig, while non-responsiveness to challenge with fluorescein-Bruculla abortus (FL-BrA) required intact anti-Ig. Two hour pretreatment of B cells with 10-100 pg/ml of cholera toxin inhibited subsequent anti-Ig mediated B cell proliferation or FL-BrA induced differentiation, however, cholera toxin had little effect on LPS driven proliferation or differentiation. In addition, pretreatment with cholera toxin substantially inhibited delivery of a negative differentiative signal by anti-Ig. Preliminary evidence indicates that modulation of cyclic nucleotide levels or inhibition of protein kinase C (H-7, H-8 and Polymyxin B) has little effect on the delivery of the negative differentiative signal. These data suggest that the proliferative and/or differentiative signals (both negative and positive) delivered via sIg require a cholera toxin-sensitive G protein, whereas LPS driven proliferation and/or differentiation does not. (Supported by NIH grants CA41363 and T32 CA09363)

G 436 CYTOPLASMIC ACCUMULATION OF IMMUNOGLOBULIN AND CELL-DIFFERENTIATION ANTIGENS IN B CELL CHRONIC LYMPHOCYTIC LEUKEMIA, I.S. Watanabe, L. Lotuaco, L. Racela, T. Sato, J.W. Davis, E.P. Gephardt, Veterans Admisnistration Medical Center, Kansas City, MO 64128-2295.
In chronic lymphocytic leukemia (CLL), neoplastic lymphocytes resemble immunologically those at the ontogenic stage of immature B cell. To study the ultrastructural localization of immunoglobulin(Ig) and differentiation antigens, blood lymphocytes from 18 adult cases with B cell CLL were studied by immunologic electron microscopy. The methods include glutaraldehyde fixation, incubation with a monoclonal antibody, incubtion with peroxidase-conjugated antimouse goat Ig,visualization of peroxidase with H2O2 and DAB, and observation of ultrathin sections by electron microscopy. Surface receptors were represented by reaction product on the plasma membrane. Cytoplasmic accumulation of IgM was seen in the most leukemic lymphocytes in every case, while IgM was detectable only on the plasma membrane in normal B cells. The reaction product was distributed difusely in the ribosomal cytoplasm. It is absent in the nucleus, mitochondria, and rough ER cisterns. Cytoplasmic accumulation of other Ig were also seen, but less extensively than IgM. Among the B cell receptors, CD19 and CD20 stained in closely similar manners as IgM. In contrast, HLA-DR and CD5 were located only on the plasma membrane. Thus, intracytoplasmic accumulation of these proteins, which were distinctly demonstrated by us, may be a manifestation of metabolic defect of CLL cells.

G 437 NEGATIVE SIGNALLING WITH ANTI-IDIOTYPE ANTIBODIES IN MURINE B CELL TRANSFECTANTS, Carol F. Webb, Chieko Nakai and Philip W. Tucker, University Of Texas Southwestern Medical Center, Dallas, TX 75235.
The murine B lymphoma line, CH33, undergoes markedly reduced proliferation in response to cross-linking of its membrane immunoglobulin (mIg) by anti-IgM and anti-idiotype antibodies, thus providing a useful model for studying B cell tolerance[1]. Differential roles have been proposed for IgM and IgD in negative signalling[2]. To test this hypothesis we produced a series of CH33 transfectants containing IgM or IgD constructs which yielded mIg^+ cells with a T15 idiotype and phosphorylcholine specificity. Both mIgM and mIgD positive transfectants underwent negative signalling in response to anti-T15 antibodies, while CH33 was not affected. To determine the role of the cytoplasmic portion of mIg in mediating this effect, we used "mutant" constructs in which the membrane exons of $C\mu$ were replaced by the membrane exons of $\gamma 2b$, α, or the $I\text{-}A^b$ class II α chain. All of the heavy chain mutants underwent negative signalling; however, the $I\text{-}A\alpha$ mutants did not, implying that these cells are no longer capable of transmitting a negative signal through that Ig receptor. These results suggest that B cells can be tolerized through IgM or IgD receptors and that the carboxyl terminus plays a role in this effect.
1. Pennell, C.A. and D.W. Scott (1986). *Eur. J. Immunol.*, 16:157.
2. Vitetta, E.S. and J.W. Uhr (1975). *Science*, 189: 964.

G 438 ISOLATION AND CHARACTERIZATION OF THE GENE FOR THE ALPHA SUBUNIT OF THE HIGH AFFINITY Fc RECEPTOR FOR RAT IgE, Bruce Witthuhn, Randy McMillan & Charles Faust, Texas Tech Univ. Health Sciences Center, Lubbock, TX 79430.
Mast cells and tissue basophils possess a unique high affinity Fc receptor for IgE. This receptor is a heterotetrameric protein structure composed of three distinct polypeptides - one alpha, one beta and two gamma chains. The alpha subunit is a transmembrane glycopolypeptide which binds a single IgE molecule on the extracellular face of the plasma membrane. Allergic reactions are mediated through these high affinity Fc receptors, when two or more receptor bound IgE molecules are cross-linked on the cell surface. Molecular details of the IgE-Fc receptor interaction or initial signal transduction are not known. Therefore, as the beginning of an approach to gain understanding of these molecular details, the gene for the alpha subunit of this high affinity Fc receptor for IgE has been isolated from a LOU rat liver cosmid DNA library. This was achieved using two chemically synthesized oligonucleotide probes, based on the structure of a reported cDNA clone derived from the rat basophilic leukemia cell line, RBL-1 (Biochemistry 26:4605-4610, 1987). One probe is a 44-mer complementary to the 5'-end of the mRNA of the alpha subunit, and the other probe is a 42-mer complementary to the 3'-end of this mRNA, and consequently, these define the 5' and 3' limits of the high affinity Fc receptor alpha subunit gene for IgE. After screening 400,000 colonies of the cosmid DNA library, about 30 putative positive clones were found. These were colony purified and preliminarily characterized by restriction enzyme mapping and Southern blotting. Details of these preliminary characterizations will be reported.

G 439 A UNIQUE B CELL SUBPOPULATION IN DIABETES-PRONE BB/W RATS, ABSENT IN DIABETES RESISTANT SUBLINES, Penny Shockett and Robert T. Woodland, University of Massachusetts Medical School, Worcester Ma. 01605.
The Diabetes-Prone (DP) subline of Bio Breeding/Worcester (BB/W) rats spontaneously develops, at high frequency, an insulin-dependent diabetes mellitus strikingly similar to human type 1 diabetes. These rats also produce autoantibodies to various tissues, characteristic of a generalized immune dysregulation. In contrast, a Diabetes-Resistant (DR) subline of BB/W rats has also been developed, which rarely if ever develops diabetes or other autoimmune abnormalities. We have examined the B cell subpopulation of DP BB/W rats by two dimensional flow cytometry and demonstrated a $CD5^+$ (OX-19)/ sIg^+ subpopulation in the spleen and peripheral blood of these animals. B cells of this phenotype are not found in DP animals less than 1 month of age, but appear before the onset of disease at 6-8 weeks of age. In contrast, we have not observed $CD5^+$, sIg^+ B cells in the DR subline. Preliminary analysis of non-immune sera show DP animals have, on average, IgG2c immunoglobulin levels 3-6 fold higher than that found in DR animals. These observations suggest that $CD5^+$, sIg^+ B cells may be a common feature of autoimmune disorders. (supported by grants NIH AI 21858 and CA 45941)

G 440 TRANS-STIMULATION OF L-SYSTEM AMINO ACID TRANSPORT IN NORMAL AND CLL LYMPHOCYTES: PHORBOL ESTER RESTORES FUNCTION IN CLL. T.J. Woodlock, G.B. Segel, and M.A. Lichtman, University of Rochester School of Medicine, Rochester, NY 14642.
Chronic lymphocytic leukemia (CLL) B-lymphocytes have a unique and specific diminution of L-system (leucine favoring) amino acid uptake; the maximal velocity is approximately 10% of normal B-lymphocytes. Treatment of CLL B-cells with the maturational agent, tetradecanoyl phorbol acetate, results in restoration of L-system amino acid uptake to normal velocity. To further characterize the effect of phorbol ester on the L-system of CLL B-cells, we have examined the ability of normal and CLL lymphocytes to exchange intracellular for extracellular amino acids by the L-system (trans-stimulation). L-system transport was not trans-stimulated in CLL B-lymphocytes while a near two-fold increase in L-system uptake was noted in normal B- and T-lymphocytes in the presence of a high intracellular concentration of 2-amino-2-carboxy-bicycloheptane (BCH), a largely L-system-specific substrate. Phorbol ester treatment restored L-system uptake in CLL from a V_{max} of 100 umol/liter cell water per minute to a normal V_{max} of 900 after a 16 hour incubation. The V_{max} could be increased three-fold to 2400 if CLL cells were loaded with BCH. Hence, phorbol esters result not only in a normalization of L-system transport in CLL B-cells but the transport system demonstrates exchange rates comparable to normal lymphocytes.

G 441 **THE V_H REPERTOIRE OF B CELL COLONIES IN MOUSE**, Gillian E. Wu, Department of Immunology, and Christopher J. Paige, Department of Medical Biophysics, University of Toronto, Toronto, Canada.
We have developed a rapid direct method for detecting RNA present in B lineage colonies grown in semi solid agar. Using this technique we have explored the V_H gene utilization using cloned specific V_H gene fragments from 8 V_H families. We have evidence that the adult V_H repertoire in LPS stimulated B cells is genetically controlled and thus varies with different strains of mice. For example, the C57BL/6 and BALB/c strains utilize the V_HJ558 and V_H7183 families at much different frequencies that can be most easily expressed as differences in their V_HJ558 to V_H7183 ratios. Thus this ratio is 4.5 for C57BL/6 and 0.6 for BALB/c. Further studies with colonies derived from pre-B, sIg^- cells demonstrated that this strain difference is not due to selection of B cells in vivo via sIgM. Moreover, studies with mice congenic at the IgH locus have V_H utilization patterns (i.e. the V_HJ558/V_H7183 ratios) that correlate with the background of the congenic strain not the V_H locus. Thus the genetic element determining the V_H family usage is not the V_H gene segments themselves, but some genetic element unlinked to IgH elsewhere on the genome. We are currently analysing the V_H usage of recombinant inbred mice in order to locate this genetic element and analysing FACS sorted Ig^a or Ig^b B cells from F1 mice in order to determine if this genetic element is acting in cis or in trans.

G 442 ROLE OF CALCIUM MOBILIZATION IN B CELL DIFFERENTIATION INDUCED BY INTERLEUKIN 5, Naoto Yamaguchi, Takeo Takahashi, Nobuko Yamaguchi, Nobuyuki Harada and Kiyoshi Takatsu, Dept of Biol,Inst for Med Immunol,Kumamoto Univ Med Sch,Kumamoto,JAPAN.
To analyze the intracellular mechanisms of differentiative responses of B cells, we have selected an in vitro $Ly\text{-}1^+$ single clone of the murine B cell chronic leukemia BCL_1 cells at limiting dilution for its ability to differentiate into IgM-secreting cells in response to affinity-purified IL5. Subcloned BCL_1-B20 cells, which express IL2 receptors on their surface, were induced to secrete IgM in response to not only IL5 but also IL2 alone. By utilizing this clone, we thus analyzed whether the triggering of its differentiation is mediated through calcium mobilization. Neither IL5 nor IL2 had any effect on basal intracellular free calcium concentrations ($[Ca^{2+}]i$) as monitored by indo-1 whereas $F(ab')_2$ fragments of anti-Ig ($F(ab')_2$ anti-Ig) and the calcium ionophore A23187 caused a rapid increase in $[Ca^{2+}]i$. However, $F(ab')_2$ anti-Ig or A23187 did not induce the differentiation of the cells. Furthermore, $F(ab')_2$ anti-Ig and A23187 did not affect the differentiation induced by IL5 or IL2, nor was the increase in $[Ca^{2+}]i$ induced by $F(ab')_2$ anti-Ig and A23187 interfered with the presence of IL5 or IL2. IL5 also did not affect the basal levels of the rate of calcium influx and efflux across plasma membranes determined by the use of $^{45}Ca^{2+}$. These findings suggest that calcium mobilization such as an increase in $[Ca^{2+}]i$ and enhancement of calcium fluxes are not required for the differentiative response of the cells and signals mediated by IL5 and IL2 are transduced via a different intracellular pathway from mIg-mediated signaling accompanied by an increase in $[Ca^{2+}]i$.

G 443 THE ROLE OF B LYMPHOCYTES ON THE GENERATION OF ANTI-IDIOTYPIC T LYMPHOCYTE REPERTOIRE, Hiroshi YAMAMOTO, Soji BITOH and Shigeyoshi FUJIMOTO, Department of Immunology, Kochi Medical School, Kochi 781-51, JAPAN

In previous studies we showed that MHC-restricted cytotoxic T lymphocytes (CTL) specific for the cross-reactive idiotype(CRI) of MOPC104E myeloma protein could only be induced in BALB/c or BAB-14 mice which have the ability to produce the CRI, but not in C.AL-20 or C.B-20 mice which have no ability. The strong correlation between CRI-specific CTL responder strains and CRI producers supports the idea that the V_H gene products are intrinsic primary antigenic stimuli for the generation of the anti-idiotypic CTL. To investigate the role of B lymphocytes in the selection of T lymphocyte repertoire, the purified B cells of CRI producer strains were repeatedly injected into anti-CRI CTL nonresponder neonatal mice. CRI-specific CTL activity was successfully induced in the CRI nonproducer mice only when they were exposed to CRI producer strain B lymphocytes from neonatal life. When the CTL nonresponder adult mice received CRI-producer B lymphocytes, the nonresponder phenotype was not changed into the responder phenotype. Inducibility of CRI-specific CTL was also analyzed in tetraparental bone marrow chimeras. When CRI nonproducer bone marrow cells repopulated along with CRI producer bone marrow cells, the anti-CRI CTL of CRI nonproducer origin were generated. Adaptive differentiation of haplotype preference was also observed. When these observations are taken collectively, we see that the anti-idiotypic T lymphocyte repertoire is not a genetically determined one, but rather that the repertoire of T lymphocytes strongly depends on the postnatal selection process through the intrinsic idiotopic repertoire of B lymphocytes, i.e., internal images.

G 444 THE EFFECTS OF MURINE IL-4 and IL-5 ON I_{29}.B CELL LYMPHOMA CELL LINES, Israel Zan-Bar, Faculty of Medicine,Tel Aviv University, Israel

I_{29} B cell lines derived spontaneously from I mice are capable to differentiate upon stimulation with various B cell stimulating agents. The cell characterized phenotypically as IgM; LyT-1, B-200, and FcR positive cells can be stimulated to secrete IgM antibodies and or differentiate in isotype switch. In order to understand the signaling for B cell differentiation and maturation processes, the following reagents were examined; LPS, anti isotypes, anti idiotype, IL-1, IL-2, IL-4, IL-5, INF-β and TNF. Each one of the reagents were examined for its effect by itself or in combination with other reagents. No direct or indirect effect of IL-1, IL-2, or TNF on the growth or differentiation of various I29 cell lines were detected. On the contrary, both IL-4 and IL-5 induces specific Isotype switch, antibody production and changes in rate of proliferation. These effects could be obtained only on IgM bearing cell lines while none of the more differentiated cells respond. This effect of IL-4 and IL-5 could be obtained only if the cells were activated prior or at the same time of their interaction with the factors. Incubation of IgM cells with LPS and IL-4 for three days in culture induces the cells on day fifteen to differentiate to 25-35% IgG and 5-10% IgE bearing cells.Incubation of the cells in the same conditions with anti IgM plus IL-5 induces on day fifteen 15-20% of the cells to IgA bearing cells. IL-4 synergize IL-5 as incubation of the cells with the two reagents in the present of LPS induced 30-35% of the cells to differentiat to IgA bearing cells.

G 445 SYSTEMIC MAINTENANCE OF B-CELL ANERGY IN TNBS-INDUCED TOLERANCE, Margot Zöller and Giancarlo Andrighetto, Institute of Nuclear Medicine,German Cancer Research Center, 6900 Heidelberg, FRG.

It is still a matter of debate whether B-cell tolerance is the consequence of (functional) clonal deletion or suppression. To investigate the underlying mechanism in TNBS-induced humoral unresponsiveness, limiting dilution (LD) analysis of the frequencies of B-cells and regulatory cells was performed. After injection of a total of 20mg TNBS, the frequency of functionally active hapten-specific B-cells was decreased to less than 10% of controls. After an immunogenic challenge, TNP-specific B-cells of tolerized mice expanded, which was in contrast to the absence of anti-TNP plaque forming cells in freshly harvested spleen cells. Hence, the functional deletion of B-cells in vivo appeared to be sustained by regulatory cells. LD analysis revealed a transient augmentation of TNP-specific helper T-cells, continuously elevated levels of suppressor T-cells and a low level of contrasuppressor T-cells (T_{cs}). But, contrary to non-tolerized mice, T_{cs} were rather refractory to an immunogenic challenge. Hence, clonal anergy of B-cells leads to inappropriate activation of T_{cs}. Thus, in the continous presence of TNP a stable steady state of unresponsiveness is created, which depends on a cascade of events, i.e. B-cell anergy, activation and maintenance of suppression due to the interrupted counterregulation by T_{cs}, the latter being kept down by absence/shortage of antibody as their antigen.

G 446 CLONAL ANALYSIS OF HUMAN B CELL RESPONSES. R. Zubler, C. Werner, C.Straub, L. Wen. Division of Hematology, University Hospital, 1211 Geneva, Switzerland.

We have recently developed a culture system in which the majority of B cells from human peripheral blood or spleen are induced to proliferate and to generate short-term clones of a mean of 400 antibody-secreting cells (ASC). B cell stimulation is performed with mutant EL-4 thymoma cells of mouse origin in conjunction with human T cell-supernatant. By using this system we have undertaken several studies.

1) Ig-switchfrequencies: Single B (CD20+) cells from adult blood were placed into cultures with the autoclone apparatus controlled by the FACS. By ELISA for Ig's we found a cloning efficiency of 57% (122 clones per 214 cultures) with the following pattern of isotype frequencies: 49%M, 17%G, 8%A, 21%M+G, 0.8% each for M+A and G+A, 2.5% for M+G+A -clones.

2) Frequencies of antigen-specific B cells: In normal adults the mean precursor frequencies were 1/2000 for anti-BSA ASC, 1/3000 for rhumatoid factor ASC, 1/50 for anti-denatured DNA ASC, 1/3000 for anti-malaria(p. falciparum) ASC. Rheumatoid factor and anti-DNA ASC-precursor frequencies were not significantly different in individuals with rheumatoid arthritis (n=4) or systemic lupus erythematosus (n=6). However, the anti-malaria ASC-p frequency was 10 times higher in individuals with past malaria (1/300,n=6) than in controls.

3) Epstein-Barr virus (EBV)-dependent B cell immortalization: Inspite of strong proliferation of EBV-infected (EBNA+) cells, the immortalization frequency was low(0.7%), suggesting that B cells were committed or not to long-term growth already before clonal expansion.