B CELL IMMUNOBIOLOGY AND HUMAN DISEASE

Organizers: Rainer F. Storb, Carol Nottenburg and Edward Clark

February 1 - 8, 1993; Taos, New Mexico

Plenary Sessions *Page*

Poster Sessions

B Cell Immunobiology and Human Disease

Keynote Address (Joint)

F 001 GENETIC ANALYSIS OF B CELL DEVELOPMENT, Klaus Rajewsky, Institute for Genetics, University of Cologne, FRG.

B cell development is governed by cellular selection through surface immunoglobulin. This principle operates at various stages of development. When B cells develop from stem cells in the bone marrow, cells failing to assemble their immunoglobulin V region genes properly are rigorously eliminated. Gene targeting experiments have been instrumental in advancing our knowledge of the sophisticated mechanisms which control this process and allow the production of a cell population expressing a selected antibody repertoire which is further shaped by interactions of the cells with their environment. Similarly, when memory B cells are generated in T cell dependent responses in germinal centers through somatic hypermutation of their V region genes, only cells expressing Ig receptors of suitable antigen binding specificity are permitted to survive. This process of positive and, perhaps, negative selection has striking analogies to the maturation of T cells in the thymus. It can now be approached through the isolation of single B cells from various, histologically defined positions in the microenvironment of the germinal center and their subsequent molecular analysis.

Stem Cells and Early B Cell Progenitors (Joint)

F 002 STROMAL CELL CONTROL OF B CELL DEVELOPMENT Shin-Ichi Nishikawa[1], Takumi Era[1], Minetaro Ogawa[1], Shin-Ichi Hayashi[1], Takahiro Kunisda[1], Satomi Nishikawa[1], Richard. R. Hardy[2], Yuji Yamanashi[3], Tadashi Yamamoto[3], Werner Müller, Klaus Rajewsky[3], [1]Institute of Molecular Embryology and Genetics, Kumamto University School of Medicine, Kumamoto, Japan, [2]Fox Chase Cancer Research Institute, Philadelphia, USA, [3]Institute of Medical Science, Tokyo University, Tokyo, Japan, [4]Institute for Genetics, Cologne University, Cologne, FRG

Intramarrow B cell-genesis is regulated by fibroblastoid stromal cell component in mouse bone marrow. The fact that a stromal cell clone, ST2 is capable of supporting the entire process from single FACS-sorted multipotent stem cells into sIgM$^+$ B cells clearly indicates that all molecules required for this process is expressed by ST2. We have been investigated the molecular basis for stromal cell-dependent B cell genesis. Among a number of cytokines which are constitutively or inducibly expressed by ST2, IL-7 and the ligand for c-kit (Steel Factor, SLF) are indispensable for B cell-genesis. However, while no active B cell genesis occurs in the IL-7(-) microenvironment, a considerable B cell-genesis is still observed in the SLF(-) microenvironment. Thus, it is likely that some other stromal cell molecule(s) may compensate for SLF, although SLF is essential for actual intramarrow B cell-genesis. When both SLF and IL-7 are included in the culture of the earliest B progenitor cells, proliferation and maturation of B lineage cells are supported. However, the extent of B lineage cell proliferation in such a culture is far less than that in the culture with stromal cell layers. This suggests that the actual B cell-genesis is regulated by multiple cytokines, among which some, inclucing IL-7 and SLF, support basal cell proliferation, while others may modulate this basal level. Nonetheless, using recombinant IL-7 , SLF and IL-7(-) stromal cell clone, PA6, the process of early B cell-genesis is divided into three stages with different growth signal requirements. Differentiation stages of B cell development has been defined also either by the organization and expression of immunoglobulin genes or by the expression of a number of surface molecules. We next attempted to correlate the differentiation stages in terms of growth signal requiremnts with the differentiation stages defined by other criteria. While some loose correlation was present between the growth signal requiremnts and the surface differentiation markers, it could hardly be a strict correlation. Thus, the signals regulating the growth signal receptors, surface differentiation markers, and immunoglobulin gene rearrangement might be different, though overlaps to a considerable extent. To gain an insight into the signalling pathways regulating the B lineage cell-differentiation, we have isolated μ^- B cell clones which apoptose by the LPS-stimulation. The similar apoptosis is inducible by introducing activated form of *lyn* into the same cells. Using these cell lines, the molecules which are able to rescue this cell death is currently screened. Finally, the studies of ours and other groups indicate that the expression of SLF is the minimum requirements for the hematopoietic as well as B-lymphopoietic microenvirioments. To understand the molecular basis for tissue-specific and stage-specific expression of murine SLF gene, we cloned the genomic SLF gene and determine a region which renders it expressed in fibroblastoid lines but does not so in hematopoietic cell lines. The same region was ligated to bacterial β-gal gene and introduced to fertilized eggs, which eventually gave rise to a number of transgenic mouse strains. Since these mice express β-gal gene in a tissue specific manner, they will provide a powerful tool to investigate the necessary conditions which modulate the stem cell vs stromal cell-interaction in hematopoietic tissues as well as other c-kit-dependent stem cell systems.

F 003 GROWTH FACTOR REGULATION OF LYMPHOHEMOPOIETIC PROGENITORS, Makio Ogawa, Ralph H. Johnson Department of Veterans Affairs and Department of Medicine, Medical University of South Carolina, Charleston, South Carolina.

In the steady state marrow, the majority of hemopoietic stem cells are dormant in the cell cycle and only small numbers of stem cell clones provide all of the blood cells. During regeneration from marrow aplasia these cells are induced to proliferate actively by a group of early-acting cytokines. By serial observations of blast cell colony development, we have demonstrated that combinations consisting of either IL-3 or steel factor (SF) and one of IL-6, G-CSF, IL-11 or IL-12 (natural killer cell stimulating factor, cytotoxic lymphocyte maturation factor) effectively support proliferation of the primitive progenitors. In this communication, we present that similar cytokine combinations are also involved with the proliferation of lymphohemopoietic progenitors in culture

The existence of pluripotent lymphohemopoietic stem cells was postulated for many years through clinical observations such as identification of chromosomal markers in myeloid as well as lymphoid cells in the recipients of bone marrow transplantation and identification of the Philadelphia chromosome in myeloid and lymphoid cells in patients with chronic myelogenous leukemia. Recently, studies using retroviral labeling of individual hemopoietic progenitors confirmed the existence of the population of cells that are able to provide both myeloid and lymphoid cell lineages. Despite these *in vivo* evidence, it has not been possible to detect and quantitate the lymphohemopoietic progenitors until recently because of the absence of appropriate cell culture techniques. Now investigators in 2 laboratories have described culture assays for murine and human fetal bone marrow lymphohemopoietic progenitors by co-culture with murine marrow stromal cells. In our laboratory, we described a two-step methylcellulose culture system in which we unequivocally demonstrated that individual primitive progenitors from 5-fluorouracil-treated mice have a capacity for differentiation in both myeloid and B-cell lineages. By using micro-manipulation techniques we plated highly enriched primitive murine marrow cells individually in the presence of pokeweed mitogen spleen conditioned medium (PWM-SCM), SF, erythropoietin and IL-7. Resulting primary myeloid colonies were replated on day 11 of culture into secondary cultures containing SF and IL-7. Approximately 1/4 of the primary micromanipulated cells yielded pre-B cell colonies in secondary cultures. When pre-B cell colonies derived from a single lymphohemopoietic progenitor were pooled and individually injected into scid mice, donor-type IgM was detected in the serum of the mice and the spleens contained donor-type B-cells upon sacrifice on day 112. We then tested growth factor combinations that might replace PWM-SCM in the primary culture. Combinations of two factors that included SF together with IL-6, IL-11, G-CSF or IL-12 effectively supported the B-lymphoid potential of the primary myeloid colonies. Colonies supported by combinations of IL-3 plus one of the synergistic factors failed to maintain the lymphoid potential. Our observations demonstrated that many cell-cycle dormant progenitors from 5-fluorouracil-treated mice which were previously believed to be myeloid-committed, also possess B-lymphoid potential. Studies of the mechanisms regulating early-stages of lymphohemopoiesis should be facilitated by this culture system.

B-Cell Adhesion and Accessory Molecules (Joint)

F 004 B CELL TRIGGERING VIA CD38, Maureen Howard, Leopoldo Santos-Argumedo, Christopher Grimaldi, Fernando Bazan, Nobuyuki Harada and Michael Parkhouse†. DNAX Research Institute, Palo Alto, CA 94304 and †Institute for Animal Health, Pirbright Laboratory, Surrey GU24 ONF, England

We have recently isolated a cDNA encoding a murine B cell activation antigen initially identified by a growth-inducing monoclonal antibody. Sequence analysis of this cDNA revealed it was a novel murine protein exhibiting 70% homology to human CD38. Surprisingly, this putative murine CD38 exhibits a cellular distribution that is markedly disparate from that of human CD38. While that latter is expressed on human T cells, immature B cells, germinal center cells and plasma cells, the putative murine CD38 is expressed almost exclusively on all unprimed mature murine B cells. Triggering murine B cells with an agonistic anti-CD38 antibody in the presence of IL-4 induces Ca^{++} mobilization and proliferation. This proliferation is augmented by LPS or anti-CD40 antibodies, but not by anti-IgM antibodies. Recent structural analyses have suggested CD38 is related to an enzyme involved in a recently discovered pathway of Ca^{++} mobilization that is distinct from the inositol 1,4,5 triphosphate pathway of Ca^{++} mobilization.

F 005 STRUCTURE AND FUNCTION OF LYB-2/CD72, Jane R. Parnes, William H. Robinson, Han Ying, Ju-Fay Chang and Henry N. de Vegvar, Stanford University Medical Center, Stanford.

Lyb-2/CD72 is a 45-kDa cell surface glycoprotein expressed on early B lineage cells through mature B cells, but not on terminally differentiated plasma cells. Early functional studies had demonstrated that monoclonal antibodies specific for mouse Lyb-2/CD72 could block differentiation into plasma cells in response to T cell-dependent antigens and could induce B cell proliferation. We have isolated and sequenced cDNA clones encoding Lyb-2/CD72 in both mouse and man, and these studies have led to the conclusion that Lyb-2/CD72 is a type II membrane protein homologous to CD23 and asialoglycoprotein receptors, all of which belong to the C-type lectin superfamily. We have sequenced cDNA clones encoding three serologically defined alleles of mouse Lyb-2/CD72. These studies have demonstrated that the alleles are highly conserved in their cytoplasmic and transmembrane domains but exhibit a high degree of polymorphism in their extracellular domains. This polymorphism in the extracellular region involves amino acid substitutions at a minimum of 20 residues and is concentrated primarily in the membrane distal region, the region that is also least conserved between mouse and human Lyb-2/CD72. We have demonstrated that mouse and human Lyb-2/CD72 each are ligands/receptors for both mouse and human CD5, a cell surface glycoprotein expressed on all T lymphocytes and a subset of B lymphocytes. We are currently examining how this binding affects B cell/T cell interactions. To further understand the role of Lyb-2/CD72 in vivo we have generated transgenic mice in which the Lyb-2^a allele is expressed in an Lyb-2^b mouse under the control of the immunoglobulin kappa light chain promoter and enhancers. This should lead to expression of both the Lyb-2/CD72 transgene and the endogenous gene on mature B cells, but while expression of the endogenous gene is turned off upon differentiation into plasma cells, expression of the transgene should increase. We have demonstrated transgene expression is indeed restricted to $B220^+$ cells in peripheral blood, making this vector potentially useful for the study of other transgenes in B lineage cells. Further analysis of the phenotype of these transgenics will be presented.

F 006 THE ROLE OF CD22 IN LYMPHOCYTE ADHESION, Ivan Stamenkovic[1], Dennis Sgroi[1], Sten Braesch-Andersen[1], Ajit Varki[2] and Alejandro Aruffo[3], [1]Department of Pathology, Massachusetts General Hospital, and Harvard Medical School, Boston, [2]Department of Molecular Medicine, University of San Diego, La Jolla and [3]Bristol Myers Squibb Pharmaceutical Institute, Seattle.

CD22 is a B cell-specific adhesion molecule expressed on mature B cells as two isoforms of 130 and 140 kD. Expression of CD22 occurs in the cytoplasm early in B cell ontogeny and on the surface at about the same time as surface IgD. Surface expression of CD22 coincides with acquisition of B cell responsiveness to antigen, suggesting that it may play a role in early B cell activation events which are T cell-dependent. cDNAs encoding two isoforms of CD22 have been isolated, and the predicted amino acid sequence shown to contain 5 and 7 Ig domains in the extracellular region of the smaller (α) and larger (β) isoform respectively. Introduction of CD22α into COS cells promotes rosetting of erythrocytes and monocytes, whereas COS cells transfected with CD22β bind T and B cells in addition to monocytes and erythrocytes. The first three domains of CD22β contain the sequences necessary for recognition of ligands on adjacent cells. A soluble CD22-Ig fusion protein was used to identify ligands of CD22, and was found to immunoprecipitate multiple cell surface glycoproteins from both T and B cells, the major species being 115, 130 and 180-220 kD molecules. Immunoblotting experiments revealed that the high molecular weight bands correspond to different isoforms of CD45, the leukocyte common antigen. To determine whether interaction between CD22 and its T cell ligands might have an effect on T cell activation, T cells were stimulated by CD22Ig crosslinked with anti-CD3 antibody. A dramatic inhibition of intracellular calcium mobilization produced when T cells are stimulated with anti-CD3 alone was observed. Similarly, inhibition of PLCγ1 phosphorylation was also observed, closely reminiscent of the effect of coligating CD3 and CD45. CD22 interaction with CD45 and other cell surface ligands is dependent upon the presence of ligand-associated sialic acid in α2,6 linkage. Transfection of COS cells with a cDNA clone encoding α2,6 sialyltranferase alters the COS cell phenotype and promotes reactivity with CD22Ig. CD22Ig immunoprecipitates a 115 kD glycoprotein from α2,6ST-transfected COS cells but not from mock-transfected counterparts. CD22 therefore behaves as a sialic acid binding lectin, and is the first Ig-like molecule to be observed to do so. It many play an important role in T cell-B cell interaction by helping regulate T cell responses.

F 007 MULTIMERIC CD19-CELL SURFACE PROTEIN COMPLEXES REGULATE B CELL FUNCTION, Laura E. Bradbury[1], Liang-Ji Zhou[1], Shoshana Levy[2], Robert L. Evans[3], and Thomas F. Tedder[1], [1]Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA, [2]Stanford University School of Medicine, Stanford, CA, [3]Roswell Park Memorial Institute, Buffalo, NY.

CD19 noncovalently associates with CD21 and a number of other surface proteins to form a multimolecular signal transducing complex. CD19 is a member of the Ig superfamily and CD21 is a member of the complement receptor family. Both are only expressed by B cells and follicular dendritic cells. CD19 is expressed from the early stages of Ig heavy chain rearrangement, and CD21 is first expressed at the time of IgD expression. Two additional members of the CD19 complex have been identified as TAPA-1 and Leu-13, which are physically associated in many cell lineages. TAPA-1 is expressed on most cell types while Leu-13 is expressed on subsets of lymphoid cells. Precipitation of CD19 specifically coprecipitates CD21, TAPA-1 and Leu-13 from B cell lines lysed in digitonin buffers. Western blot analysis with a TAPA-1 mAb verified the identity of TAPA-1 in CD19 and CD21 immunoprecipitated materials. In addition, when TAPA-1 or Leu-13 were crosslinked and patched on the cell surface, CD19 comigrated with them. Furthermore, mAb binding to CD19, CD21, TAPA-1 and Leu-13 on B cell lines induced similar biologic responses, including the induction of homotypic adhesion, inhibition of proliferation, and an augmentation of the increase in intracellular Ca^{++} induced by suboptimal crosslinking of surface Ig on B cell lines. Together, these data suggest that TAPA-1 and Leu-13 are broadly expressed members of a signal transduction complex in which lineage-specific proteins, such as CD19 and CD21, provide cell specific functions.

CD19 has two extracellular Ig-like domains, a transmembrane domain, and a highly charged ~240 amino acid cytoplasmic tail. Significant evolutionary pressure for the conservation of amino acid sequences within the cytoplasmic tail suggests that it has a critical role in CD19 function. Therefore, the role of the cytoplasmic domain in signal transduction and the formation of intermolecular associations was examined using a series of cytoplasmic tail deletion mutants and a chimeric fusion protein containing only the extracellular portion of CD19. Deletion of all but the first 11 amino acids of the cytoplasmic tail had no affect on the ability of CD19 to associate with TAPA-1 and Leu-13, but the CD19 transmembrane domain was required. In addition, the induction of homotypic adhesion through CD19 or CD19 deletion mutants was equivalent, although homotypic adhesion was not induced through the CD19 chimeric molecule lacking the CD19 transmembrane and cytoplasmic domains. In contrast, growth arrest or Ca^{++} flux induced through CD19 was decreased, but not ablated, by removal of the last 117 amino acids of the cytoplasmic tail. Thus, although all the CD19 deletion mutants retained the ability to associate with the other members of the CD19 complex on the cell surface, some, but not all, signaling events were impaired. Therefore, CD19 may regulate cell growth and Ca^{++} flux via the cytoplasmic tail and other intracellular signals via its associated proteins.

To further examine the *in vivo* significance of CD19 expression, a series of eight transgenic mouse strains were produced that express differing levels of CD19. Ectopic expression of the human CD19 gene in transgenic mice was completely B lineage-restricted. As occurs in humans, CD19 was only expressed by B cells and was expressed by all mature B cells in blood and spleen. Each mouse line expressed varying amounts of human CD19, ranging from low to high levels of expression, depending on gene copy number. In general the phenotype of these mice was normal with one major exception. There was an inverse correlation between high expression of human CD19 and the number of circulating B cells in blood. This may correlate with increases in the number of B cells found in other tissues. It is possible that CD19 either directs the migration of B cells or that overexpression of CD19 makes B cells more susceptible to factors/stimuli that recruit them from the circulation. Further characterization of the unique features of these mice in conjunction with additional *in vitro* studies should provide considerable insight into the function of the CD19 signal transduction complex.

Development of Germinal Centers

F 008 AFFINITY MATURATION OF IMMUNE RESPONSES: MOLECULES AND MICROENVIRONMENTS, David Gray[1], Sandra Jainandunsing[1], Eva Källberg[2] and Tomas Leanderson[2], [1]Royal Postgraduate Medical School, Hammersmith Hospital, London, U.K. and [2]University of Lund, Lund, Sweden.

The antigen-driven foci of proliferation that are germinal centres represent the clonal expansion of memory B cells. The accumulation of somatic mutations in the V genes of memory B cells followed by the selection of high-affinity mutants was therefore proposed to take place in the germinal centre. Over the last few years experiments have borne out this idea and provided a molecular basis for for the antigenic selection and programmed *differentiation* of memory cells and plasma cells in these sites. We have studied the kinetics of somatic mutation in germinal centre and recirculating B cell populations by PCR amplification of the VκOx 1 gene, from cells in the lymph node draining a footpad injection of phenyl-oxazolone (phOx) ovalbumin. The results show that in lymph nodes mutations can be detected as early as the second day after immunisation. While this is at least 2 days prior to the appearance of small accumulations of cells in primordial germinal centres, these early mutations are seen only in cells with a germinal centre phenotype. Both the onset of mutation and the formation of germinal centres is accelerated by about 3 days in the lymph node compared to the spleen. Detailed analysis of the observed mutations in relation to the contact residues that bind phOx reveal that the early burst of mutation between day 2 and 4 occurs without associated antigenic selection. Selection of the somatic mutants for high affinity binders seems to be initiated sometime between day 4 and 7. This early burst of mutation might correlate with the initial explosive phase of B blast proliferation that occurs prior to the appearance of centrocytes in early germinal centres (described by MacLennan and colleagues). These results will be discussed in the context of ideas about memory B cell lineages.

Following the antigenic selection of the mutated B cells by cross-linking of their Ig receptors with antigen bound as immune complex on follicular dendritic cells (FDC) the B cells are programmed for further differentiation, to memory cells or to plasma cells during their exit from the germinal centre. The signal for memory cell differentiation seems to be delivered via CD40. In other experimental systems CD40 seems to be a maintenance factor for B cells (ie. prolonged growth in vitro). We have evidence that the continued survival of memory B cells in vivo requires, in addition to antigenic stimuli, non-cognate signals from T cells (either lymphokines or contact mediated interactions). We will describe adoptive transfer experiments in which the role of CD40 and other molecules as survival signals for B cells are tested.

F 009 SIGNALS INVOLVED IN B CELL SELECTION AND DIFFERENTIATION IN FOLLICLES, Ian C. M. MacLennan, Gerald D. Johnson, Montserat Casamayor-Palleya, Yong-Jun Liu, Deborah Hardie and J. Gordon. Department of Immunology, University of Birmingham Medical School, Birmingham B15 2TT, England.

Increase of affinity of antibody produced in responses to T cell -dependent antigens is associated with the appearance of B cells which have developed mutations in their Ig V- region genes. These mutations are induced by a site-directed hypermutation mechanism which acts on the rearranged V-region genes of centroblasts - proliferating B-lineage cells of germinal centers. It appears that centrocytes, the progeny of centroblasts, are selected within germinal centers on the basis of their ability to receive signals dependent on interaction with antigen held in the germinal center on follicular dendritic cells. Centrocytes which fail to receive this signal destroy themselves by apoptosis. Most positively-selected cells on the other hand leave the germinal center as plasmablasts or memory B cells. A range of signals have now been identified which induce isolated centrocytes to differentiate. These include soluble CD23, which in combination with IL-1α induces centrocytes to differentiate to IgG-containing plasmablasts. The B cell surface molecule recognized by CD40 antibody results in centrocytes adopting the phenotype of small B cells, while the combination of CD40 and IL-4 produces B blasts which express high levels of surface CD23, CD25 and surface immunoglobulin. IL-2 induces a small proportion of germinal center cells to become IgM-containing plasmablasts. This cytokine is also a candidate for maintaining proliferation among centroblasts. These processes take place in four distinct microenvironments within the germinal center. These are the dark zone the basal light zone, the apical light zone and the outer zone. The first three of these zones respectively appear to relate to: (i) proliferation with Ig V-region-gene hypermutation; (ii)antigen-driven selection and (iii) the delivery of signals which induce centrocytes to differentiate to memory B cells or plasmablasts.

F 010 B CELL PROLIFERATION: COSTIMULATORY ACTIVITY OF FOLLICULAR DENDRITIC CELLS, John G. Tew[1], Daniel H. Conrad[1], Dahui Qin[1], Andras K. Szakal[2] and Gregory F. Burton[1], [1]Departments of Microbiology and Immunology and [2]Anatomy, Division of Immunobiology, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia.

Follicular dendritic cells (FDC) trap Ag in the form of immune complexes and are capable of presenting Ag in these complexes to specific B cells. These events occur in germinal centers where B cells are rapidly proliferating. We reasoned that FDC might not only provide specific Ag (the primary B cell signal) but also additional co-stimulatory signal(s) which would enhance the ability of Ag to stimulate B cell proliferation in germinal centers. To test this, FDC were cultured with B cells activated by a sIg dependent (anti-μ-dex) or independent (LPS) pathway and their proliferation measured using ^{3}H-thymidine incorporation. The addition of FDC markedly augmented B cell proliferation in a dose dependent fashion. Depletion of FDC from cultures abrogated the increased proliferation. Addition of highly purified FDC obtained from cell sorting resulted in B cell co-stimulation whereas addition of other sorted cells was without effect. The FDC accessory activity was apparent over a 5 day culture period and over a wide range of either polyclonal B cell activator. When B cells and activators were cultured in the absence of FDC, only about one-fourth of the cells remained viable after 3 days. In contrast, virtually all cells in cultures containing FDC, B cells and activator were viable. FDC from nude mice also provided normal co-stimulation in the presence of either activator. The co-stimulatory activity of the FDC could not replace either the anti-μ-dex or IL-4 and was not MHC restricted. B cells proliferated best when in direct contact with FDC, however, FDC could be separated from the B cells using an 0.4 micron filter and still deliver a marked co-stimulatory signal when anti-μ-dex was used as the primary signal. Similarly, supernatants from the cultured FDC had co-stimulatory activity although much of this activity could be removed by centrifugation suggesting that the co-stimulator may be membrane associated (Perhaps on immune complex coated bodies or iccosomes). Furthermore, the co-stimulatory activity of both the intact FDC and the FDC supernatant could be significantly inhibited by an anti-ICAM-1 monoclonal antibody (MK-1) suggesting that some of the co-stimulatory activity may be attributable to ICAM-1. It is known that mouse FDC label with MK-1 far more intensely than any other cell type in lymph nodes suggesting that they are very rich in ICAM-1 (Maeda, Kosco, Burton, Szakal, and Tew, Unpublished). In short, these data indicate that FDC provide not only Ag but also facilitate B cell proliferation by means of other co-stimulatory interactions which contribute to make the microenvironment in the germinal center favorable for B cell proliferation. Supported by NIH Grant # AI 17142.

F 011 THE CYTOKINE DEPENDENCE OF NORMAL AND LYMPHOMATOUS GERMINAL CENTER CELLS IN MICE, Vincent K. Tsiagbe and G. Jeanette Thorbecke, New York University School of Medicine, New York, NY 10016.

A comparison was made of the properties of germinal center (GC) derived lymphoma cells ("RCS" from SJL mice) with those of GC cells isolated from normal immune lymph nodes (LN). Proliferation of both the normal and the abnormal GC cells requires help from T cells: neither proceed in nu/nu mice and both are helped by added T cells in vivo and in vitro. While the continued cytokine requirements for growth of tissue culture cell lines derived from the SJL lymphomas (cRCS) vary somewhat, 3/3 respond to IL-5, 1/3 to IL-4 and 1/3 to IL-2 with increased proliferation. None are inhibited by IFN-gamma, all by TGF-B. Normal GC cells are costimulated, similar to other B cells, by IL-4 in proliferation to anti-IgM, but to costimulation by chondroitin sulfate C and IL-4, and by dextran sulfate and IL-5, GC B cells respond much better than other LN B cells. While peritoneal B cells are also costimulated by IL-5 and dextran sulfate, they are very sensitive to inhibition by IFN-gamma, whereas GC cells are not. Normal GC cells probably stimulate T cells to produce cytokines used for their own growth via antigen presentation, and need costimulation which they may receive from the surrounding follicular dendritic cells and/or T cells. RCS cells do not require the costimulation, and have found a novel way of stimulating the T cells to produce the needed cytokines. They all express very high levels of a 1.5 kb mRNA for MMTV-LTR, and their ability to stimulate syngeneic T cells is inhibited by antisense S-oligo-nucleotides to this mRNA. The sequence of the MMTV-LTR cloned from RCS differs in the 3' region from MMTV-LTRs previously sequenced. The strong stimulation of normal SJL T cells by SJL lymphomas thus appears to be due to a superantigen coded for by this MMTV-LTR, as is also suggested by our finding that the T cell response to RCS is confined to Vβ16$^+$ cells. We suggest that the production of IL-5 and other cytokines by Vβ16$^+$ T cells, possibly in equilibrium with anergy (or apoptosis) in these cells regulates the varying growth rates of primary RCS in vivo.
Supported by USPHS Grant # CA-14462.

B-Cell T-Cell Interactions

F 012 REGULATION OF B-CELL FUNCTION BY CD40 LIGAND, Richard J. Armitage, Melanie K. Spriggs, Charles R. Maliszewski, Mark R. Alderson, Kenneth H. Grabstein, and William C. Fanslow, Immunex Research and Development Corporation, Seattle.

B-cell activation mediated by activated helper T cells has been shown to be dependent on direct cell contact. Several groups have found that soluble constructs of the CD40 molecule can effectively inhibit this B-cell response. Recently murine and human ligands for CD40 were identified and cloned. CD40 ligand (CD40L) is a Type II membrane protein transiently expressed by activated, but not resting, T cells. Maximal expression of CD40L on T helper clones occurs 8-12 hours post-stimulation with only low levels of ligand still detected after 24 hours. Fixed CV1/EBNA cells expressing recombinant CD40L had mitogenic activity for human and murine B cells in the absence of costimulus. The proliferative response of murine B cells was enhanced by addition of IL-4 + IL-5, while the response of human cells was augmented by IL-2, IL-4 or IL-10. CD40L-induction of human and murine IgE secretion was dependent on IL-4 being present in cultures. In the murine system, secretion of Ig isotypes, including IgE, from purified splenic B cells required IL-4 + IL5 in addition to CD40L. In contrast, purified human tonsil B cells cultured with CD40L alone secreted modest amounts of IgM, IgG1-3 and IgA. Production of these isotypes was greatly increased in the presence of IL-2 or IL-10. Secretion of IgG4 was observed only in the presence of CD40L and IL-4. Secretion of all human Ig isotypes in response to CD40L was inhibited by TGFβ in a dose-dependent manner. In addition to the induction of B-cell proliferation and differentiation, CD40L was found to modulate the expression of class II MHC, CD23 and surface IgM on B cells prior to their entry into cell cycle. Taken together, these data indicate a pivotal role for CD40 ligand in B-cell function.

F 013 **STRUCTURE AND FUNCTION OF RECEPTORS THAT MEDIATE LEUKOCYTE ACTIVATION AND HOMING.** **Ellen Pure', Wistar Institute, Philadelphia, PA, 19104.**

Anti-Ig antibodies induce the rapid accumulation of tyrosine phosphorylated proteins. We have found that mIg also mediates the upregulation of tyrosine and serine/threonine kinase activities associated with anti-P-tyr immune complexes from lysates of B cells. B lymphoblasts generated under T cell independent and T cell dependent conditions are primed for enhanced mIg mediated stimulation of the tyrosine kinase activity. We have identified a prominent endogeonous substrate of the *in vitro* tyrosine kinase activity as the 72-kDa protein tyrosine kinase, PTK72, that associates with mIg. The induction of PTK72 phosphorylation is dependent on receptor crosslinking and is induced synergistically by anti-Ig and interleukin-4. Although the critical tyrosine phosphorylated protein(s) induced *in situ* has not yet been identified, we have pharmocologic evidence that tyrosine phosphorylation is required for ligand-induced internalization of the mIg receptor. This suggests that tyrosine phosphorylation may be important for B cells to function as specific antigen presenting cells. The second receptor to be discussed is CD44. CD44 may play a role in lymphocyte extravasation, leukopoiesis and tumor metastasis, mediated in part by its affinity for hyaluronic acid and/or endothelial cell counter-receptors. Expression of high lvels of CD44 distinguish virgin from antigen primed B cells and is characteristic of "memory" T cells. It has become evident that the adhesion function of CD44 is regulated. We have demonstrated a cell type and activation state dependent variation in the N-linked glycosylation, phosphorylation and cytoskeletal association of CD44. *In vivo* experiments indicate that CD44 expression is not required for normal leukocyte extravasation, but is necessary for optimal contact allergic responses.

F 014 HELPER T CELL SUBSETS AND THEIR INTERACTION WITH B CELLS, Susan L. Swain, Linda Bradley, Michael Croft, and Ahmad Montazer. University of California, San Diego, La Jolla, CA 92093-0063.

Helper T cells (Th) regulate the activation, proliferation and differentiation of B cells. Signals delivered both during the direct interaction of Th and B cells and by Th produced cytokines promote all phases of B response and determine the development of B cells able to secrete different isotypes of Immunoglobulin (Ig). During T-B interaction, co-receptors on T and B cells interact and become clustered in the area of interaction between the cells. Some molecules such as LFA-1 and ICAM-1 show redistribution regardless of whether the interaction involves cognate recognition whereas other(s) such as CD4 on T cells only cluster during cognate interaction. Distinct subsets of helper T cells can be generated in vitro and these subsets regulate B cell response quite differently. Using TCR transgenic mice we have evaluated the helper activity of several subsets of Th. Naive CD4 T cells can only help B cells when they interact via cognate recognition. They promote only an IgM response with little isotype switch. Effector Th cells, with Th0 or Th1 phenotypes give good help to B cells in cognate responses, promoting a range of isotypes. Some bystander help is apparent, but Ab produced without cognate interaction represents a small fraction of the amount generated when a cognate interaction occurs. In contrast, Th2-like effector cells give very impressive help in cognate interactions and also promote more vigorous bystander responses. Activation of all Th by plate bound anti-CD3 results in increased ability of the population to deliver bystander help. As expected, production of IFN-γ is associated with enhanced IgG_{2a} and decreased IgG_1 and IgE responses, while IL-4 and IL-5 production are associated with enhanced production of all isotype other than IgG_{2a}. In vivo, a unique population of memory effectors can be generated. Memory effectors are the most potent Ag-specific helper providing much more help than resting memory Th or effectors generated from naive T cells. It is clear that the particular Th subsets that are initially available, and that are generated during an immune response, will determine in large measure the magnitude and isotype of the antibody response that will occur.

Immunoglobulin Rearrangements (Joint) (Session Sponsored by Abbott Laboratories, Diagnostics Division)

F 015 HOW IS THE LYMPHOKINE-PRODUCING PHENOTYPE OF AN IMMUNE RESPONSE ESTABLISHED, William E. Paul, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892 USA

The protective value of immune responses is largely determined by the set of lymphokines produced by T cells in that response. CD4+ T cells can develop into cells that principally produce IL-2 and IFNγ or into cells that principally produce IL-4, IL-5, IL-6 and IL-10. In order to examine the factors that control which set of lymphokines will be produced, we carried out *in vitro* priming of CD4+ T cells from mice transgenic for genes for the α and β chains of the T cell receptor for peptide 88-104 of pigeon cytochrome C in association with the class II MHC molecule I-E^k. These cells produced little IL-4 or IFNγ upon initial *in vitro* challenge with 88-104 and appropriate antigen-presenting cells (APC) although they proliferated vigorously. Upon rechallenge 4 days later, they produced either IL-4 or IFNγ (and IL-2) depending upon the conditions during the priming culture. The key determinant of the lymphokine-producing phenotype was IL-4 itself. If IL-4 was present during the priming culture, the cells were excellent producers of Il-4 upon challenge but made little or no IFNγ or IL-2. By contrast, if IL-4 were omitted, the cells produced IFNγ and IL-2 but no IL-4. Varying the type of APC changed the magnitude of the stimulation but did not effect the pattern. The effect of IL-4 in determination of lymphokine-producing phenotype was verified by in vivo experiments in which mice were treated with anti-IL-4 at the time of priming. Such treatment diminished the production of IL-4 upon in vitro challenge with antigen and APC for at least 75 days. By contrast, anti-IL-4 administered at the time of secondary challenge did not diminish IL-4 production in cells from mice that had been primed in the absence of anti-IL-4. The effect of IL-4 determining lymphokine-producing phenotype was correlated with a potent inhibitory action of IL-4 on IL-2 and IFNγ production in response to stimulation of naive T cells with soluble anti-CD3 and APC, concanavalin A and APC or antigen and APC. This inhibitory effect of IL-4 was not observed on long term T cell lines, on T cell hybridomas or on transgenic T cells that had been primed with antigen and were restimulated with antigen and APC in the presence of IL-4. Although IL-4 failed to inhibit IL-2 and IFNγ production by naive T cells in response to immobilized anti-CD3 or to calcium ionophore and phorbol ester, studies indicated that its effect was not at the level of blocking the CD28/B7 accessory signals delivered through the interaction of B7+ APC with CD28+ T cells. These results establish that IL-4 present at the time of priming is the principal factor that determines lymphokine-producing phenotype of CD4+ T cells and establish an experimental system in which the mechanism of IL-4 action can be studied.

F 016 ANALYSIS OF IG GENE REARRANGEMENT IN A TEST SUBSTRATE IN TRANSGENIC MICE, Ursula Storb, Peter Engler, Emily Klotz and Andrew Weng, Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL 60637.

We have produced a transgenic mouse line with seven head-to-tail copies of an artificial V-J recombination substrate, pHRD. This test gene contains the heavy chain enhancer and metallothionein promoter. The methylation of the transgene is under the control of a dominant strain-specific modifier gene, *Ssm-1* (1). When the transgene is methylated (in C57BL/6), no recombination can be detected, but when it is unmethylated (in DBA/2), rearrangement is easily seen in spleen, bone marrow, lymph nodes and Peyer's patches, but not in thymus or nonlymphoid tissues (2). In mice with a partial methylation phenotype, rearrangement is mainly seen in hypomethylated copies. Furthermore, only unmethylated pHRD transgenes are transcribed, and only in lymphoid organs. Thus, methylation interferes with both transcription and rearrangement, but undermethylation alone is not sufficient for expression. The brain may be an exception and is under further investigation.

The pHRD test gene does not encode a selectable Ig or TCR. Therefore, the V-J joints should persist as a pool of randomly created rearrangements. Over 70, mostly different, V-J joints were sequenced from fetal liver and adult lymphoid organs. In the fetus, no N regions were detected, whereas 74% of the joints in the adult contained N regions (3). Thus, the absence of N regions in endogenous fetal Ig genes is apparently due to a mechanistic difference between fetal liver and early pre B cells, presumably caused by differing levels of terminal transferase.

Since the pHRD test genes in this transgenic line are aligned as seven identical head-to-tail copies it was possible to determine whether the spacing between rearrangement signal sequences plays a role in rearrangement. It was found that recombination occurs randomly between any two recombination signal sequences within the transgene array (2). The significance of this finding with respect to the mechanism of V(D)J recombination will be discussed.

Supported by NIH grants HD23089 and AI24780. P.E., E.K. and A.W. were supported by NIH training grants CA09594, GM07183 and GM07197 respectively.

1. P.Engler, D.Haasch, C.Pinkert, L.Doglio, M.Glymour, R.Brinster & U.Storb, Cell 65,939, 1991.
2. P.Engler, A.Weng & U.Storb, Mol.Cell.Biol., in press, Jan. 1993.
3. P.Engler, E.Klotz & U.Storb, J.Exp.Med.,176,1399, 1992.

B-Cell in Marrow Transplantation

F 017 HOW DO B CELLS RECOVER AND FUNCTION AFTER T CELL DEPLETED BONE MARROW TRANSPLANTATION?, Malcolm K. Brenner, St. Jude Children's Research Hospital, Memphis, TN 38101

T cell depletion (TCD) of donor marrow is probably the most effective way of preventing graft versus host disease, a major cause of morbidity and mortality after allogeneic bone marrow transplantation. One concern about TCD was that B cell growth and development in the recipient would be severely compromised by the absence of T helper cells. We have investigated this issue by using a human adoptive transfer system, in which donors, or recipients, or both are immunized before transplantation and antibody is measured in the recipient. We found that responses to recall antigens were readily transferred, but that efficient transfer required both donor and recipient to be immunized 7-10 days prior to BMT. At this time antibody producing B cells are present in the donor marrow and are transferred to the recipient where they secrete large quantities of antibody, provided antigen remains available. Allotyping and spectrotyping confirmed that the antibody comes from engrafting donor cells. The highest titers of antibody are found by about 21 days post BMT, when helper T cells are undetectable. Stimulation of antigen activated B cells appears to come instead from non-cognate interactions with circulating CD56+CD16+/- large granular lymphocytes (LGL) which numerically dominate lymphocyte recovery in the early post transplant period. These LGL secrete IL6, TNF and other B cell growth and differentiation factors and may contribute to the antibody responses seen. LGL cannot provide help to B cells responding to a primary stimulus; responses to such antigens appear to require cognate interactions with helper T cells and can only be transferred to recipients after a period of B cell maturation and sometimes re-stimulation in the original, T replete, host. Thus, even without helper T cells, it is possible to use the donor graft to transfer protective antibody responses to a range of pathogens, including Hepatitis B virus, Pseudomonas and Klebsiella.
Unexpectedly, it is a lack of cytotoxic T cells that is proving more problematic for B cell function after T cell depleted BMT. Up to 10% of TCD marrow recipients develop fatal Epstein-Barr virus induced B cell lymphoproliferation. We are currently using adoptive transfer of virus specific cytotoxic T cells to try to overcome this problem.

F 018 RECONSTITUTION OF THE B CELL REPERTOIRE AFTER BONE MARROW TRANSPLANTATION, Carol Nottenburg[1], Laurie Pfister[1], Ivy Suzuki-Jaecks[2], and Eric Milner[2], [1]Fred Hutchinson Cancer Research Center, Seattle WA, and [2]Virginia Mason Research Center, Seattle, WA.

Bone marrow transplant recipients, especially those with chronic graft-versus-host disease (GVHD) often fail to mount a specific antibody response. The extent of immunological recovery of these recipients can be determined by examining the utilized antibody gene repertoire. First, we have examined the relative usage of the seven V_H gene families in peripheral B cells isolated from a marrow recipient, the corresponding marrow donor, and an unrelated normal individual. Libraries of heavy chain gene rearrangements were made by polymerase chain reaction (PCR) amplification and were probed with radiolabeled fragments specific for the seven V_H families. V_H family usage between the marrow recipient at 90 and 458 days after transplant and the marrow donor was statistically significantly different; V_H family usage in the recipient between 90 and 458 days after transplant was also statistically significantly different. The greatest fluctuation in usage occurred in the V_H3 and V_H4 families. Furthermore, DNA sequences of isolated V_H3 gene rearrangements from the marrow recipient showed a large proportion of identical DNA sequences at the rearrangement junction, suggesting that peripheral B cells may arise from a limited pool of B-cell precursors.

Not all members of individual families are expressed equivalently during development and during antigen responses. We have examined and compared the expression of individual genes within the V_H3 and V_H4 families in normal adults and in marrow recipeints. Libraries of V_H3 and V_H4 gene rearrangements, as well as libraries of unrearranged V_H3 genes from the same individuals, were generated by PCR amplification. The resulting clones were hybridized with radiolabeled oligomer probes that detect specific V_H genes. Comparison of V_H3 gene expression between a library of the rearranged genes to a library of unrearranged genes from a normal adult showed statistically significant differences in usage among three of 18 V_H3 genes examined. Only one of these overexpressed genes is a fetal-expressed gene. More generally, there is a random utilization of V_H3 genes in peripheral B cells. In contrast, B cells isolated from a marrow recipient show a very distinct usage pattern of V_H3 genes; one V_H3 gene is not expressed at all and another V_H3 gene is expressed in very high proportion.

Cytokine Interactions with B-Cells (Joint)

F 019 CYTOKINE CONTROL OF HUMAN B LYMPHOCYTE DIFFERENTIATION, Jacques Banchereau, Dominique Blanchard, Francine Brière, Serge Lebecque and Françoise Rousset. Schering-Plough, Laboratory for Immunological Research, Dardilly, France.

INTRODUCTION : Antigens breaking the cutaneous or mucosal barrier are rapidly taken up by Dendritic/Langerhans cells which migrate to the draining lymph nodes where they initiate the development of T and B cell reactions. Antigen specific B cells proliferate and differentiate into plasma cells. Some B cells migrate within the follicles to generate germinal centers where they expand, undergo somatic mutations, isotype switching, antigen selection and differentiation into either memory cells or plasmablasts secreting antibodies of higher affinity and specificity. In order to understand the mechanisms controlling these steps, we have studied the role of cytokines in the growth and differentiation of B cells activated in two different systems : the CD40 system and the CD3 system.

THE CD40 SYSTEM: Resting B cells proliferate when cultured in the presence of anti-CD40 and a fibroblastic cell line (L cells) expressing an IgG Fc receptor ($Fc\gamma R_{II}$/CDw32). Additional triggering of the sIg results in T cell-independent differentiation of B cells. Naive $sIgD^+$ B cells produce mostly IgM and isotype commited $sIgD^-$ B cells produce mostly IgG and IgA. IL-4 strongly stimulates the proliferation of B cells cultured in the CD40 system and factor dependent B cell lines could be maintained for up to 10 weeks. Single B cells can give rise to colonies of 50-500 cells within two weeks. IL-10 enhances short term B cell proliferation and combinations of IL-4 and IL-10 result in strong multiplication of B cells. Naive B cells undergo isotype switching towards IgE in response to IL-4. This is demonstrated by using the clonal growth of G8 idiotype positive single B cells and by sequencing of the $VDJC_H$ products of single daughter B cells. B cells secrete large amounts of IgM, IgG and IgA in response to IL-10 following differentiation into plasma cells. Naive $sIgD^+$ $sIgM^+$ B cells, which produce essentially IgM in response to IL-10, are induced to secrete both IgA_1 and IgA_2 following addition of TGFß. In contrast, TGFß suppressed the IL-10 mediated IgG, IgM and IgA secretion by $sIgD^-$ B cells. The CD40 system displays many of the features of B-cell immunopoiesis, including intense proliferation, isotype switching and plasma cell differentiation, but does not to induce somatic mutations.

THE CD3 SYSTEM: Some $CD4^+$ clones, activated with immobilized anti-CD3, induce growth and differentiation of resting B cells. The proliferation is limited because cells readily differentiate into non proliferating plasma cells. These clones also induced isotype switching in naive B cells. Addition of IL-4 antagonists to cultures result in inhibition of IgE production while the secretion of other isotypes and the proliferation is poorly affected. Addition of IL-2 antagonists inhibits B cell proliferation and production of IgG, A, M without inhibiting that of IgE. T cell clones activated for 24-28 h and washed free of cytokines fail to induce B cell proliferation and differentiation. Addition of IL-2 (but neither of IL-4 nor of IL-10) to such cultures results in strong B cell growth and Ig secretion and IL-10 further enhances these effects.

PERSPECTIVES : Identification of culture conditions yielding somatic mutations will represent a major step towards the "*in vitro* immunization" of naive B cells. This would ultimately permit us to generate human monoclonal antibodies of desired specificity and unavailable in the human repertoire.

F 020 THE TNF/NGF SUPERFAMILY OF RECEPTORS AND THEIR LIGANDS, Raymond G. Goodwin, Richard J. Armitage, Douglas E. Williams, Brian Gliniak, Kenneth Grabstein, William C. Fanslow, Terry Farrah, Ian B. McAlister, Ben Falk, Terri Davis, Wenie S. Din, and Craig A. Smith, Immunex Research and Development Corp., Seattle

A growing superfamily of receptor molecules has recently emerged which includes the two receptors for TNF, the low affinity receptor for NGF, the B cell antigen CD40, the lymphoid activation antigens CD27 and CD30, the rat T cell antigen OX40, the murine T cell antigen 4-1BB, the surface antigen Fas, as well as the predicted protein products of several viral open reading frames. Homology between the various family members is primarily confined to their cysteine-rich extracellular domains. Ligands for these receptor-like molecules have been identified for the TNF and NGF receptors, and recently for CD40. The CD40 ligand, like pro-TNFα, is a type II membrane protein with sequence similarity in its extracellular domain to TNFα and TNFβ. Using the extracellular domain of CD30 linked to the constant domain of human IgG1 as a probe, we have identified a ligand (CD30L) on the surface of an induced murine T cell clone. A cDNA encoding this protein was isolated by a direct expression method, the sequence of which revealed the ligand to be a 239 amino acid type II membrane protein with significant homology in its C-terminal, extracellular domain to TNFα, TNFβ, and the CD40 ligand. These results suggest the emergence of a ligand superfamily parallel to the receptor superfamily with which they interact. A human CD30L cDNA isolated from a peripheral blood T cell library by cross hybridization encodes a protein of 234 amino acids which is 72% identical to its murine homologue at the amino acid level. Investigations of the possible role of CD30, a surface marker on Reed-Sternberg cells, and CD30L in the pathogenesis of Hodgkin's Disease will be discussed.

Modulation of B-Cell Response in Vivo

F 021 **INDUCTION OF T CELL ACTIVATION OR TOLERANCE BY ANTIGEN PRESENTING B LYMPHOCYTES *IN VIVO*,** **Fred D. Finkelman[1], John J. Ryan[2], and Suzanne C. Morris[1], [1]Department of Medicine, Uniformed Services University of the Health Sciences, and [2]Naval Medical Research Institute, Bethesda, Maryland, 20814**

Injection of mice with a rat IgG2a mAb that effectively crosslinks B cell membrane IgD stimulates both polyclonal and rat IgG2a-specific IgG1 antibody responses. In contrast, injection of rat IgG2 mAbs to CD23, $Fc\gamma RII$, or complement receptor 1, which also bind to B lymphocytes, fails to stimulate specific or polyclonal antibody production. The failure of mice injected solely with rat IgG2a anti-CD23 mAb to make anti-rat IgG antibody was accompanied by the induction of tolerance to rat IgG2a. Anti-CD23 pretreatment completely blocked the development of specific anti-rat IgG2a responses to challenge with rat IgG2a anti-IgD antibody or rat IgG2a in complete Freund's adjuvant, and inhibited the polyclonal response to rat IgG2a anti-IgD by ~85%. In contrast, there was no inhibition of the polyclonal response to allo-anti-IgD mAbs or to goat anti-mouse IgD. This selective inhibition of the polyclonal response to rat IgG2a anti-IgD indicates that T cell tolerance was induced. Considerable tolerance induction was observed within 4 days of anti-CD23 mAb injection, and with doses of mAb as low as 100 ng. Simultaneous injection of mice that express Ig of the *a* allotype with any of these rat IgG2a anti-CD23 plus a mouse alloantibody that crosslinks IgD of the *a* allotype did not induce tolerance to rat IgG2a, but instead, stimulated both polyclonal and rat IgG2-specific antibody responses. These T cell-dependent antibody responses were observed even when both the rat IgG2a mAb and the mouse allo-anti-IgD^a antibody were injected into *a* x *b* allotype heterozygous mice. In these allotype-heterozygotes, the anti-IgD^a mAb, which is seen as self, fails to induce an antibody response if given alone, and most of the polyclonal IgG1 response that is induced by injection of both anti-IgD^a and rat IgG2a mAb is of the *a* allotype. Avoidance of tolerance induction by coinjection of allo-anti-IgD antibody was not simply a result of activating B cells, since one rat IgG2a anti-IgD antibody that modulates most IgD from the B cell surface, but poorly activates B cells, induced a small anti-rat IgG2a response rather than tolerance, and injection of rat IgG2a anti-CD23 mAb with an allo-anti-IgD mAb-dextran conjugate that effectively activates B cells, but poorly modulates B cell membrane IgD, was tolerogenic. Taken together, these observations provide evidence that: 1) crosslinking of B cell Ig contributes to the generation of T cell-dependent antibody responses, even once T cell activation has already occured; 2) B lymphocyte presentation of antigen to T lymphocytes can either activate or tolerize the T lymphocyte; and 3) the degree of activation of the antigen presenting B lymphocyte is not the only determinant of whether T lymphocyte activation or tolerance is induced.

Stem Cells and Early B Cell Progenitors

F 100 CYTOKINE RESPONSE PATTERNS OF A HUMAN PRE-B CELL-LIKE CELL LINE (SMS-SB) OVER-EXPRESSING THE c-*fos* ONCOGENE.

W. Cushley[1], R.G. Smith[2] & B.W. Ozanne[3]. [1]Department of Biochemistry, University of Glasgow, Glasgow, Scotland, UK, [2]Department of Internal Medicine, Southwestern Medical School, Dallas, Texas, USA, & [3]Beatson Institute for Cancer Research, Glasgow, Scotland, UK.

The ability of recombinant cytokines to influence the autonomous growth of the SMS-SB cell line, a human pre-B lymphoma-like line which over-expresses the c-*fos* oncogene, was investigated. Cells were seeded at low density (1000 or 5000 cells/well) under serum- and protein-free culture conditions, and proliferation assessed by tritiated thymidine incorporation after appropriate culture periods. IL-1α, IL-1β, IL-2, IL-6 and IL-11 had no effect on SMS-SB growth, but IL-3, IL-5 and, to a lesser extent, IL-4 and IL-7 could promote proliferation of the cells. No cytokines appeared to be growth inhibitory towards SMS-SB. Similar data were obtained in short (24hr) and long-term (3 day) proliferation assays. IL-4 also induced morphological changes in SMS-SB cells in protein-free cultures within 2 hours of addition. Leukaemia Inhibitory Factor (LIF) was also found to act as a mitogen for SMS-SB cells, particularly at high ($\geq 10^3$ U/ml) dose. Some mitogenic activity was also observed at very low concentrations (<1U/ml) of LIF, suggesting that two different affinity LIF receptors might deliver proliferative signals to SMS-SB cells. Addition of anti-LIF antibody to SMS-SB cultures did not inhibit the growth of the cells suggesting that, if SMS-SB cells synthesise and secrete LIF, then the cytokine is neither growth-inhibitory nor capable of potentiating proliferaton of the cells. Studies employing anti-sense oligonucleotides to abolish any LIF synthesis by SMS-SB cells are in progress to confirm that LIF plays no autocrine regulatory rôle in SMS-SB proliferation.

F 101 B AND T LYMPHOCYTE PROGENITORS ARE SELECTIVELY MAINTAINED IN VITRO BY SOLUBLE FACTORS DERIVED FROM MOUSE BM ADHERENT CELLS

Irving Goldschneider and Sean D. McKenna, Department of Pathology, School of Medicine, University of Connecticut Health Center, Farmington, CT 06030

We have reported that the selective *in vitro* generation of rat BM terminal deoxynucleotidyl transferase-positive (TdT^+) early lymphoid precursor cells in our long-term BM lymphoid culture system is dependent upon the presence of a mouse BM adherent cell feeder layer or medium conditioned by these cells. Most of these immature lymphoid cells and their TdT^- precursors have not yet undergone Ig or TCR gene rearrangement, and none express cIg, sIg or TCRαβ. Here we demonstrate that rat BM lymphoid cells from these cultures can, in the absence of detectable pluripotent hemopoietic stem cells (PHSC), reconstitute both the T- and B-cell compartments of irradiated recipient rats upon *in vivo* transfer. Both donor-origin thymocytes and sIg^+ splenocytes were observed after three weeks when as few as one million cultured cells were adoptively transfered intravenously. In addition, functional T and B cell progenitors, but not PHSC, were maintained selectively in suspension cultures of rat BM incubated with medium conditioned with mouse BM adherent cells. These *in vivo* adoptive transfer results correlated with the ability of conditioned medium to maintain the precursors of TdT^+ lymphoid cells, as determined by serial passage *in vitro*. The experiments demonstrate the feasibility of selectively generating and maintaining lymphoid progenitor (and possibly lymphoid stem) cells in the presence of stimulatory cytokines for subsequent *in vitro* manipulation and/or transplantation *in vivo*. Inasmuch as this culture system has been successfully adapted for the generation of TdT^+ lymphoid cells from normal human bone marrow, the present findings suggest that it may also be possible to stimulate the selective outgrowth of developmentally normal human lymphoid progenitor cells in suspension cultures. (Supported in part by ACS Grant IM-645 and NIH Grant AI-32752)

F 102 DEVELOPMENTAL AND EVOLUTIONARY CONTROL OF THE EXPRESSION OF V_H11 TO ENCODE ANTI-PHOSPHATIDYL CHOLINE (PTC) ANTIBODY. Geoffrey Haughton, Jessica K. Booker and Larry W. Arnold. Department of Microbiology and Immunology , Univ. of North Carolina, Chapel Hill, NC 27599.

Naturally occurring anti PtC in mice is encoded predominantly by $V_H11/V\kappa9$ or $V_H12/V\kappa4$. The obligatory heavy/light chain combinations and extreme constraints on sequence encoded by the VDJ assemblies, are evidence of antigen driven clonal selection. We have studied the starting population of B cells on which this process of selection operates. We selected hybridomas made from neonatal mice for production of V_H11 protein, sequenced the expressed Ig genes and tested specificity of the antibody. We used PCR to define rearrangements of V_H11 in 18 day fetal liver and in spleens of neonates. We found that the diversity of VDJ assemblies generated is much less than would be expected of a random process. There is a strong (DNA) bias in favor of those productive rearrangements of V_H11 needed to code anti-PtC, a bias that would probably be disabled by addition of N nucleotides, and there is evidence of negative selection against cells expressing "inappropriate" rearrangements of V_H11. Neonatal mice contain a low, but detectable proportion of cells expressing $V_H11/V\kappa9$ and secreting anti-PtC, but in most cases V_H11 is associated with a different light chain and secreted antibody does not bind PtC. Thus, molecular mechanisms controlling VDJ rearrangement in the fetus and neonate serve to heighten the probability that cells producing anti PtC will be generated during this period and will be available for antigen driven clonal expansion.

We also sequenced multiple alleles of functional V_H11 and V_H12 genes from inbred strains of mice and found a large excess of silent over replacement point mutations, as compared to alleles of the S107 family of V_H genes indicating that the protein products of V_H11 and V_H12 have high survival value for the mouse and have been evolutionarily conserved. The data imply that anti-PtC is highly beneficial and are sufficient to explain the fetal origin of cells producing this antibody, without any need to postulate discrete lineages of B cells.

F 103 PHYSICAL APPROACHES TO THE ISOLATION OF THE X-LINKED AGAMMAGLOBULINEMIA GENE (XLA), Christine Kinnon, Marie-Anne O'Reilly, Angela Sweatman, Linda Bradley, Lesley Alterman, Ruth Lovering, Sue Malcolm, and Roland Levinsky, Institute of Child Health, London WC1N 1EH, UK.

XLA is a rare immunodeficiency disorder, whereby affected boys have no circulating antibodies and thus, no humoral immunity. The nature of the XLA gene product is not known but it is postulated to play some role in the maturation of B cells, since affected boys have normal numbers of pre-B cells in their bone marrow. Attempts are being made to identify the gene by positional cloning.

So far genetic linkage analysis has mapped the XLA gene to Xq22 and identified proximal and distal flanking markers separated by 2cM (see abstract by Lovering et al.). Our physical maps of the region extend over 6.5Mb. DXS178, the locus which shows no recombination with XLA in over 70 informative meioses, lies on a 1.5Mb *MluI* fragment, together with DXS265 and DXS101. The DXS265 locus is almost coincident with DXS178, and while at least one copy of DXS101 is within 540kb of DXS178, there are at least another two copies of this sequence within Xq22, one of which lies near to the PLP gene. By mapping a series of YAC clones we estimate that the polymorphic copy of DXS101 lies within 1Mb of DXS178 and that DXS442 lies within 1.2Mb of DXS178, thus substantiating the estimated genetic distance between these two markers. We have identified a number of CpG islands, indicating the presence of transcribed gene sequences, in the region of the DXS178 locus. We are now using YACs which span the DNA between the flanking markers to isolate pre-B cell specific cDNA clones as candidates for the XLA gene.

F 104 GERMLINE KAPPA PROTEINS EXPRESSED BY PRECURSOR B CELLS ASSOCIATES ON THE CELL SURFACE WITH IgM. Hector Martinez-Valdez, Veronique Frances, Dominique Pandrau, Christiane Guret, Stephen Ho, Sem Saeland, and Jacques Banchereau, Schering-Plough, Laboratory for Immunology Research, Dardilly, France

We have cloned the cDNA encoding a JC kappa (JCk)germline transcript expressed by both normal and leukemic B cell precursors. Analysis of its sequence revealed an open reading frame, which predicted a polypeptide of(Mr.) 13,700 daltons (13.7 Kd). By using a panel of polyclonal and monoclonal anti-kappa antibodies we found by flow cytometry that polyclonal but not monoclonal antibodies, could detect intra-cytoplasmic kappa in B cell precursors with proven light chain loci in germline configuration. Furthermore, In Vitro translation of cRNA generated from the cloned germline kappa cDNA template, revealed a unique protein of the predicted molecular weight. Immunoprecipitation with anti-kappa antibodies, however, revealed that in addition to the 13.7 Kd molecule, another protein of approximately 16 Kd could be identified.Consistent with PCR data, this 16 Kd protein may represent a germlineVk which would associate to the JCk germline product. Two dimension electrophoresis, following immunoprecipitation of surface iodinated proteins demonstrated the presence of the two proteins in which only JCk appeared covalently associated to m. We thus propose, that during maturation, the signal transduction delivered through the association on the cell surface of rearranged m with a pseudo-light chain may not be resticted to l5 and VpreB.

F 105 GENERATION OF CELLS WITH PROPERTIES OF MICROGLIA FROM CLONED EBV-TRANSFORMED LYMPHOID PROGENITOR CELLS DERIVED FROM HUMAN FETAL LIVER, Atsushi Muraguchi, Hiromi Tagoh, Yasuhisa Ichigi, and Masao Kimoto, Department of Immunology, Toyama Medical and Pharmaceutical University, Toyama, Japan

Single cell clones from the Epstein Barr virus (EBV)-transformed lymphoid progenitor-like cell line established from human fetal liver at 8 wk gestation, have been derived and characterized. These clones retained immunoglobulin (Ig) and T cell receptor (TCR) genes in their germ line configuration. They expressed HLA-DR and some B lymphoid markers such as CD19, CD20, and in some, the T lymphoid marker, CD2. They did not express surface Igs, CD3, CD4, CD8 or TCRs. A sensitive RT-PCR assay revealed that they did not express mRNA for a recombination activating gene (RAG)-1, which is expressed after commitment to lymphoid cells. However, RAG-1 mRNA expression was induced in the cloned cells by co-culturing the cells with normal bone marrow-derived stromal cells. These results suggest that the established cloned lines are very early lymphoid progenitors that have not yet been committed to lymphoid cell lineage. In one of the lines, FL8.2.1.4, a marked morphological change that resembled microglia was induced when the cells were cultured in the presence of phorbol myristate acetate (PMA). After 72 hs of culture, 5-10% of FL8.2.1.4 cells developed a microglial morphology when stimulated with 10 to 100 ng/ml PMA. The newly generated cells with microglial morphology expressed HLA-DR and stained with *Recinus communis agglutinin-1*(RCA-1), which has been reported to bind specifically to brain microglia. In contrast, expression of lymphoid markers on cells with microglia-shaped morphology was remarkably diminished by PMA stimulation. Thus, the early lymphoid progenitor cells have the capacity to differentiate into cells with the morphological and antigenic properties of microglia cells. This system might be useful for further understanding of the characteristics and functions of microglia cells distributed in the central nerve system(CNS).

F 106 GERMLINE TRANSCRIPTS OF ALL 6 HUMAN VH GENE FAMILIES FROM NORMAL FETAL LIVER, Jerrold Schwaber, Department of Pathology, Hahnemann University, Philadelphia, PA 19106.

Transcription of the germ line gene elements that form the variable region of mature immunoglobulin heavy chains has been proposed to represent the process controlling access for regulation of the recombination enzymes in their sequential steps of catalysis. Evidence for germline transcription of VH gene elements, as part of VH to DJH recombination, has been limited to transcripts of only a few gene elements. We have examined normal fetal liver by Northern blotting, and present evidence for candidate germline transcripts from all of the six human VH gene families. The candidate VH4 transcripts have been confirmed as germline transcripts by hybridization with 3' flanking sequences that would have been removed by recombination from mature VHDJH genes. In addition polymerase chain reaction using primers for the VH4 gene elements has been used to amplify VH4 germline transcripts from normal bone marrow as well as fetal liver RNA. Direct sequence determination of these PCR products confirms their identity as VH4 germline transcripts.

F 107 EFFICIENT INTRODUCTION OF EXOGENOUS GENES INTO MURINE LYMPH NODE B CELLS AND LONG-TERM BONE MARROW CULTURE PRE-B CELLS AND RECONSTITUTION OF SCID MICE Natalie Sutkowski[1,2], Ming-Ling Kuo[1,2], Joseph Dougherty[1], and Yacov Ron[1], [1]Department of Molecular Genetics and Microbiology, University of Medicine and Dentistry of New Jersey, Piscataway, NJ 08854. [2] Graduate Program in Microbiology and Molecular Genetics, Rutgers University, New Brunswick, NJ 08903.

Efficient introduction of exogenous genes into primary lymphocytes is useful for studies on lymphocyte biology and for gene therapy purposes. Hence, murine lymph node (LN) B cells and long-term bone marrow culture (LTBMC) pre-B cells were used as target cells for gene transfer. Retroviral vectors containing both the neomycin phosphotransferase II (*neo*) and human adenosine deaminase (*ada*) genes mediated efficient transfer of these genes into the target cells by co-cultivation with irradiated vector virus-producing helper cells for 24 hours in the presence of interleukin-7 for LTBMC pre-B cells and LPS for LN B cells. Very efficient gene transfer approaching five proviral copies per target cell was detected by Southern blot analysis 2 days post-infection. Expression of the exogenous human ADA protein was also detected by enzymatic assay. Human ADA activity was at levels similar to the endogenous murine ADA protein.

Infected LTBMC pre-B cells from BALB/c cultures were injected into Igh congenic CB17 SCID mice. Proviral sequences were detected 5-8 weeks later by Southern blot analysis of genomic DNA isolated from whole spleen. Human ADA protein was also detected at a significant level in spleen cell lysates. Flow cytometric analysis of recipient CB17 SCID spleen cells showed that the BALB/c pre-B cells differentiated into mature B cells bearing surface IgM, IgD, and class II MHC. Similar studies using infected, mature LN B cells adoptively transferred into SCID recipients are ongoing.

F 108 REGULATION OF δ mRNA SYNTHESIS BY TRANSCRIPTIONAL TERMINATION.
Dorothy Yuan, Jing Tang, Tam Dang, and Bethany Moore. Department of Pathology, University of Texas Southwestern Medical Center, Dallas, Tx. 75235

The base level of membrane IgD expressed by bone marrow B lymphocytes is correlated with a low abundance of mRNA for the δ heavy chain. The low abundance is a reflection of transcriptional termination which has been shown to require a 1200 bp gene segment located between the μ and δ heavy chain genes. Using early B cells generated in long-term bone marrow cultures we have found that the transcriptional block can be alleviated by stimulation of the cells with phorbol ester, resulting in the increased production of δ mRNA. The ability to synthesize an anti-termination factor appears to be lost in transformed tumor cells because most of the stable transfectants of an intact μ-δ transcriptional unit synthesize no detectable δmRNA. However, one of these stable transfectants was found to express δmRNA, suggesting that this clone expresses an anti-termination factor(s). The effect of anti-termination can be mediated in trans because transient transfection of a second vector containing the termination segment also resulted in transcriptional readthrough. In contrast, control transfectants which terminated were not able to bypass the termination region in the transient transcriptional unit. Using these otherwise identical transfectants we hope to clone the gene(s) responsible for anti-termination.

B Cell Adhesion and Accessory Molecules

F 109 CD43 (LEUKOSIALIN) EPITOPES ARE DIFFERENTIALLY EXPRESSED ON MURINE B CELLS.
Cory G. Best, John D. Kemp, Lorraine T. Tygrett and Thomas J. Waldschmidt, Department of Pathology, University of Iowa College of Medicine, Iowa City, IA 52242

Murine CD43, as defined by the monoclonal rat antibody S7, has been previously shown to be a marker for a subset of pro-B cells, and for terminally differentiated B cells. Accordingly, CD43 was thought to be restricted to distinct stages of B cell differentiation. S7 also recognizes most T cells and granulocytic cells. When utilizing S11, a second rat anti-CD43 antibody, different results are obtained. S11 recognizes all B220+, IgM- pre-B cells, and all IgM^{hi} immature B cells. Similar to S7, S11 does not stain IgD^{hi}, CD23+ mature conventional B cells. In the peritoneum of adult mice, S7 recognizes only a portion of the Ly1+ B cells, whereas S11 stains all of these cells. Thus, CD43 has a broader expression on B cells than previously determined, although the epitope recognized by S7 is only accessible on distinct subsets of CD43+ B cells. On CD4+ splenic T cells, a similar phenomenon is observed. Although S11 stains CD4+ T cells with a uniform bright intensity, S7 stains a portion of these cells brightly, some moderately, and some not at all. Together, these observations imply that CD43 either exists in isoforms defined by post-translational modification, or that certain epitopes on the molecule are cryptic or hidden at distinct points in differentiation.

F 110 CD23 FUNCTIONS AS AN ADHESION MOLECULE INVOLVED IN HOMOTYPIC AGGREGATION OF HUMAN B LYMPHOCYTES, Pia Björck, Carina Elenström§, Eva Severinson§, Anders Rosén§ and Staffan Paulie, Department of Immunology, Stockholm University, Stockholm, Sweden and §) Department of Medical Cell Biology, Karolinska Institute, Stockholm, Sweden.

Antibodies to a number of B cell surface antigens including CD19, CD20, CD40 and MHC class II have been shown to induce homotypic aggregation of resting B cells and B cell lines. We have previously found that interleukin-4 (IL-4) is a strong potentiator of this process. The aggregation is clearly dependent on LFA-1, but the small clusters of cells remaining after blocking with LFA-1 antibodies suggest the involvement also of other adhesion system/s. We show here that antibodies to CD23 were able to inhibit aggregation to a similar extent as were LFA-1 antibodies. Inhibition was restricted to the MHM6 epitope of CD23 and antibodies to other epitopes (EBV CS-1, EBV CS-2, EBV CS-5) or occupation of the Fc-binding site with IgE had no or a slightly enhancing effect. This was also the case with antibodies to CD21 (THB5), the recently defined ligand for CD23. When combining antibodies to LFA-1 and CD23 aggregation was almost completely inhibited suggesting that these are the major adhesion systems responsible for homotypic B cell aggregation. The possible *in vivo* consequences of these findings are discussed.

F 111 CHARACTERIZATION OF MOUSE HOMOLOGUES OF HUMAN COMPLEMENT RECEPTORS 1 AND 2, Michael Holers, Taroh Kinoshita, Carol Brenner and Hector Molina, HHMI and Dept. of Med., Washington Univ. Sch. of Med., St. Louis, MO 63110

Complement receptors 1 and 2 (CR1 and CR2) have been proposed to play roles in localization of antigen to follicular dendritic cells, antigen presentation, modulation of B cell proliferation induced by surface Ig cross-linking, and the promotion of B cell responses to T dependent antigens. In order to further develop an in vivo animal model of CR1 and CR2 activities, we have characterized mouse homologues of these two proteins. Through combined cDNA and genomic cloning, in addition to the analysis of expressed recombinant proteins, we have previously characterized mouse CR1 (MCR1) and CR2 (MCR2). Surprisingly, as opposed to humans in which these proteins are the products of unique genes, MCR1 and MCR2 are the products of alternatively spliced transcripts from a common gene. Like human CR1 and CR2, though, these proteins are built of repeating modules of approximately 60 amino acid domains called short consensus repeats (SCRs). By deletion analysis, we now find that MCR1 contains a C3b binding domain in the amino terminal 4 of 21 SCRs. Expression of these 4 SCRs on a background of the membrane proximal 13 of 15 SCRs of human CR2 (which by themselves have no C3b binding activities) results in a chimeric molecule capable of rosetting with erythrocytes coated with mouse C3b, but not C3d. These four SCRs also contain the binding site for monoclonal antibody 8C12, which blocks mouse C3b binding to MCR1. Recombinant MCR1 also contains cofactor activity for factor I mediated cleavage of mouse C3b, an activity which human CR1 expresses for human C3b. Therefore, in spite of significant differences in the structure and molecular origin of MCR1 and MCR2, the molecules maintain several general characteristics of their human counterparts.

F 112A SUBTRACTED cDNA CLONE DIFFERENTIALLY EXPRESSED IN B CELL DEVELOPMENT AND ORGANS IS EVOLUTIONARY CONSERVED

H. Illges*§, A. Radbruch* and U. Krawinkel#
*Institute for Genetics, Cologne, Germany. #Faculty of Biology, Konstanz, Germany. §Present address: University of Alabama, Birmingham, USA.

In an attempt to search for genes involved in the activation and recombination of murine B cells we analysed cDNA clones derived from a subtraction of LPS activated murine spleen cells minus the plasmacytoma PC140. One clone analysed showed a differential expression in B cell development. The corresponding mRNA is expressed in two forms. While pre B cell lines express both forms in equivalent amounts, plasma cells express predominantly the larger form. Sequence analysis showed a strong homology to B7 (1), a GPI linked molecule expressed on B and T cells upon activation.

A probe derived from the cDNA not homologous to B7 detects several bands in southern blots, homologous in different strains of mice. Zoo-Blots hybridised with the same probe showed 1-3 bands in genomic DNA from human, horse, Xenopus, salm, zebra-fish and Drosophila thus suggesting an important function of the corresponding gene. However, we do not know the function of the cDNA. Presently we are analysing the two forms of the RNA in detail.

(1) Kubota et al. (1990) Identification and gene cloning of a new phosphatidylinositol-linked antigen expressed on mature lymphocytes. J.Immunol. 145:3924.

F 113 ORGANIZATION OF THE GENE LOCUS ENCODING MURINE CD22, *Cd22*: MAPPING TO CHROMOSOME 7 AND CHARACTERIZATION OF TWO ALLELES, *C.-L. Law, *R. M. Torres, *H. A. Sundberg, R. ^M. E. Parkhouse, #C. I. Brannan, #N. G. Copeland, #N. A. Jenkins, *E. A. Clark, *Dept. Microbiol., Uni. Washington, Seattle, WA 98195, ^Instit. Animal Health, Pirbright, England, #Mammalian Genetics Lab., Frederick Cancer Res. Devel. Ctr., Frederick, MD.

We recently isolated a full length cDNA clone encoding the murine homologue of the B cell associated adhesion protein CD22 (Torres, Law, et al., J. Immunol. 149, in press). Murine CD22 (mCD22) is a polypeptide with seven extracellular Ig-like domains that is 62% identical to human CD22. We also identified a rat mAb NIM-R6 that recognized a BHK transfectant expressing this full length mCD22 cDNA. Southern analysis on genomic DNA isolated from a panel of murine B cell lines and tissues from several mouse strains demonstrated that *Cd22* is a single copy gene ≤30kb. Moreover, digestion of genomic DNA preparations with four restriction endonucleases revealed the presence of RFLP between BALB/c, C57BL/6, and C3H strains versus DBA/2J and NZB strains suggesting the presence of two or more *Cd22* alleles. Using the BALB/c mCD22 cDNA clone, we isolated genomic clones from a DBA/2J genomic library which contain all the exons necessary to encode the full length mCD22 cDNA. Fourteen exons including exon 3 that encodes the translation start codon have been identified, and each Ig-like domain is encoded by a single exon. A comparison between the nucleotide sequences of the BALB/c mCD22 cDNA and the exons of the DBA/2J genomic clones revealed an 18-nucleotide deletion in exon 4(Ig-like domain 1) of the DBA/2J genomic sequence in addition to a number of substitutions in other exons. We confirmed these nucleotide differences by sequencing a mCD22 cDNA clone isolated from the RNA of LPS-activated DBA/2J splenocytes by reverse transcription-PCR using primers that flank the coding region of the full length BALB/c mCD22 cDNA. Similar deletions have been identified in *Cd72* alleles (Robinson et al., J. Immunol. 149: 880, 1992). We also mapped the *Cd22* locus to the proximal region of chromosome 7, a region sytenic to human chromosome 19q, close to the previously reported loci, *Lyb-8* and *Mag*, a homologue of *Cd22*. An antibody (CY34) against the Lyb-8 B cell marker reacted with a BHK transfectant expressing the full length mCD22 cDNA demonstrating that Lyb-8 and CD22 are identical. The mCD22 mAb NIM-R6 binds to DBA/2J B cells, confirming the expression of a CD22 protein by this allele. (Supported by NIH grant GM42508 and NCI, DHHS, under contract N01-C0-74101 with ABL)

F 114 REGULATION OF PROSTAGLANDIN E_2 RECEPTOR LEVELS BY LPS ON "IMMATURE" AND "MATURE" NORMAL AND MALIGNANT B LYMPHOCYTES. Richard P. Phipps and Deborah M. Brown, Cancer Center and Department of Microbiology and Immunology, University of Rochester School of Medicine, Rochester, N.Y. 14642

Macrophages, follicular dendritic cells and fibroblasts, all inhabitants of the B cell microenvironment, synthesize prostaglandin E_2 (PGE_2), a powerful regulator of lymphocyte activation and differentiation. The purpose of this investigation was to determine the levels and affinity of prostaglandin E_2 (PGE_2) receptors on PGE_2-sensitive and resistant murine B cell lymphomas. CH31 is a Ly 1^+ B cell lymphoma which undergoes apoptosis in response to treatment with μM concentrations of PGE_2, but not $PGF_2\alpha$. CH31 lacks surface IgD and is representative of immature B cells due to its exquisite sensitivity to anti-IgM mediated growth-inhibition. The CH12 lymphoma differs from CH31 in that it expresses IgD and is not growth-inhibited in response to anti-Ig reagents. CH12 also does not undergo apoptosis after PGE_2 treatment. To determine whether sensitivity to PGE_2 was due to the level or affinity of prostaglandin receptors on these cells, a receptor binding assay utilizing ^3H-PGE_2 was employed. Surprisingly, the PGE_2-resistant CH12 lymphoma bound 2-3 times more ^3H-PGE_2 to its surface than CH31, indicating that the resistant lymphoma displays more receptors than its PGE_2 sensitive counterpart. Scatchard analysis indicates that the CH31 lymphoma possesses a higher affinity PGE_2 receptor than does CH12. LPS upregulates various B cell activation antigens such as Class II and CD23. To determine whether LPS had any effect on the upregulation of PGE_2 receptors, CH12 and CH31 lymphomas were incubated with 5μg/ml LPS for 18-20 h. Scatchard analysis revealed that LPS upregulates PGE_2 receptor numbers on CH12 cells (B_{max} values change from 9.4×10^{-12} M for the untreated group to 3×10^{-11} M for LPS treated CH12). Conversely, treatment of the CH31 lymphoma with LPS increases the affinity of the PGE_2 receptor while having little effect on receptor number. The B_{max} values remain relatively constant, however, the dissociation constants change from 4×10^{-9} M for CH31 to 8.8×10^{-10} M for LPS treated CH31. Finally, we have determined that PGE_2-sensitive and resistant populations also exist amongst normal B lymphocytes. For example, a subset of neonatal normal B cells undergo apoptosis after treatment with PGE_2, unlike their mature counterparts. Activation of normal lymphocytes by LPS or other activating agents, may also increase PGE_2 receptor levels thus rendering them more sensitive to regulation by PGE_2. This research was supported by CA42739 and CA11198.

F 115 EXPRESSION OF SYNDECAN REGULATES MYELOMA PLASMA CELL ADHESION TO TYPE I COLLAGEN, Ralph D. Sanderson, Ronnie C. Ridley, Huiquing Xiao, Hiroyuki Hata, Jeff Woodliff and Joshua Epstein, Dept. Pathology, Anatomy and Medicine, Univ. Arkansas for Med. Sci., Little Rock, AR 72205

The syndecans comprise a family of integral membrane proteoglycans that regulate cell behaviors by binding to extracellular matrix and binding growth factors. In mouse blood cells, syndecan expression is restricted to cells of the B cell lineage where it is expressed by pre-B cells and plasma cells, but is absent from circulating B cells. This regulated expression of syndecan apparently plays a role in the adhesive changes that occur during B cell differentiation. In the present study, the expression, structure and function of syndecan by human myeloma cells was investigated using 10 myeloma cell lines and 14 myeloma patient bone marrow aspirates. On human myeloma cells, syndecan is a small (modal $M_r = 120$ KD) heparan sulfate proteoglycan localized at the cell surface. Syndecan was detected by immunodot blotting on seven of ten human myeloma cell lines and by reverse transcriptase PCR on ten of fourteen myeloma patient samples. Cell binding assays demonstrate that myeloma cells expressing syndecan bind to type I collagen via heparan sulfate chains, while those cell lines not expressing syndecan do not bind to collagen. Furthermore, the cell lines expressing syndecan were negative for CD19 and CD45, while those lines not expressing syndecan were positive for CD19 and CD45, suggesting that syndecan expression is restricted to those tumors having a well differentiated phenotype. These data indicate that the expression of syndecan by human myeloma plasma cells regulates their adhesion to type I collagen and may participate in directing their localization to specific sites. (Supported by NIH CA55879, CA37161, CA28771 and the Arthritis Foundation).

F 116 CLONING OF A NOVEL B CELL ACTIVATION ANTIGEN RELATED TO HUMAN CD38, Leopoldo Santos-Argumedo, Noboyuki Harada, Ray Chang, Andrew W. Heath, Christopher Grimaldi, R.M.E. Parkhouse* and Maureen Howard, DNAX Research Institute, Palo Alto CA, USA and *Institute for Animal Health, Pirbright Laboratory, Surrey, U.K.

A novel murine B cell activation antigen has been cloned. The antigen is a 42 KDa type II protein, 68% homologous with human CD38, which is recognized by the rat monoclonal antibody NIM-R5. NIM-R5 enhances the expression of class II and ICAM-1, and induces proliferation of small resting B cells; the proliferation is significantly enhanced with IL-4. NIM-R5 is not costimulatory with anti-Ig and neither induce apoptosis in WEHI-231 or CH-31 nor rescue these cells from the apoptosis induced by anti-IgM. However, NIM-R5 induces a small but significant rescue from apoptosis on *in vitro* activated B cell blasts; the rescue is significantly increased with IL-4. NIM-R5 stains several B cell lymphomas and mature resting B cells. Its expression is increased upon activation. In contrast, NIM-R5 recognizes a very small subpopulation of T cells in the thymus, and is negative on resting or activated splenic T cells. The distribution of the staining is different from that described for human cells because human CD38 is expressed on T cells, but not on resting B cells. All these results suggest that the epitope recognized for NIM-R5 is related but may be different from the human CD38. Finally, this molecule has been shown to be important in the activation of normal resting and activated B cells in the mouse.

F 117 CO-STIMULATION AND -DIFFERENTIATION OF HUMAN B CELLS THROUGH CD72, Hilde Van de Velde, Karin Mels, Wei Luo and Kris Thielemans, Department of Hematology and Immunology, Free University of Brussels, Brussels, Belgium

Recently we have found that CD5 (Ly-1), a 67 kDa glycoprotein expressed on the cell surface membrane of T lymphocytes and a subpopulation of the B cells, is a counter-receptor for CD72 (Lyb-2), a 45 kDa protein present on the cell surface membrane of B cells. The functional importance of the CD5/CD72 interaction still remains to be determined. The role of CD72 in the activation and/or differentiation of human B lymphocytes has not yet been studied thorhoughly. Therefore, we have generated two new anti-CD72 mAb, JT3 and WL225 (respectively IgG1 and IgG2a). These mAb have been used in *in vitro* stimulation experiments with small resting tonsillar B cells. Soluble anti-CD72 mAb induce very little (if any) B cell proliferation, but they provide an important co-stimulatory signal for B lymphocytes that have been activated by immobilized anti-sIgM mAb and/or soluble anti-CD40 mAb. Since anti-CD72 mAb excert similar effects on B lymphocyte proliferation as anti-CD40 mAb, we have used our CD72-specific mAb in a stimulation system where anti-CD40 mAb have been shown to induce B cell proliferation and differentiation. In this assay, cell surface antigens are cross-linked using CDw32 (high affinity receptor for IgG) transfected L cells. The anti-CD72 mAb alone and the combination anti-CD72 + anti-sIgM mAb do not excert any important effect on the growth of the B cells in this type of cell culture. However, our anti-CD72 mAb act synergistically with the CD40-specific mAb 89 and this effect can still be observed after 6 days of cell culture. We have shown that through CD72 a co-stimulatory signal can be delivered to resting human B cells that have been activated via sIgM and/or CD40. We are currently investigating the contribution of CD72 in the final differentiation of B lymphocytes. Therefore, we will determine the isotype and the amount of immunoglobulins produced in the B cell cultures.

F 118 *Abstract Withdrawn*

F 119 FUNCTION OF CD40 EXPRESSION ON A HUMAN MULTIPLE MYELOMA CELL LINE, J. J. Westendorf, G. A. Ahmann, J.A.Lust, P.R.Greipp, J.A.Katzmann, and D.F.Jelinek, Depts. of Immunology and Hematology, Mayo Clinic, Rochester, MN, 55905.

In these studies, we have analyzed the activation and growth requirements of a recently derived interleukin 6 (IL6) - dependent human multiple myeloma (MM) cell line, ANBL-6. These cells morphologically resemble plasma cells, and contain clonally rearranged immunoglobulin heavy and light chain gene loci. The ANBL-6 cells also express high levels of the plasma cell antigen, CD38, as well as markers (CD10, CD44, and CD54) frequently associated with MM cells. Although plasma cells have been reported to lack CD40 expression, the ANBL-6 cells surprisingly expressed the 50 kD glycoprotein, which is normally found on B cells, as well as epithelial cells, and some carcinomas. In functional studies, others have shown that antibodies to CD40 can deliver activation signals to resting B cells, including the induction of low levels of IL6 secretion. To address the role of CD40 expression on the ANBL-6 cells, we have examined the responsiveness of the cells to a CD40-specific monoclonal antibody (MoAb) (G28.5; kindly provided by Dr. E. Clark, U. of Washington, Seattle). Initial experiments examining the response of ANBL-6 cells to a wide variety of cytokines demonstrated that only IL6 was capable of supporting proliferation of these cells. This proliferation was completely blocked by a neutralizing MoAb to IL6. Of interest, the anti-CD40 MoAb also induced proliferation of the ANBL-6 cells. The level of proliferation observed was about 40-60% of the IL6 driven response. Moreover, the IL6 neutralizing MoAb substantially inhibited the anti-CD40 induced proliferation by 70-80%. In addition, analysis of ANBL-6 cell culture supernatants by ELISA demonstrated that the anti-CD40 stimulated cells secreted significant levels of IL6, whereas unstimulated cell culture supernatants contained undetectable levels of IL6. Furthermore, semi-quantitative analysis of mRNA levels by PCR showed an increase in IL6 message in anti-CD40 stimulated ANBL-6 cells over unstimulated cells. These results therefore suggest that the mechanism of anti-CD40 stimulated proliferation results primarily from the induction of autocrine IL6 production, followed by cell growth. This study provides further evidence that CD40 and IL6 signals in B cells are functionally linked. To extend these findings, we have begun preliminary studies examining the expression of CD40 on freshly isolated MM cells. Of 5 bone marrows from MM patients tested thus far, all contain plasma cells ($CD38^+CD45^-$) that express CD40. These data suggest that the expression of CD40 on malignant plasma cells may play a role in tumor cell expansion, possibly by inducing autocrine secretion of IL6.

Development of Germinal Centers

F 200 IN VITRO ANALYSIS OF SOMATICALLY MUTATED B CELLS. Debra J. Decker, Phyllis-Jean Linton, Thomas R. Gingeras, and Norman R. Klinman, The Scripps Research Institute and Life Sciences Research Lab of Baxter Diagnostics, La Jolla, CA 92037

Whereas the importance of somatic hypermutation in the generation of high affinity antibodies is well established, the mechanism of somatic mutation and the cells involved in the mutation process are less well understood. In an attempt to define the cell subpopulations that are involved, we have used an adaptation of splenic fragment culture technology which permits the generation and stimulation of memory B-cells in vitro. Through the use of RNA amplification (3SR) as well as PCR amplification and DNA sequence analysis, we have now obtained the sequence of immunoglobulin genes of antibodies generated from various subpopulations of B cell precursors after antigenic stimulation in fragment culture. These studies enable us to analyze several precursor populations with respect to both the prior accumulation of somatic mutations and the ability of their progeny to somatically mutate. The results indicate that the immunoglobulin genes of primary B cells and of naive progenitors of secondary B cells were not somatically mutated whereas the immunoglobulin genes of secondary B cells and precursors of memory B-cells obtained from immunized mice were mutated. We are currently assessing the capacity of these four cell subpopulations to somatically mutate in vitro.

F 201 INTERLEUKIN-4 INDUCES A GERMINAL CENTER PHENOTYPE ON B CELLS, Jeannine M. Durdik and Satyajit Rath, Departments of Medicine and Microbiology and Immunology, University of Colorado School of Medicine, Denver, CO 80262, and National Institute of Immunology, Shahid Jit Singh Road, New Delhi 110067, India

The surface phenotype of the germinal center B cell can be generated in a noncognate fashion by Th2 cell products. While the induction of high levels of class II is a well established effect of IL4, we report that Ig down-modulation and the high PNA reactivity characteristic of germinal center B cells is concomitantly observed. Splenic B cells convert to a germinal center phenotype upon culture in recombinant IL4 or Th2 culture supernatants, as do two other B lineage cell lines. One of these, 70Z/3 demonstrates that the Igκ locus is one of the genes affected by this treatment. Transcription of Igκ in 70Z/3 is induced by lipopolysaccharide. Addition of IL4 strongly inhibits this induction as judged by steady-state κ RNA levels. As the generation of the germinal center cell surface phenotype is mitigated by anti-interleukin 4, this lymphokine is capable of inducing the germinal center phenotype without other factors.

F 202 SPECIFIC ANTIBODY-PRODUCING CELLS IN PERIPHERAL BLOOD AND TONSILS AFTER ORAL IMMUNIZATION. Gilbert C.FAURE, Carole ZANIN, Philippe PERRIN, Anne M.PERRUCHET, Marie C.BENE. GRIP, Laboratoire d'Immunologie, Faculté de Médecine, Université de Nancy I, BP 184, 54500 Vandoeuvre les Nancy, France. Laboratoires INAVA, 81106 Castres, France.

Oral immunization with microbial antigens is used in clinical practice to enhance immune defenses against chronic and recurrent ENT infections. To document the physiological mechanisms involved, specific antibody producing cells (SAPC) were investigated in peripheral blood and tonsils from 14 children who had received prior to tonsillectomy a treatment with Ribomunyl®. This oral vaccine is commonly prescribed in France, composed of ribosomal fractions from *H.influenzae, S.pneumoniae, S.pyogenes and K.pneumoniae*, supplemented with *K.pneumoniae* proteoglycans. Isotype-specific and SAPC directed to each of these 4 microorganisms were detected in peripheral blood lymphocytes, before and after treatment and in tonsillar eluted lymphocytes from treated patients and 10 controls using an Elispot technique. SAPC, at the plasma cell level, were detected using a three step immunofluorescence technique and enumerated in subepithelial and interfollicular areas.

The levels of plasma cells elaborating antibodies specific for the 4 microrganisms were significantly higher in tonsils from treated patients, compared to untreated subjects, confirming earlier results.

SAPC in peripheral blood were significantly elevated after treatment and mostly of the IgM isotype. SAPC in treated tonsils were also significantly higher compared to controls, this time with a significant elevation of IgA secreting cells.

These results confirm the theory of a mucosal immune system in man, involving tonsils. Specific-antibody producing B lymphocytes, sensitized in the gut, probably in Peyer's patches, can be detected in the blood. They can also be detected in the mucosal tissues at two different levels of differenciation, as lymphocytes and plasma cells. These results also confirm that Ribomunyl® acts as an oral vaccine stimulating at a distance ENT mucosal sites.

F 203 HETEROGENEITY OF RESPONSES OF HUMAN B CELL SUBSET POPULATIONS, Manlio Ferrarini, Mariella Dono, Simona Zupo, Giuseppe Taborelli and Nicholas Chiorazzi, IST, GE, Ist. G. Gaslini, GE, Italy, Cornell Univ., N.Y., USA.

The capacity of human B cell subsets to respond to different stimuli in vitro was investigated. Tonsillar B cells were separated into CD5+ and CD5- B cells. The CD5+ B cell fraction was comprised of > 90% CD5+ cells with the features of mantle zone cells (IgM+ IgD+ CD39+ CD38- CD10-) and gave a vigorous response to a-μ-ab, SAC or anti CD40 mAb in the presence of IL2 or IL4 and produced IgM polyreactive antibodies. The CD5- cell fractions were fractionated further on Percoll density gradients. The 60% Percoll fraction was comprised of resting (CD23- CD69- CD71-) cells that did not express typical lymphoid follicle markers (CD38, CD10 or CD39). They were activated by the stimuli used for CD5+ cells but failed to enter into cycle or secrete Ig molecules. Cell activation was not followed by apoptosis. Proliferation was observed by co-culturing the CD5- B cells in close contact with murine EL-4 thymoma cells suggesting that they required interaction with an accessory cell. The 50% percoll fractions that were comprised of 25-40% cells expressing surface activation markers (CD23, CD69 and CD71) proliferated and produced Ig molecules in response to SAC, IL2, anti CD40 mAb with the help of IL2 or IL4 in the absence of any accessory cell. Removal of the cells with activation markers abrogated the proliferative response. The lighter Percoll fractions contained primarely cells with the features of germinal center cells (CD38+ CD10+), that undervent spontaneous apoptosis in vitro but could be rescued and recruited into cycle by exposure to anti CD40 mAb and IL4. These observations indicate an heterogeneity of responses by human B cells, which is emphasized further by the observation that resting peripheral blood B cell of the CD5+ and CD5- fractions both respond equally well to a variety of stimuli.

F 204 THE GERMINAL CENTER REACTION: PURIFICATION OF MURINE CENTROBLASTS FROM AN EARLY PRIMARY IMMUNE RESPONSE.

John E. Ford, Michael G. McHeyzer-Williams and Michael R. Lieber. Laboratory of Experimental Oncology, Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305.

Formation of secondary lymphoid follicles and the subsequent germinal center reaction is an early consequence of T cell dependent immune responses. These histologically-distinct microenvironments arise during the first week after primary immunization. Initially, they contain rapidly dividing centroblasts, the progeny of antigen-specific B cells activated in a T cell-dependent manner. Somatic hypermutation of immunoglobulin (Ig) genes is thought to proceed within this population of surface Ig (sIg) negative B cells. Centroblasts give rise to sIg positive centrocytes which can be selected by antigen on follicular dendritic cells in the light-zone of the germinal center. The selection of clones expressing higher affinity antigen receptors and the establishment of long-term memory are the critical outcomes of this antigen-dependent phenomenon. Although our understanding of the histological changes accompanying this process is extensive, the detailed analysis of regulatory mechanisms remains poorly understood. Isolation of the cells within this pathway in a biologically-active state may offer a means of studying these most complex processes.

Using 6-parameter flow cytometry it was possible to isolate cells drawn into the germinal center pathway following T cell-dependent immunization. Primarily, differential surface expression of "markers" for homing, activation and site-specificity were used to identify populations of cells involved in the ongoing response. The germinal center cells were defined as those expressing B220 (the B cell form of CD45R), binding peanut agglutinin and down-regulating both sIgD and L-selectin (the Mel 14 antigen). Within this population, lack of expression of kappa or lambda light chain allowed discrimination of centroblasts from centrocytes.

F 205 ADHESION RECEPTOR INTERACTIONS PREVENT GERMINAL CENTER B CELLS FROM ENTRY INTO APOPTOSIS, Gerrit Koopman*, Robert M.J. Keehnen*, Walter Newman+ and Steven T. Pals*, *Department of Pathology, Academic Medical Center, University of Amsterdam, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands, and +Otsuka America Pharmaceutical, Inc., Rockville, MA 20850.

Germinal centers play a key role in the maturation of the B cell immune response. In the germinal center, B cells are either selected to become memory cells or plasma cells, or if their Ig receptors do not meet the antigen binding affinity requirements are eliminated through the process of programmed cell death. FDC which are intimately associated with the germinal center B cells are thought to be important in this selection process. FDC can trap antigen in the form of immune complexes, and present the antigen to the B cells. FDC may also deliver growth factors, like CD23, to the B cells. Previously we showed that the LFA-1/ICAM-1 and VLA-4/VCAM-1 adhesion pathways are involved in FDC - B cell interaction. Triggering of adhesion molecules is known to be important in lymphocyte activation. We therefor investigated whether adhesive interactions could also contribute to the B cell selection process by studying the effect of these molecules on apoptosis of germinal center B cells. We observed that adhesion of B cells to plastic coated with purified ICAM-1 protein (ligand of LFA-1) or purified VCAM-1 protein (ligand of VLA-4) could prevent B cells from entry into apoptosis. However the VLA-4 ligand fibronectin did not prevent B cell entry into apoptosis. Addition of mAb directed against LFA-1, ICAM-1, VLA-4 or VCAM-1 also had no effect, which may indicate that ligand interaction provides a stronger signal to the B cell than interaction with mAb. Thus adhesive interactions may provide an additional mechanism through which FDC can affect the B cell selection process.

F 206 LYMPHOKINE CROSSTALK DURING THE GERMINAL CENTER (GC) REACTION, Marie H. Kosco, Zoher Kapasi, Doris Scheidegger, Franca Ronchese and Michael Wiles, The Basel Institute for Immunology, CH-4005 Basel, Switzerland

The interactions of cells during a gc response appear to be dynamic, complex and not dependent upon a single cytokine. Recently, we have designed a system to isolate cells involved during its different phases and examine their interactions. As evaluated by neutralizing mabs directed against various interleukins, follicular dendritic cell - B cell - T cell cultured clusters displayed a certain pattern of sensitivity which differed during the course of the response. To obtain these clusters, Balb/c mice were primed and boosted with DNP-OVA. Cells were isolated by enzyme digestion from the draining lymph nodes 2.5, 5 and 10 days post 2' immunization. The low density nonadherent fraction of cells was cultured for 48 hrs in the presence of the neutralizing mabs. B cell proliferation was measured in the last 18 hrs of culture by 3H-thymidine uptake. Anti-mouse IL 1 alpha (IP-110), IL 2 (S4B6), IL 3 (1623-01), IL 4 (11B11), IL 5 (TRFK5), IL 6 (1650-01) and IL3/IL5 (R.52) were used separately or in combinations. No individual anti-IL showed any effect. At day 2.5, combinations containing anti-IL 2, 3 or 6 inhibited the B cell proliferation by 25-50%. R.52 (anti-IL 3+5) also caused a 27% decrease. At day 5, anti-IL 1+3 blocked the most (30-50%), while other combinations had little effect (0-15%). At day 10, the level of proliferation was generally unaffected by the mabs (0-10% inhibition). By RT-PCR analysis, significant levels of mRNA for IL 1 alpha, IL6, IL 3 and IL 2 were detected from cells isolated at day 2.5. Messenger RNA for IL 4, IL 5 and IL 7 were not detectable. Supernatants from these cultures revealed that a soluble factor was released which could augment B cell proliferation. Upon analysis using IL 2 (HT-2), IL 3 (FDC-1) or IL 4 (CT4S) dependent cell lines, the supernatant was shown to contain IL 3. The presence of other factors and the effect of IL 3 on FDC function are being assessed. Together these results reveal the intricate interaction of cytokines and the transient levels being produced in this microenvironment.

F 207 FOLLICULAR DENDRITIC CELLS RESCUE GERMINAL CENTRE B LYMPHOCYTES FROM DEATH BY APOPTOSIS, Ernst Lindhout, M.L.C.M. Mevissen, J.M. Tager and C. de Groot, Laboratory of Cell Biology and Histology, University of Amsterdam, Meibergdreef 15, 1105 AZ Amsterdam, The Netherlands.

Germinal centres (GC) can be divided into two main compartments, a dark zone populated by rapidly dividing centroblasts and a light zone containing non-dividing centrocytes (progeny of centroblasts) and a dense network of follicular dendritic cells (FDC). It is generally believed, that during GC reactions, B cells in the light zone are selected for their capacity to compete for binding to antigen present in limited amounts on FDC. Those cells that lose this competition rapidly die by apoptosis whereas the binding cells subsequently are rescued from apoptosis by receiving an as yet unknown positive signal. Although it is supposed that FDC have a pivotal role in this selection process it has never been demonstrated directly that the interaction between FDC and B cells prevents B cells from entering apoptosis. To investigate if cell-cell contacts between B cells and FDC in vitro prevent B cells from dying by apoptosis, we studied FDC and B cells by direct observation of cultures in vitro.

FDC were isolated from human tonsils and cultured in vitro together with GC B cells. FDC and B cells rapidly formed spherical clusters when brought into culture. T cells were not observed inside these clusters. After 48, 60 or 120 hours, cultures of FDC and B cells were supravitally stained with Hoechst 33258 and examined directly in their culture wells by fluorescence microscopy. Viable cells appeared to be exclusively present in clusters, whereas single B cells all had an apoptotic appearance. By addition of monoclonal antibodies against LFA-1 or VLA-4, the formation of FDC-B cell clusters could be prevented, resulting in an apoptotic appearance of virtually all B lymphocytes. The present data demonstrate that a physical interaction between FDC and B cells is a prerequisite to rescue the latter from apoptotic cell death.

F 208 FUNCTIONAL SEPARATION OF GERMINAL CENTERS, MEMORY AND ANTIBODY-FORMING B CELL RESPONSES. Jaroslav Stedra, Carla Miller, Garnett Kelsoe and Jan Cerny, Department of Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, Maryland 21201.
Following a primary immunization with T-dependent antigen, the responding B cells appear in two anatomically and functionally distinct compartments, foci of antibody-forming cells (AFC) and germinal centers (GC). GC B cells, which secrete little Ab in situ, are the source for mutants which dominate the memory response. The present studies have explored the relationship between the GC and AFC developmental pathways during the response of BALB/c mice to phosphocholine-6-0-(hydroxyhexanoate) coupled to keyhole limpet hemocyanine (PC-KLH). Athymic (nu/nu) mice immunized with a single dose of soluble PC-KLH developed large numbers of GC in the splenic lymphoid follicles but, unlike the euthymic mice, they had no detectable AFC (by immunochemical staining in situ and by a single cell suspension AFC assay). The GC B cells in nu/nu mice expressed the PNA marker as well as the T15 Id that is associated with the canonical Ab to PC, suggesting that they represented a specific reaction to PC. The PC-KLH-primed nu/nu did not produce AFC upon secondary challenge. However, when these mice received normal $CD4^+$ cells (10^7) from unprimed donors before (1 d.) the challenge they developed a vigorous AFC response that exceeded a typical memory response of euthymic mice to PC-KLH. The single, PC-specific secondary GC were dissected and the somatic diversity of the T15 H chains was studied by the amplification and sequencing of genomic VD7 rearrangements. This technique was used previously to demonstrate the mutations of IgV genes in GC B cells in response to other antigens. However, the rescued sequences did not show evidence of somatic hypermutation. Together with other data from our laboratories, these results show that (1) the residual T cells in nu/nu mice sustain the development of GC and priming for B cell memory independently of AFC development, and (2) an antigen stimulation of B cells within GC may not always be accompanied by somatic hypermutation of IgV genes.

B Cell-T Cell Interactions

F 209 MAb TO CELL INTERACTION ANTIGENS BLOCK HUMAN T-DEPENDENT B CELL ACTIVATION. Christopher D. Benjamin, Donna M. Hess and Barbara A. Wallner. Department of Cell Biology and Immunology. Biogen Inc., 14 Cambridge Center, Cambridge, MA 02142.

We have used a mitogen-free human culture system to evaluate the effect of mAb to cell interaction antigens, on T-dependent B cell activation to Ig secretion. We remove monocytes by adherence and culture human peripheral T and B cells in round bottom wells in the absence of mitogen or antigen. We quantify Ig secreted into the culture medium by ELISA, between days 7 and 12 of culture. Ig production, predominantly IgM depends on the presence of T cells. To determine the role of adhesion molecules in these T-B interactions, mAb to CD2, LFA3, ICAM and VLA4, were added at various times after the initiation of culture. We found that mAbs to CD2, LFA3 and ICAM, but not VLA4, potently block T dependent B cell activation to Ig secretion when added at the beginning of culture. Mab added after two days of culture had little or no effect suggesting that the adhesion molecules which participate in cell interactions are important early in this system. We have used this in vitro system to evaluate the immunological competence of animals treated in vivo with mAbs to LFA3 and found that in vivo treatment also blocks in vitro generation of Ig. Together, these results indicate that T and B cell interactions resulting in Ig production in vitro involve multiple adhesion pathways which each contribute to productive cellular cooperation.

F 210 ANTIBODY ACTS AS A "CARRIER" FOR A HAPTEN-SPECIFIC IMMUNE RESPONSE BY IMMUNGLOBULIN TRANSGENIC MICE. Michael J. Caulfield and Deborah Stanko, Department of Immunology, Research Institute of the Cleveland Clinic Fndn., Cleveland, OH 44195.

M167 (μ, κ) TG mice encode an IgM antibody that binds phosphorylcholine (PC) determinants on the pneumococcal cell wall polysaccharide (PnC). Although ~ 75% of B cells from these mice express the transgene, they respond poorly in a primary immune response to PnC, a type II thymus independent antigen. However, when the PnC antigen is presented as a complex with anti-PC antibodies, a vigorous immune response occurs in which the TG mice produce 10 - 50 fold more anti-PC antibody than when immunized with antigen alone. Three different IgA anti-PC antibodies were compared for the ability to mediate the enhanced response to PnC. Both TEPC-15 and McPC-603 antibodies coupled with PnC stimulated a twentyfold higher anti-PC response than mice immunized with PnC alone. Interestingly, MOPC-167, an IgA myeloma protein that expresses the VH and VL regions used to encode the transgene antibody, was a relatively poor "carrier" for PnC. The results indicate that M167 TG mice may be tolerant to PnC, despite the fact that 75% of their B cells possess receptors for this antigen. This "tolerance" is broken by presenting the antigen in the form of an immune complex in which the antibody "carrier" expresses a different idiotype from that encoded by the M167 transgene. We propose that the immune complex mimics a classical hapten-carrier system in which the B cells recognize the antigen and the T cells recognize the idiotype of the TEPC-15 carrier antibody. It will be of interest to determine whether the putative Id-specific helper T cells recognize native Id or "idiopeptides" that result from processing the TEPC-15 antibody by antigen-specific B cells.

F 211 CONTACT-DEPENDENT INDUCTION OF $P34^{CDC2}$ KINASE ENZYMATIC ACTIVITY IN B LYMPHOCYTES, Lisa Boucher Clark, Michael Hung-Hwa Yen, Lisa S. Marshall, and Randolph J. Noelle, Department of Microbiology, Dartmouth Medical School, 1 Medical Center Drive, Lebanon, N. H. 03756.

The serine-threonine protein kinase $p34^{cdc2}$, in association with cyclin proteins, regulates the entry of eukaryotic cells into mitosis. The kinase activity of the p34/cyclin complex fluctuates in a cell-cycle dependent manner, with kinase activation resulting from an abrupt dephosphorylation of the complex at mitosis. We have shown that purified resting splenic murine B lymphocytes constitutively express low levels of tyrosine phosphorylated $p34^{cdc2}$ and cdc2 mRNA. Following in vitro exposure to various mitogens including plasma membranes from preactivated helper T cell clones and anti-Ig antibodies, p34 mRNA, protein levels and tyrosine phosphorylation state are upregulated within 16-24 hours. Lymphokines, including IL-2, IL-4 and γ-IFN in the absence of a mitogenic signal are not sufficient for $p34^{cdc2}$ upregulation. We are presently investigating the involvement of the CD40 ligand, gp39, in cdc2 kinase induction with current studies aimed at determination of which contact-dependent signals are involved in activation of cdc2 kinase enzymatic activity.

F 212 VARIATIONS IN ASSEMBLY LEAD TO TWO DISCRETE BIOLOGICALLY ACTIVE IgM POLYMERS, Ronald B. Corley, Joseph W. Brewer, and Troy D. Randall, Department of Immunology, Duke Medical Center, Durham, NC 27710

Secreted IgM is classically thought of as a pentameric molecule containing a single joining (J) chain. However, we and others have demonstrated the existence of a second IgM polymer, a hexamer lacking J chain. IgM hexamers are produced by various B cell lymphomas and normal B cells. Little is known about the function of the hexameric form of IgM, but since a hexamer activates the complement cascade 20-times more efficiently than the canonical pentamer, a thorough understanding of its contribution to normal and abnormal immune responses is warranted. We have found that differences in the secretion of IgM hexamers is related to the abundance of J chain in the secreting B cell. A direct role for J chain in IgM polymer formation was shown by transfecting J chain into cells that expressed no endogenous J chain and secreted predominantly (>90%) IgM hexamers. Following transfection, these cells secreted predominantly IgM pentamers. Using inducible B cell lymphomas, we also found that the relative abundance of J chain could be altered by varying the stimuli used to induce antibody secretion, with lymphokines such as IL-2, IL-5, and IL-6 stimulating high levels of J chain transcription, while LPS stimulated low or negligible increases in J chain. This resulted in variations in the abundance of hexamers in the secreted IgM, leading to substantial differences in the lytic capacity of the IgM secreted. These results suggest that the biologic function of the IgM secreted may vary depending on the involvement of T cells in the primary immune response. Experiments are underway to evaluate the contribution of IgM hexamers to T-dependent and T-independent primary immune responses, and to determine the basis for the differential ability of IgM hexamers and pentamers to activate the classical complement cascade. Current experiments are also focusing on understanding the assembly of IgM and defining when J chain is added to the polymer during this process, and determining the abundance of IgM hexamers in various disease states. (Supported by NIH grant AI31209)

F 213 IMMUNOHISTOCHEMICAL ANALYSIS OF B7/BB-1 EXPRESSION USING ANTI-B7 MAB B7-24.

Mark de Boer[1], Peter Vandenberghe[2], Jan Delabie[3], Chris De Wolf-Peeters[3] and Jan Ceuppens[2]. [1]Dept. of Immunology, Innogenetics, Industriepark Zwijnaarde 7, box 4, B-9052 Ghent, Belgium, [2]Laboratory for Clinical Immunology and [3]Laboratory of Histo- and Cytochemistry, University of Leuven, Belgium.

For optimal activation of T cells, binding of their TCR to antigenic peptides in the context of MHC molecules on APC is not sufficient. Accessory signals, provided by accessory cells, are needed to induce proliferation and clonal expansion of normal T cells. It has been shown previously that the B7 molecule, present on the cell surface of activated APC, can provide the secondary signal by binding to the CD28 molecule on T cells. Here we describe a novel anti-B7 mAb, B7-24. This mAb binds to a functionally important epitope of the B7 molecule. Fab fragments of B7-24 can almost completely block anti-CD3-induced, B7-dependent T-cell proliferation when tested in a model system where purified T cells are co-cultured with 3T6 cells expressing the human FcγRII and human B7 in the presence of anti-CD3 mAb. Using mAb B7-24, we have examined the expression of the B7 molecule in a number of normal and pathological tissues by immunohistochemistry. Dendritic cells in the skin (Langerhans cells) and in T-cell-dependent zones of spleen and lymph node were strongly positive for B7. In addition, dendritic cells in the thymic medulla of fetal tissue, but not in adult tissue, were positive for B7. A subset of B-cell blasts and follicle center cells in B-cell zones of lymph node and spleen were positive for B7. We further found that B7 is expressed on a subset of infiltrating macrophages in granulomatous inflammation. The expression of B7 on APC in lymphoid tissue and in non-lymphoid tissue under inflammatory conditions, combined with functional data on B7, strongly points to an important role of the B7-CD28 interaction in T-cell activation.

F 214 T HELPER CELL FUNCTION INVOLVED IN THE HUMORAL IMMUNE RESPONSE INDUCED BY MURINE CHRONIC GRAFT- VERSUS- HOST DISEASE. Fiona H. Durie and Randolph J.Noelle, Department of Microbiology, Dartmouth Medical School, Lebanon, NH, 03756.

Graft versus Host Disease (GVHD) results when immunocompetent cells are transfered into a genetically different immunocompromised host. GVHD occurs in humans after bone marrow transplantations, and can be found in acute and/ or chronic forms. Acute GVHD takes place whithin 60 days post- transplantation and is the result of damage to the skin, liver, and gut by cytolytic lymphocytes. Chronic GVHD occurs later and is a systemic autoimmune disease that effects primarily the skin. Although differences in the MHC are an important prognostic indicator in the development of GVHD, a large percentage of recipients receiving MHC-matched bone marrow inoculum still develop GVHD. Thus non- MHC loci play an important role in the development and regulation of GVHD.

Once helper T cells (T_h) are activated by antigen-presenting B cells, they express the ligand for CD40, p39, and as a result p39 binds to CD40 on the B cell. The interactions between p39 and CD40 play an essential role in the initiation of thymus-dependent humoral immune responses. The *in vivo* role of p39-CD40 interactions in the development of antibody-producing cells was investigated in terms of the development of chronic GVHD. Injection of parental spleen cells into unirradiated F1 hybrid recipients is frequently used for the induction of murine graft- versus- host disease. Chronic GVHD was induced by the injection of DBA/2 spleen cells into (C57BL/6 x DBA/2) F_1 hybrid recipient mice. The disease was allowed to progress in the presence and absence of i.p. injections of anti-p39 antibody (MR1). The mice were anaesthatised and killed by cervical dislocation. Spleen cells were isolated and cultured ($5x10^6$/ ml) for 3 days in complete media and then the supernatants were collected. Eliza assays were performed for the detection of polyclonal Ig production

Results indicate the hyperproduction of Ig upon induction of GVHD and the subsequent reversal of this via the administration of the anti- p39 (MR1) antibody indicating the relevance of helper T cells function in the humoral immune response in autommune disease conditions.

It is proposed to study the effects of treatment of GVHD induced mice with the anti- p39 antibody (MR1) on (a) proliferative responses of spleen cells in the presence of various mitogens, (b) the ability to suppress mitogen or MLR responses of normal spleen cells and (3) the CTL activity of the spleen cells. This will help to determine if T:B cognative help is required for any immunodeficiency elicited by GVHD.

F 215 PRIMARY IMMUNIZATON WITH KILLED *BRUCELLA ABORTUS* INDUCES IFN-γ AND IL-10 GENE EXPRESSION BY BOTH TCR-α/β AND TCR-γ/δ T CELLS. W.C. Gause[1], A. Svetic[1], P. Lu[1], Y.C. Jian[1], and F.D. Finkelman[2]. Departments of [1]Microbiology and [2]Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD 20814.

Immunization of mice with killed *Brucella abortus (BA)* increases serum IgG2a levels. This elevation is decreased by administration of anti-IFN-γ antibodies. T cells cloned from BA-immunized mice generally secrete a Th1 pattern: IFN-γ but not IL-4 or IL-5. Analysis of cytokine gene expression by RT-PCR showed a highly specific pattern with elevations in both IFN-γ and IL-10 as early as 24 hours after immunization, but no changes in IL-2, IL-4 or IL-5 expression: a pattern maintained up to 10 days after BA injection. To a large extent these results were verified by protein assays including ELISPOT, and ELISAs on supernatants of cultured cells from immunized mice. Intervention experiments with anti-IFN antibodies showed elevations in Th2 cytokines including IL-4, IL-5, and IL-9. Cell sorting analysis revealed that by day 1 Thy-1^+ cells were the source of the increased IL-10 and IFN-γ mRNA. Further analysis showed that these cytokines were elevated in $CD4^+$ T cells from BA-immunized mice compared to $CD4^+$ T cells from untreated mice. FACS analysis of the total spleen cell population revealed that the frequency of TCR-γ/δ^+ T cells in the spleen increased from approximately 1% of total lymphocytes at 1 day after immunization to 15% of total lymphocytes at five days after immunization. Cell sorting analysis revealed that the TCR-γ/δ^+ T cells were expressing high levels of both IL-10 and IFN-γ by five days after immunization, demonstrating that the TCR-γ/δ^+ T cells, in addition to the $CD4^+$ T cells, are important contributors to the elevations of both IL-10 and IFN-γ in BA-immunized mice, and suggesting that both of these populations regulate the isotype of the antibody response.

F 216 ROLE OF CD40 AND CD40 LIGAND IN T CELL-DEPENDENT B CELL ACTIVATION, Marilyn R. Kehry, Brian E. Castle, Karen Kishimoto, Maryanne L. Brown and Della White, Department of Molecular Biology, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT 06877

Activated helper T (Th) cells deliver contact-dependent signals to resting B lymphocytes that initiate and drive B cell proliferation. A ligand for the B lymphocyte membrane protein CD40 has recently been identified and may deliver contact-dependent Th cell signals to B cells. A soluble form of CD40 fused to the Fc region of human IgG1 was capable of blocking Th cell-dependent B lymphocyte proliferation but not B lymphocyte responses to LPS or anti-IgM. Both CD40 ligand and IL-2 were transiently synthesized between 1 and 12 h after Th cell activation and followed similar kinetics of synthesis. Newly synthesized CD40 ligand exhibited a high initial rate of turnover (1.5 h half life) but became more stable after 5 h of Th cell activation (10 h half life). Ligation by CD40 markedly stabilized expression of CD40 ligand on the Th cell surface. Newly synthesized CD40 ligand appeared to not be solely responsible for delivery of Th cell-dependent contact signals to resting B cells. Biochemical experiments supported this finding and suggested that oligomerization of CD40 ligand and/or accessory proteins were needed to stimulate resting B cell proliferation. The induction of CD40 ligand expression by antigen presenting B cells was also examined on several subsets of Th cells. These findings suggest that regulation of CD40 ligand expression may contribute to the high degree of specificity in B cell responses. Although the interaction of CD40 and its ligand on activated Th lymphocytes may represent a major signaling pathway in the activation of resting B lymphocytes, other components appear to be involved.

F 217 ANTI-CD3 ACTIVATED HUMAN T CELLS STIMULATE B CELL GROWTH VIA CD40 DEPENDENT AND INDEPENDENT PATHWAYS, Stephen J. Klaus*, Richard J. Armitage,+ William C. Fanslow+, Ken H. Grabstein+ and Edward A. Clark*, Department of Microbiology*, University of Washington and Immunex Corporation+, Seattle WA.

Freshly isolated, mitomycin C-treated, $CD4^+$ T cells from peripheral blood induce vigorous B cell proliferation when stimulated with insoluble anti-CD3 . Using soluble CD40.Fc (the extracellular domain of human CD40 fused to the Fc portion of human IgG_1), we detect CD40 ligand (CD40L) expression on anti-CD3 activated T cells, with peak expression between 16-24 hours after activation. When soluble CD40.Fc is added to our B-T cultures however, it only partially inhibits B cell growth. This was true even when activated T cells are incubated with CD40.Fc first, to ensure that binding to CD40 ligand was not inhibited by conjugation with B cells. This suggested that other molecules on B and T cells may mediate the delivery of cognate T cell help. We examined the role of cyclosporin A (CsA), which inhibits induction of T cell help, and anti-CD28, which overcomes CsA inhibition of IL-2 production, for their effects on T cell help and CD40L expression. Costimulation of T cells with anti-CD3 plus anti-CD28 did increase the magnitude of B cell growth without concomitant increases in CD40L expression. Anti-CD28 costimulation also overcame CsA inhibition of T dependent B cell growth, without rescuing CD40L expression on T cells. Addition of exogenous IL-2 alone did not substitute for anti-CD28 stiumulation. These data suggest that T cell surface proteins other than CD40 ligand regulate B cell growth, and that this regulation may involve signaling through CD28. Experiments in progress are focusing on kinetic analyses to determine if the kinetics of CD40L expression is altered. We are also continuing to look for additional molecules (by mAb blocking experiments, etc.) on the surfaces of B and activated T cells that mediate the delivery of T cell help.

F 218 Soluble CD40-ligand delivers one of at least two signals received by B cells in T cell dependent activation.

Peter Lane, Antonio Lanzavecchia, and Fiona McConnell
Basel Institute for Immunology, Grenzacherstrasse 487, Basel CH-4005, Switzerland.

Using the cDNA for the murine T-cell ligand of the B cell antigen CD40, we constructed a soluble chimeric fusion protein between the mouse CD8α chain and the mouse CD40 T cell ligand. This protein binds to both human and murine B cells and is biologically active. Alone, this molecule induced a modest degree of B cell proliferation, but was potently synergistic in combination with an anti-Ig stimulus, inducing a 10 to 20 fold increase in DNA synthesis and a concomitant increase in B cell numbers. We also identified a CD4 positive T cell clone which did not express the CD40 ligand constitutively or after stimulation. When activated, this T cell clone induced some B cell proliferation, which was augmented by our chimeric protein but not inhibited by a soluble form of the CD40 molecule. In contrast, activated CD40-ligand positive T cell clones induced strong B cell proliferation which was inhibited by soluble CD40. These data provide evidence that activated T cells provide at least 2 signals to drive B cell proliferation: one provided by CD40 ligand, and another which can be mimicked by anti-Ig.

F 219 ANTIBODY RESPONSE OF MURINE PERITONEAL B CELLS TO A T-DEPENDENT ANTIGEN, Laura A. Ling, Colleen A. Metzger, and Dennis W. Metzger, Department of Microbiology, Medical College of Ohio, Toledo, OH 43699

The murine peritoneal cavity contains large numbers of B-1 (CD5) B cells which have been reported to produce autoreactive, multispecific IgM, but incapable of responding specifically to an exogenous T-dependent antigen. In this study, we directly tested the ability of peritoneal exudate cells (PEC) to produce antibodies against hen egg white lysozyme (HEL). PEC from normal BALB/c mice (Igh^a) were transferred into irradiated SCID mice (Igh^b) alone or together with 10^7 primed T cells from CB-17 mice (Igh^b). One week after transfer the sera of the recipient mice contained 0.25-2.0 mg/ml of donor IgM^a. All mice produced multispecific IgM of the donor allotype. Mice that had also received T cells produced HEL-specific IgG1 of the donor allotype. Cross-reactivity of the IgG1 antibodies with various related lysozymes suggested that there is a difference in the antibody repertoire of peritoneal B cells in comparison to conventional B cells. These results demonstrate that PEC B cells are able to respond specifically to a T-dependent antigen, when appropriate T cell help is provided. (Supported by ACS IM-579)

F 220 MOLECULAR ANALYSIS OF SINGLE ANTIGEN-SPECIFIC B CELLS FOLLOWING PRIMARY T CELL-DEPENDENT IMMUNIZATION.

Michael G. McHeyzer-Williams, M. J. McLean, P. A. Lalor and G.J.V Nossal. Howard Hughes Medical Institute, Stanford University, Stanford, CA 94305

Secretion of specific antibody and the development of germinal centers is an early response to T cell-dependent immunization. Both features of the primary response are crucial to the process of affinity maturation and the establishment of B cell memory. Using multi-parameter flow cytometry we have isolated single B cells expressing isotype-switched, antigen-binding immunoglobulin receptors from the developing primary response to a T cell-dependent form of (4-hydroxy-3 nitrophenyl)acetyl (NP). The majority of primary antigen-specific clones express the lambda antibody light chain gene, consistent with information from previous sera and hybridoma analyses. The emergence of both antigen-specific antibody-secreting and germinal center cells was followed directly using syndecan expression and peanut agglutinin (PNA) binding respectively. Total RNA extraction from single cells, cDNA synthesis from their rearranged VH gene mRNA and subsequent DNA amplification allowed direct DNA sequence analysis from individual clones. The presence of somatic mutation and the extent of CDR3 diversity in clones bearing the dominant VH186.2 was assessed. The first signs of mutation were evident by day 7 of the response where up to 25% of clones contained single point mutations. By day 12, however, up to 70% of the clones expressed a tryptophan to leucine change at amino acid position 33 which leads to an affinity increase and is indicative of positive selection. Mutation potential assorted with the ability to bind high levels of PNA while cells expressing syndecan in the early response exhibited no sign of mutation. Analysis of junctional diversity in the third complementarity determining region of antigen-specific clones from the antibody-secreting and germinal center pathways of development revealed no significant differences in clonotypes. These data argue for a common clonal origin of B cells drawn into an early primary T cell-dependent response.

F 221 IN VITRO SECRETION OF CD23 AND Ig BY HUMAN B-CELL HYBRIDOMA CLONES GENERATED FROM PATIENTS WITH RHEUMATOID ARTHRITIS OR NORMAL CONTROLS REFLECTS METHODS OF IN VITRO STIMULATION, Marianna M. Newkirk[1], Rebeca Renzonzew[1], Alicia Ferraro[1], Marie Sarfati[2], and Guy Delespesse[2], [1]Department of Medicine, McGill University and [2]Department of Immunology, University of Montreal, Montreal, Quebec.

In order to more efficiently generate human hybridoma clones from normal and diseased individuals, we have established a protocol of *in vitro* stimulation of peripheral blood B-cells by anti-CD3 activated T-cells. Using the CD4+ T-cell clone HUT78, we can generate 7 clones per $3x10^7$ normal PBMCs after fusion with a heterohybridoma line SP2/HPT, compared to 0.8 clones for the same number of cells in the absence of activated T-cells. With PBMCs isolated from patients with RA, the results however, are different as there was no additional efficiency in generating clones with stimulation by T-cells, (4.6 and 5.8 clones were generated from $3x10^7$ PBMCs in the T-cell stimulated, or not stimulated cultures respectively). Characterization of the clones for secretion of soluble CD23, an autocrine factor for B-cells, revealed that exposure to activated T-cells prior to fusion resulted in a 2-fold increase in sCD23 secretion by the clones from both sources. Interestingly, sCD23 secretion was 2-fold higher by clones derived from normal individuals compared to the RA derived clones ($p=0.06$). Production of IgM was highest in the low sCD23 secreting clones which were generated primarily from patients with RA. This study demonstrates that *in vitro* culture conditions can clearly alter the population of human B-cells immortalized.

F 222 EFFECTS OF mIg RECEPTOR AFFINITY AND DENSITY ON THE ABILITY OF A B CELL TO FUNCTION AS ANTIGEN PRESENTING CELL. Steven J. Penner, Julia George, Janice Berry, and J. Latham Claflin. Dept. of Microbiology and Immunology, The University of Michigan, Ann Arbor, MI 48109-0620

The biologically significant activity of mIg on a resting B cell is the capture of antigen, thus effecting cellular activation signalling mechanisms and selection of B cells with highest affinity for antigen. To examine what molecular parameters contribute to selection, we co-transfected anti-phosphocholine (PC) H chains containing 0 to 2 mutations and unmutated L chain constructs into an mIg negative B cell line M12.4; stable transfectants expressing a range of mIgM comparable to splenic B cells were selected. We evaluated the ability of lines to function as antigen presenting cells upon stimulation with specific PC-OVA by co-culturing with the OVA-specific T cell hybridoma, DO11.10. The amount of IL-2 production in this system reflects the B cell's ability to capture Ag. Our results showed that both mIg receptor density and affinity contribute significantly to B cell ability in the uptake and presentation of Ag in a PC-inhibitable and MHC class II restricted manner. In particular, a B cell with low mIg receptor density and high affinity for PC was able to induce an IL-2 response as well as a low affinity clone with 25-fold greater mIg density. However, at low receptor density a low affinity clone was not able to elicit an IL-2 response by DO11.10. The results demonstrate that the mIg receptor density on stable B-cell transfectants has a profound effect on the ability of an antigen-specific B cell to process/present antigen to T cells. In light of the importance given to B-T collaboration in expansion of B-cell clones and the observation of decreases in germinal center B cell mIg, we suggest that our results may explain B cell selection and mutation-derived affinity maturation at the fundamental level of the mIg Ag receptors of B cells. The findings obtained with mIgM are being extended by studying B cells co-expressing mIgM and mIgD. (Supported by ACS Grant #IM 664)

F 223 ICAM-1, MHC II AND IL-5 SYNERGIZE TO INDUCE B CELL RESPONSIVENESS TO IL-2, Johanne Poudrier, Diane Heath and Trevor Owens, McGill University, Montreal, Quebec, Canada. H3A 2B4

During cognate interactions between B cells and Th cells, B cells become responsive to T-derived cytokines. We have previously shown that sustained interaction with an activated Th1 clone induced allogeneic small resting B cells to enter cycle and secrete Ig. B cell activation was contact and IL-2 dependent. We were unable to detect expression of IL-2R protein or mRNA by small resting B cells. Expression of both IL-2Rα and β was upregulated by contact with activated T cells. T-independent B cell responsiveness to IL-2 was induced only if IL-5 was included in the T:B coculture. IL-5 alone did not induce B cell responsiveness to IL-2, but synergized with T-contact to enhance responsiveness. IL-5 also synergized with suboptimal LPS to induce IL-2R expression and IL-2 responsiveness. By contrast, the induction of functional IL-2R expression by cross-linking anti-Ig was independent of any co-stimulus. To identify cell surface molecule(s) that signal to induce B cell responsiveness to IL-2, we cultured B cells with sepharose beads that were coupled with anti-ICAM-1 and anti-MHC II. IL-2Rα and β were upregulated when B cells were cultured with beads coupled with both mAb, in the presence of IL-5, whereas each mAb alone, with or without cytokines, was ineffective. These B cells responded to IL-2 by Ig secretion. Co-cross-linking of ICAM-1 and MHC II in conjunction with IL-5 therefore upregulates a functional IL-2R on B cells. These data indicate a role for ICAM-1/MHC II co-aggregation in signalling during T:B contact.

F 224 B7 IS INDUCED ON NORMAL OR LEUKEMIC B CELLS BY ACTIVATED T CELLS VIA A CD40 DEPENDENT SIGNAL.

E. A. Ranheim and T. J. Kipps. Department of Medicine, University of California, San Diego, La Jolla, CA, 92093-0663

Normal T cells, when activated through TCR crosslinking by monoclonal antibodies (mAbs), are able to induce proliferation, differentiation, and immunoglobulin secretion by B cells in an MHC-unrestricted manner which is contact dependent. Because of the potential importance of B cells as antigen presenting cells (APCs) in normal and autoreactive immune responses, we wished to determine what effect the interaction with activated T cells has on the expression of important B cell accessory molecules and subsequent APC function. We find that co-culture with anti-CD3 activated human T cells induces increased expression of a number of accessory molecules, including CD11a, CD54, CD58, and B7 on allogeneic normal or chronic lymphocytic leukemia (CLL) B cells. The upregulation of B7 is of particular interest given the crucial role of B7-CD28 interactions in determining whether APCs induce T cell activation or anergy. The induction of greater than a five-fold increases in B7 expression is much greater than that seen following surface IgM or HLA-DR crosslinking by mAbs, and requires direct T cell contact. In order to discern which cell surface molecules may be responsible for B7 induction, we added a variety of mAbs specific for T or B cell surface accessory molecules to co-cultures of anti-CD3 activated T cells and CLL B cells. We find that only anti-CD40 mAbs can block the induced B7 expression, suggesting that the ligand for CD40, expressed on activated T cells, may be an important inducer of B7 expression. To further test this hypothesis, we examined the phenotypic and functional effects of CD40 crosslinking on normal and CLL B cells by culturing them with anti-CD40 mAb (G28-5) presented on murine fibroblast feeder cells transfected with human IgG FcRII. The resulting phenotypic changes were nearly identical to that seen with activated T cells, including dramatic induction of B7. These phenotypic changes have important functional consequences for the ability of B cells to act as stimulatory APCs. While CLL B cells normally are very poor stimulators of allogeneic T cells in the mixed lymphocyte reaction (MLR), pre-activation with anti-CD40 antibodies results in up to a 30-fold increase in T cell proliferation against the CLL B cells, which is 80% inhibited by blocking B7/CD28 interaction. These studies demonstrate a novel, sIg independent mechanism for inducing high levels of B7 on normal or leukemic B cells. The subsequent increase in APC capacity of B cells upon interaction with activated T cells has important implications for the amplification of the normal immune response and the initiation and/or maintenance of autoimmune disease.

F 225 CD40 ENGAGEMENT ACTIVATES *LYN* KINASE AND PHOSPHORYLATES PLC-γ2. Clement L. Ren and Raif S. Geha; Division of Immunology, Children's Hospital; Boston, MA 02115.

CD40 plays an important role in the induction of IgE synthesis and maintenance of germinal center B cells. We have recently shown that CD40 engagement in normal B cells results in protein tyrosine kinase (PTK) activation. However, the specific PTK involved has not yet been identified. We have studied the role of *lyn* kinase in CD40 signal transduction using an *in situ* phosphorylation system to measure autophosphorylation and the immune complex kinase assay to measure tyrosine phosphorylation of the exogenous substrate, enolase. In Daudi cells stimulated with anti-CD40 mAb 626.1, an increase in *lyn* autophosphorylation and phosphorylation of enolase was noted within 1 minute and remained sustained for 20 minutes. This effect was not dependent on binding of mAb 626.1 to Fc receptors, since biotinylated Fab fragments of mAb 626.1 cross-linked with avidin were equally capable of activating *lyn* kinase. CD40 engagement also results in inositol phosphate generation, protein kinase C translocation, and calcium flux, suggesting involvement of phospholipase C (PLC). Since tyrosine phosphorylation of PLC-γ can result in activation of this enzyme, we also analyzed the effect of CD40 engagement on tyrosine phosphorylation of PLC. Stimulation of Daudi cells with mAb 626.1 also resulted in tyrosine phosphorylation of PLC-γ2 with maximal phosphorylation observed 10-20 minutes after stimulation. These results provide evidence that CD40 signal transduction utilizes lyn kinase, and that PLC-γ2 is a substrate for CD40-mediated PTK activity.

F 226 IN VIVO PRIMING OF IL-4 AND γ-IFN PRODUCING T LYMPHOCYTES IN THE ABSENCE OF B CELLS. Franca Ronchese*, Barbara Hausmann* and Graham Le Gros*. *Basel Institute for Immunology and *Pharma Research, Ciba-Geigy, Basel, Switzerland.

A mouse chimera model was used to establish whether different populations of cells expressing MHC class II (dendritic cells, DC; macrophages, M; B cells) are able to present antigen in vivo and stimulate antigen specific T cell responses, and whether such different antigen presenting populations are able to determine which lymphokines are secreted by the responding T cells.

SCID mice (H-2d) were reconstituted by intravenous injection of purified T or T+B cells from cB6 F1 (H-2dxH-2b) donors. In these mice antigen presented by M or DC (of host origin) will induce a T cell response which is only H-2d restricted, while antigen presented by B cells (of donor origin) will elicit a response which is at least in part H-2b restricted. The results suggested that B cells do not contribute to the induction of a primary antigen specific response, as SCID mice reconstituted with T cells or T+B cells generated comparable frequencies of antigen specific, IL-3 producing T cells. Also, no H-2b restricted T cells could be detected in SCID mice reconstituted with naive T cells, even when in the presence of B cells bearing H-2b. In contrast, H-2b restricted T cells could be readily isolated from SCID mice reconstituted with T cells and B cells from previously primed cB6 F1 donors, suggesting that B cells are able to stimulate T cells in secondary responses.

Analysis of the lymphokine profile of antigen specific T cells showed that SCID mice reconstituted with T cells only generated γ-IFN and IL-4 responses comparable to the ones detected in normal cB6 F1 mice after in vivo immunization with protein antigen in alum adjuvant. The lymphokine response to the helminth parasite *N. brasiliensis* is currently being investigated.

F 227 ANTIGEN SPECIFIC NON-RESPONSIVENESS INDUCED BY CTLA-4IG, Mark A. Tepper, Cherry Singh, Philip M. Wallace, Jennifer Johnson and Peter S. Linsley, Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, WA 98121 and Wallingford, CT 06492

CTLA4, a structural homologue of CD28, a costimulatory molecule on the surface of T cells, binds B7, its natural ligand present on the surface of antigen presenting cells (APC). The interaction of CTLA4 with B7 delivers a costimulatory signal which in combination with a signal from T-cell receptor engagement results in T-cell proliferation. CTLA4Ig, a soluble genetic fusion protein between the extracellular domain of CTLA4(CTLA-4Ig) and an immunoglobulin C gamma chain is a potent inhibitor of immune responses dependent upon T and B cell interaction *in vitro*. In this paper we report that soluble CTLA4 abrogates antibody responses to T-cell dependent antigens in a dose dependent manner and induces a state of non-responsiveness which is both "antigen specific" and "long lasting". These results suggest that blocking the interaction of CD28/CTLA4 with B7, using a soluble CTLA4, can result in a state of clonal anergy which is a consequence of inactivation of antigen specific T-cells. CTLA-4Ig may be useful for the treatment of auto-immune disease and organ transplantation.

F 228 DIFFERENTIATION OF PURIFIED MALIGNANT B CELLS INDUCED BY PMA OR BY ACTIVATED NORMAL T CELLS. Cees van Kooten*, Irma Rensink, Lucien Aarden and Rien van Oers. CLB and Laboratory for Experimental and Clinical Immunology, University of Amsterdam, Amsterdam and the Department of Hematology, Academic Medical Center, Amsterdam, the Netherlands.

We studied the in vitro differentiation (immunoglobulin production) of purified malignant B cells of 21 patients with different B cell malignancies, including Chronic Lymphocytic Leukemia (CLL), Prolymphocytic Leukemia (PLL), Hairy Cell Leukemia (HCL) and Non-Hodgkin Lymphoma (NHL). Direct activation of purified malignant B cells with PMA resulted in the differentiation of most CLL cells, but not of the other types of B cell malignancies. This differentiation required the presence of IL-4. In contrast, with the use of anti-CD2-stimulated normal T cells and IL-2, IgM could be detected in the supernatant of all but one of the purified malignant B cell populations. However, by analysis of the light chains of the IgM produced, monoclonality could be demonstrated in only 13/21 cases: 8/11 CLL, 3/3 PLL, 0/3 HCL and 2/4 NHL. In two patients additional proof that the malignant B cells were the source of the IgM production, could be obtained in an idiotype-specific ELISA. Apart from IgM, also the production of IgG antibodies could be detected. However, only for 2/3 HCL patients, we could confirm a monoclonal IgG production. Since HCL is a malignancy of mature B cells, already carrying IgG on the membrane, this IgG production is not the result of a switch process. In all other cases where IgG production was polyclonal, we have no indications for the induction of Ig switch. The fact that more mature B cell malignancies were T cell-dependent for their differentiation might be a reflection of the in vivo situation. The efficient induction of malignant B cell differentiation described in this paper allows investigation of the antigen specificity of these antibodies.

*present address: Schering Plough, Dardilly, France.

Immunoglobulin Gene Rearrangements

F 300 PHYSICAL MAPPING IN THE VICINITY OF THE X-LINKED AGAMMAGLOBULINEMIA (XLA) LOCUS USING YEAST ARTIFICIAL CHROMOSOME CLONES, R.Cutler Allen[1], Ornella Parolini[3], Mary Ellen Conley[3], A.Craig Chinault[1], and John W. Belmont[1,2], [1]Institute for Molecular Genetics, [2]Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX 77030; [3]University of Tennessee, St. Jude Children's Research Hospital, Memphis, TN 38101

X-linked agammaglobulinemia (XLA) is an X-linked recessive disorder that has been mapped to Xq21.3-22 by linkage analysis. The defect appears to cause a lineage-specific cell autonomous defect in the maturation of B lymphocytes. Therefore, affected individuals are characterized by an absence of B lymphocytes in the peripheral circulation which results in extremely low levels of immunoglobulins. The XLA locus is known to be distal to DXS442 and proximal to DXS94 (approximately 3.5 cM). We have isolated 33 yeast artificial chromosome (YAC) clones from both the Washington University (St. Louis, MO) and the CEPH YAC libraries which are positive for a number of probes that map to the vicinity of the XLA locus. The ends of the YACs have been isolated by Alu-end PCR and the single stranded "vectorette" procedure. Chimerism for the isolated YACs (approximately 60%) has been assessed by fluorescence in situ hybridization (FISH) or mapping of the ends isolated from the YACs on an X chromosome hybrid panel. Probes used to screen these libraries include DXS87, DXS94, DXS101, DSX178, DXS442, and PLP. One of the YACs positive for DXS178 has been very well charcterized: both of the ends have been found to map back to Xq21.3-22; a CpG island has been identified and isolated in a lambda phage clone; and a CA repeat with a heterozygosity of 75% has been isolated. A number of the YACs isolated have also been found to be positive for other probes from the Xq21.3-22 region: a 240 kb YAC positive for DXS178 is also positive for DXS265; a 450 kb YAC and a 1.3 Mb YAC both positive for DXS101 are also positive for DXS328; a 400 kb YAC positive for DXS442 is also positive for DXS366; and three YACs positive for DXS87 are also positive for DXS17. YAC ends which map back to Xq21.3-22 have been used to rescreen the YAC libraries, enabling the construction of a large (approximately 1.5 Mb) contig in the vicinity of XLA. Completion of a contig map between the closest flanking markers will provide an important step toward XLA gene cloning.

F 301 PREFERENTIAL Vλ GENE USAGE IN A HUMAN MATURE B CELL LINE UNDERGOING SECONDARY REARRANGEMENTS, Neil L. Berinstein and Niclas B.J. Stiernholm, Department of Immunology, University of Toronto, Toronto, ON M4Y 1L8, CANADA.

In order to study the process of Ig light chain gene rearrangement in the absence of selective *in vivo* forces, a human sIg^+ B lymphoma cell line was used. We have previously shown that several sIg^- variant clones of this cell line undergo secondary Igλ rearrangements to the Cλ7 gene segment, and thereby regenerate sIg expression. Eleven subclones of one such sIg^- variant were independently propagated, and a single sIg^+ clone from each was established. Southern analysis demonstrated that all these sIg^+ variants had carried out secondary Igλ rearrangements, and surface immunofluorescent staining showed that they all expressed Igλ proteins of the Oz^- Cλ isotype, which is consistent with rearrangements to Cλ7. Strikingly, ten of eleven clones had new rearranged bands of identical size. Two of the ten had carried out additional rearrangements on an alternate Igλ allele, whereas the eleventh clone had a different sized rearrangement altogether. The utilization of what appears to be the same Vλ gene segment in 10/11 independent rearrangement events suggests that Igλ rearrangement is likely a highly regulated process. In order to identify the mechanism(s) behind this apparent restriction, we are currently sequencing and mapping the Vλ gene segments of these sIg^+ variants, as well as extending the analysis to other sets of sIg^+ variants, generated from different sIg^- subclones.

F 302 REGULATION OF HUMAN IMMUNOGLOBULIN CLASSES AND IgG SUBCLASSES: A DUAL ROLE FOR IL-4.
Robin E. Callard and Karolena Kotowicz, Cellular Immunology Unit, Institute of Child Health, 30 Guilford St., London WC1N 1EH, G.B.

The effect of IL-4 on immunoglobulin class (IgM,G,A,E) and IgG subclass (IgG1,2,3,4) production by human tonsillar B cells stimulated with EBV was investigated. Antibody production in response to EBV was biphasic. An early peak at day 12 due to polyclonal activation of the B cells was followed by a second peak after day 21 due to B cell transformation. The polyclonal response consisted of all Ig classes and subclasses except for IgE. Addition of IL-4 at concentrations of 100 U/ml induced the production of IgE and enhanced IgG4 secretion, but either had no effect or, more often, inhibited production of the other isotypes. In contrast, low concentrations of IL-4 (1-5 U/ml) greatly enhanced the production of IgM, IgA and IgG 1, 2, & 3, but had no effect on IgG4 or IgE. Only high density (resting) B cells responded to EBV and IL-4 indicating that IL-4 was not acting simply as a late differentiation factor. IL-4 increased IgG and IgA production by EBV stimulated surface IgM^+ B cells. This result is consistent with heavy chain switching. In some experiments, however, IL-4 enhanced IgA, and IgG1,2,3 production by $sIgM^-$ B cells and IgM production by $sIgM^+$ B cells suggesting that IL-4 may have an additional BCDF activity which was not isotype specific. The different effects of high and low concentrations of IL-4 were similar to those reported by us in B cell activation experiments, and may be due to the existence of high and low affinity receptors for IL-4.

F 303 DISPARATE FREQUENCIES OF 5' AND 3' FLANKING REGION MUTATIONS IN AN EXPRESSED VK12 GENE. Stephen Clarke and Robert Rickert, Department of Microbiology and Immunology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599.

We have identified hybridomas of two clones that have acquired >10 fold fewer somatic mutations in their expressed Vκ genes than in their co-expressed VH genes. The hybridomas of one clone express the Vκ1A gene and those of the second clone express the Vκ12.37 gene. To gain insight into the basis for these disparate frequencies, we have determined the frequency of mutation in non-coding DNA flanking these expressed Vκ genes. The Vk1A genes have a low frequency of mutation in both flanking regions, consistent with the frequency of mutation in the coding region. In contrast, among the 6 members of the Vκ12.37 expressing clone, there are 31 mutations 3' of the Vκ exon, and no mutations 5' of the Vκ exon. Moreover, coding region mutations are skewed to the 3' end. None of the 31 3' mutations are shared by two or more of the hybridomas indicating that they occurred late in clonal expansion. These results suggest that different mechanisms are responsible for the disparate frequencies of VH and Vκ mutation in these two clones, and suggest that Vκ and Jκ mutation are not inextricably linked.

F 304 TIMING OF SOMATIC GENE CONVERSION OF RABBIT VDJ GENES, Mary A. Crane and Katherine L. Knight, Department of Microbiology and Immunology, Loyola University Chicago, Chicago, IL, 60153

The mammalian genome contains multiple V_H, D and J_H gene segments that are rearranged during B cell differentiation. Normally, various V_H gene segments combine with various D and J_H gene segments to create a vast repertoire of antibody VDJ genes. We found that rabbits utilize one V_H gene segment (V_HI) in 70-90% of their VDJ gene rearrangements. We now know that rabbits diversify this V_HI gene segment by somatic gene conversion in which upstream V_H gene segments serve as donor genes to "convert" the utilized V_HI gene segment. Chickens also diversify their V_H and V_L genes by somatic gene conversion, and this process occurs in the bursa during fetal development. We do not know where or when somatic gene conversion occurs in rabbit. We sought to address the question of when gene conversion occurs by analyzing RNA isolated from peripheral blood lymphocytes of rabbits varying in age from 3 to 7 weeks. We cloned multiple VDJ gene rearrangements after amplification by PCR of cDNA made from the RNA. We sequenced the cloned VDJ genes and analyzed them for similarity to germline V_HI. At 3 weeks of age, rabbit VDJ genes were very similar to germline V_HI. By 7 weeks, however, the VDJ genes were highly diversified. The diversification included base pair changes as well as codon insertions or deletions. We searched our gene bank of known rabbit V_H gene segments for potential donor genes containing sequences that were identical to the diversified regions of the VDJ genes. We found potential donor genes for each of several clusters of nucleotide differences. Taken together, our studies indicate that heavy chain immunoglobulin genes in the rabbit begin to diversify at approximately 3 to 4 weeks of age and by 7 weeks of age are indistinguishable from the diversified VDJ genes of adult rabbits. We suggest that somatic gene conversion is involved in the development of the antibody repertoire during a narrow window of time beginning at approximately one month of age.

F 305 UNUSUAL DELETIONS WITHIN THE IMMUNOGLOBULIN HEAVY CHAIN LOCUS IN ACUTE LEUKEMIAS
M J S Dyer, J M Heward, V Buccheri and D Catovsky, Academic Haematology, Institute of Cancer Research/Royal Marsden Hospital, Downs Road, SUTTON, Surrey SM2 5NG, United Kingdom.

We have investigated the structure of the immunoglobulin (Ig) heavy chain locus in 316 cases of acute leukemia, including 71 cases of B-cell precursor ALL (BCP-ALL) and eight cases of biphenotypic leukemia. In six cases of BCP-ALL and in one case of biphenotypic acute leukemia with t(9;22)(q34;q11) which co-expressed myeloid and B-cell differentiation antigens, deletion of IgJ_H sequences in the presence of cytogenetically normal chromosome 14's was observed. Five of the seven cases analysed showed deletion of IgJ_H sequences from both chromosomes. These deletions were acquired somatically during the development of the neoplastic clone as shown by the presence of normal IgJ_H fragments in remission bone marrow samples.

Since these deletions may have contributed to the pathogenesis of the disease we have attempted to define their boundaries. Using probes which map both 5' and 3' of IgJ_H, the 3' (centromeric) boundary of the deletions was mapped to an approximately 20kb central region of the 60kb IgCδ - IgCγ3 intron in ten of the twelve deleted alleles. In the remaining two alleles, the 3' boundary mapped to the switch region associated with IgCμ. The 5' (telomeric) boundary could not be defined. However, all cases with biallelic deletion of IgJ_H showed biallelic deletion of the most proximal IgV_H (V_H6 and V_H4) sequences, indicating that the deletions span over 200kb. No gross deletions were, however, observed using a V_H3 sub-family specific probe, indicating that the 5'boundary mapped within the IgV_H locus. Furthermore, a probe (D14S20) which maps telomeric to the IgH locus was retained in germline configuration in all cases.

In one case with biallelic IgJ_H,Cμ and Cδ deletions no RNA transcripts could be detected using IgV_H3,IgIγ3 or IgCγ3 probes.

Chromosomal translocations involving the IgH locus are rare in BCP-ALL. However, we have demonstrated that pathologic deletions of the crucial 5' portion of the IgH locus including IgJ_H,Cμ and Cδ occur in about 10% of acute leukemias with immunophenotypic evidence of commitment to the B-cell differentiation pathway. The possible pathogenic consequences of the deletions remain to be determined. The clustering of the deletions to IgSμ and to a specific region within the IgCδ-IgCγ3 intron suggests a distinct pathogenic mechanism.

F 306 MURINE T CELL HYBRIDOMAS RESPOND TO SOMATIC MUTATIONS IN ANTIBODY VARIABLE REGIONS.

Margaret C..Eyerman and Larry Wysocki. Division of Basic Sciences, Dept. of Pediatrics, National Jewish Center for Immunology and Respiratory Medicine and Dept. of Microbiology and Immunology, University of Colorado Health Sciences Center, Denver CO 80206.

During an immune response, specific antibodies and B cells that are infrequently represented in the immune repertoire become amplified to abundance. Potentially novel T cell epitopes are also created through the physiologic process of somatic hypermutation in antibody genes. This presents a challenge for T cell self tolerance since many potential epitopes are either rare or nonexistent during the maturation of the T cell repertoire in the thymus. To explore the potential for T cell recognition of antibodies, we have immunized A/J mice with somatically mutated mAbs generated from the same strain. We have produced 10 T cell hybridomas to mAb36-71 and 3 T cell hybridomas to mAb45-49. These T cell hybridomas are derived from 6 mice. All of the hybridomas express αβ T cell receptors and CD4 by flow cytometry. On the basis of both antibody blocking studies and transfected antigen presenting cells, all of the hybridomas specific for mAb36-71 are restricted to I-A^k, while all of the hybridomas specific for mAb 45-49 are restricted to I-E^k. Since glutaraldehyde-fixed APC can present tryptic digests of the immunogen, but not the native form of the antibody, we conclude that processing is required. For each mAb, therefore, the response is a typical class II MHC-restricted response in which $CD4^+$ hybridomas use αβ TCR to respond to processed mAb in the context of either I-A^k or I-E^k. None of the T cell hybridomas responded to germline or constant region epitopes of the control unmutated mAb36-65. Using genetically engineered antibodies, in which mutated and unmutated H or L chains were paired in all combinations, we found that each of the 13 T cell hybridomas responded to the mutated mAb45-49 and mAb36-71 light chain V regions but not to unmutated versions of these same V regions. Since every hybridoma responds to somatic mutations, we hypothesize that the T cell repertoire is tolerant of germline antibody V regions. Our results show that T cells are able to recognize syngeneic mutated antibody variable regions in a conventional class II MHC-restricted manner. Whether tolerance to autologous somatically mutated V regions is achieved during antigen-driven stages of B cell differentiation is unknown.

F 307 RECURRENT ISOLATION OF A NOVEL IMMUNOGLOBULIN HEAVY-CHAIN VARIABLE (V_H5) *GENE FROM HUMAN FOLLICULAR B CELLS*, Rick M. Fairhurst, Mehran Neshat, Yadira Valles-Ayoub, and Jonathan Braun, Department of Pathology, UCLA School of Medicine and Molecular Biology Institute, UCLA, Los Angeles, CA 90024

Somatic hypermutation has been shown to be targeted exclusively to the rearranged immunoglobulin (Ig) variable (V) region, particularly to the complementarity-determining regions (CDRs), and is localized to germinal center B cells. In the course of studying hypermutation-related UV repair in germinal center and mantle zone B cells, we repetitively cloned a V_H5 sequence (67-71) which differs from the prototype V_H5 gene (V_H251) in 13 nucleotide positions over 285 bases of coding region DNA. Of those mutations (7) within CDR1 and CDR2, all were replacement mutations, whereas silent mutations predominated within framework regions (FRs). As such, this 67-71 sequence appears to be a somatically mutated derivative of V_H251. A similar sequence (WS1) was reported in a patient with chronic lymphocytic leukemia (CLL), a disease noted for preferential rearrangement of V_H251 and expression of near-germline transcripts. The WS1 sequence was originally interpreted to *represent a somatically mutated V_H251 derivative. Using V_H5 family-*specific primers (VH50 and JH45) in a polymerase chain reaction (PCR), we have amplified and cloned the 67-71 gene from the rearranged Ig V_H locus of follicular B cells. Forty such V_H5 amplifications have been carried out on several tissues which differ from CLL: neonatal spleen, adult tonsil subjected to UV irradiation, and a T cell lymphomatous tonsil. A total of five 67-71 sequences (at least one from each tissue) were obtained as determined by either direct sequencing or a discriminating *DdeI* site. Using primers VH50 and VH2513F (in the heptamer-nonamer spacer region) to amplify the unrearranged V_H251 gene, however, we have accumulated evidence which suggests that the WS1-like sequence may also be present in the unrearranged DNA of lymphoid cells. As the somatic hypermutation mechanism is thought to be operative only on rearranged Ig V genes, we postulate that the 67-71 sequence may not be a somatically mutated V_H251 derivative but that we may have identified a novel member of the V_H5 gene family. Alternatively, 67-71 may be a widely divergent allelic polymorphism of V_H251 or may in fact represent a recurrent somatic mutation of V_H251 suggestive of templated mutagenesis (e.g. gene *conversion). Currently, we are in the process of cloning the 67-71* sequence from non-lymphoid DNA.

F 308 PRESENCE OF THE SECRETABLE FORM OF μ HEAVY CHAIN mRNA IN CVID B CELLS THAT ARE UNABLE TO SECRETE IGM, John Farrant, Anne L. Newell, John McVey, Alfa Sa'adu and A. David B. Webster, Immunodeficiency Diseases Research Group, Clinical Research Centre, Harrow, HA1 3UJ, U.K.

B lymphocytes from patients with common variable immunodeficiency (CVID) show a differential response on stimulation with anti-μ and IL-2 in vitro. Cells from group A patients are unable to secrete IgM or IgG. We have now investigated whether this failure in secretion is reflected at the mRNA level. We also asked whether these cells could express membrane μ at both the protein and mRNA levels. After Ficoll separation, total RNA was prepared from aliquots (40 ml) of peripheral blood obtained from 8 patients and 4 normal donors. cDNA was prepared by reverse transcription and amplified by PCR using primers for the secretable or membrane forms of μ heavy chain. The identity of positive PCR fragments was confirmed by sequencing. Cells from all patients and normal donors contained mRNA for both the secretable and membrane forms of μ heavy chain. In all patients studied, surface IgM expression was detected by flow cytometry. This data shows that the block in the secretion of IgM by B cells from the most severely affected CVID patients is not due to a post-transcriptional defect.

F 309 BONE MARROW CELLS IN X-LINKED AGAMMA-GLOBULINEMIA (XLA) EXPRESS PRE-B SPECIFIC GENES (λ-LIKE AND V PRE-B) AND PRESENT IGH V-D-J GENE USAGE STRONGLY BIASED TO A FETAL-LIKE REPERTOIRE. Michel Fougereau, Michèle Milili, Françoise Le Deist*, Geneviève de Saint- Basile*, Alain Fischer* and Claudine Schiff. Centre d' Immunologie de Marseille-Luminy, and *U 132 Hopital des Enfants malades, Paris. France.

Expression of Ig and Ig-related genes has been studied in bone marrow cells from 2 patients with severe form of X-linked agammaglobulinemia (XLA). Phenotypic analysis revealed the presence of pre-B cells, in the absence of mature B cell markers. The pre-B specific genes, λ-like and V pre-B were normally transcribed. Sequence analysis of 48 distinct V-D-J cDNA clones directly derived from XLA bone marrow cells indicated that they had characteristics of an early fetal pre-B repertoire. All VH families were identified, with a strong bias in the gene usage within each family. A few VH genes were largely overexpressed, either germline or slightly mutated. Most germline genes that had been located 3' of the VH locus were used. Short D regions, (resulting from D-D fusion, usage of all D genes in both orientations and utilization of the 3 reading frames), restricted N diversity and a fetal JH usage pattern were also observed. Taken together our data suggest that the XLA defect does not alter V-D-J *rearrangements nor the expression of μ, λ-like and V* pre-B transcripts and most likely results in a poor efficiency of some critical steps of the B cell maturation.

F 310 CLONAL DIVERSITY IN AIDS-ASSOCIATED B-CELL NEOPLASIA, David F. Friedman, Michael Scully, June Goldman, and Leslie E. Silberstein, Departments of Pediatrics and Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA. 19104

B-cell neoplasms occur at higher frequency in humans with acquired immunodeficiency syndrome (AIDS) than in the general population, and differ in site of origin, histologic type, and prognosis from B cell neoplasms seen in non-HIV infected individuals. Both polyclonal and oligoclonal B cell expansions have been demonstrated in AIDS, but it is not known if these expansions are precursors for, and/or arise by the same mechanisms which give rise to the B cell neoplasms seen in AIDS patients. To investigate the clonality and the possible role of antigen mediated clonal *selection in the pathogenesis* of AIDS-related B cell neoplasia, we have studied the expressed immunoglobulin heavy chain genes in two such neoplasms by nucleotide sequencing of PCR-amplified immunoglobulin cDNA. Both neoplasms appeared to be monoclonal by Southern blot for immunoglobulin heavy chain gene rearrangements. From an indolent multiple myeloma in an HIV infected adult, sequencing of 16 independent colony isolates revealed 7 sequences derived from the same B cell clone on the basis of identity within CDR3, 3 sequences from a second B cell clone, and 6 unrelated sequences. From an aggressive B cell leukemia in a child infected with HIV, 8 sequences revealed 2 pairs of clonally related sequences and 4 unrelated sequences. Unexpectedly, in both cases we identified significant *intra*clonal sequence diversity by comparison of V gene sequences from colonies which share CDR3 sequences. One isolate of the 7 clonally related sequences from the myeloma differed from the other 6 by 25 bases, and the V region sequences in one pair of clonally related isolates from the leukemia differed by 38 bases. This degree of intraclonal diversity has been observed previously only in follicular B cell lymphomas. Both B cell leukemias and multiple myeloma arising in non-AIDS patients have previously been associated with little or no intraclonal diversity. These findings confirm that oligoclonal B cell expansions also occur in AIDS-associated B cell neoplasms. Furthermore, the intraclonal sequence diversity suggests that a phase of somatic mutation, possibly ongoing within the clonal population at the time of sampling, or alternatively, occuring earlier in the evolution of the clone, may be a feature of the pathogenesis of AIDS-related B cell neoplasms.

F 311 ENDONUCLEASE AND EXONUCLEASE ACTIVITIES IN B-CELL NUCLEAR EXTRACTS, Patricia J. Gearhart, Paul Dempsey, and Peter Schweitzer, Department of Biochemistry, Johns Hopkins University School of Hygiene and Public Health, Baltimore, MD 21205

After stimulation with antigen, B cells undergo a high rate of somatic mutation in their rearranged variable genes. Mutation may be caused by error-prone repair of DNA around the V(D)J gene, and involve an endonuclease, which makes nicks in the DNA, and an exonuclease, which creates gaps from the nicks. To test for these activities, nuclear extracts were prepared from several cell sources: splenic B cells taken 6 days after immunization when mutation is ongoing, a hybridoma B-cell line, a T-cell line, and a Chinese hamster ovary (CHO) cell line. A quantitative endonuclease assay was performed by incubating extracts with ^{3}H-labeled plasmid, denaturing the nicked plasmid in pH 12 buffer, and filtering it through a nitrocellulose filter. Nicked DNA is retained on the filter, which is then counted. Extracts from splenic B cells, hybridoma cells, and the T-cell line had high levels of endonuclease compared to CHO cells, which had one-quarter the amount of activity. The exonuclease assay was performed by incubating extracts with a 20-mer primer labeled on 5' end with γ-^{32}P, and separating the products on a sequencing gel. The splenic B-cell extract produced a ladder of smaller labeled fragments, consistent with a 3' to 5' exonuclease activity. The other cell extracts showed no evidence of a ladder, and the reaction products indicated that the cells had a 5' to 3' exonuclease. Thus, both B and T cells express high levels of endonuclease relative to CHO cells, but only splenic B cells have a 3' to 5' exonuclease activity.

F 312 SINGLE CHAIN MONO- AND BI-SPECIFIC ANTIBODIES WITH NOVEL BIOLOGICAL PROPERTIES, Martha S. Hayden[*], Margit A Gayle[&], Lisa K. Gilliland, Jurgen Bajorath, Jeffrey A. Ledbetter, and Peter S. Linsley[$], [*]Johnson and Johnson Biotechnology Center, San Diego, CA 92121, [&]Immunex Corporation, Seattle, WA 98121, and [$]Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, WA 98121

The eukaryotic expression vectors pCDM8 and piLNXAn were modified to express single chain antibody molecules as individual protein domains encoded on interchangeable cDNA cassettes Individual heavy and light chain variable domains from the hybridoma expressing the antibody of interest were isolated and fused using a short $(Gly_4Ser)_3$ linker. This cassette was expressed as a single chain antibody molecule when inserted into the modified vector Two different single chain antibodies were constructed, one which binds the L6 tumor associated antigen, and the other which binds the CD3 T-cell surface receptor molecule.

These novel fusion proteins were expressed in a COS cell transient transfection system. Antigen specific binding activity to cells expressing antigen was detected in crude supernatants or affinity purified proteins from transfected cells. The functional properties of the single chain molecules were investigated using both physical and biological assays. Several mutant derivatives of the Fc domain were used to test the single chain L6, and demonstrated varying capacities to mediate ADCC and CDC responses. The CD3 single chain molecule exhibited stronger signalling and a different spectrum of proliferative responses in T cells when compared with native antibody.

The two binding domains for L6 and CD3 were fused using a short helical peptide linker to produce a bispecific single chain cassette. The CD3-L6 bispecific fusion protein was capable of mediating adhesion between tumor cells expressing the L6 antigen and T cells expressing the CD3 receptor. In vitro assays demonstrate that this protein stimulates high levels of T cell proliferation and cytotoxicity against L6 positive tumor cells. The bispecific molecule therefore possesses novel properties and capabilities with therapeutic application that were not present in either isolated single domain molecule alone.

F 313 GENE CONVERSION CONTRIBUTES TO HUMAN ANTIBODY DIVERSITY, Richard A. Insel, Willam S. Varade, Elides Marin, and Ann M. Kittelberger, Department of Pediatrics, University of Rochester School of Medicine, Rochester, NY 14642.

Somatic gene conversion is an important mechanism for generation of antibody diversity in the chicken and rabbit. To determine whether human variable region (V) gene diversity can arise from targeted, templated mutations, we have determined whether the conserved human V_{H5} pseudo (Ψ) gene, which lacks normal recombination signal sequences, can act as a donor of templated mutations into the more 3' conserved functional (fx) V_{H5} gene in $V_{H5}DJ_H$ rearrangements. We have cloned and sequenced $V_{H5}DJ_H$ rearrangements from human spleen that contain both large regions of sequence identity to ΨV_{H5} as well as regions with sequence identity to fxV_{H5}. These rearrangements have an open reading frame, show additional somatic point mutations away from the germline sequence in both their ΨV_{H5} and in their fxV_{H5} regions, can be isotype-switched to IgG, and can diversify. Multiple clones with unique as well as shared somatic point mutations arising from the same rearrangement with ΨV_{H5} and fxV_{H5} regions have been isolated. The finding of somatic point mutations in the $\Psi V5$ regions, as well the ability to generate the identical rearrangements and sequences in independent experiments and after only 35 cycles of PCR amplification rule out PCR artifact for their generation. The results suggest that a mechanism for templating V gene mutations, such as gene conversion, exists also in man.

F 314 *IN VIVO* EXPRESSION OF HUMAN Ig GERM-LINE mRNA AND ITS CORRELATION TO CLASS SWITCHING, Khalid B. Islam[1,2], Berivan Baskin[1,2], Birger Christensson[2], Erna Pettersson[2], Lennart Hammarström[1,2] and C. I. Edvard Smith[1,2] [1]Center for BioTechnology and [2]Department of Clinical Immunology, Karolinska Institute, NOVUM and Huddinge Hospital, 141 57 Huddinge, Sweden

The human Ig heavy chain locus is composed of 9 functional genes located on chromosome 14. During immunoglobulin isotype switching the VDJ complex is transferred from its physical association with Cμ to another constant gene through a recombinatorial process involving switch regions. This phenomenon is preceded by transcription from exonic regions located upstream of all constant region genes with the exception of Cδ, designated I-regions according to the generally accepted nomenclature. The RNA emanating from these regions, being devoid of VDJ, are normally referred to as germ-line (GL) transcripts. We have previously been involved in the isolation of human Iγ and Iα genes (Int Immunol 1:631, 1989; 3:1099 and 1107, 1991). However, the function of the GL transcripts is not known. To understand the *in vivo* role of this transcriptional activity we have adapted the reverse transcription-polymerase chain reaction to analyze the GL transcripts from unstimulated mononuclear cells in different human organs and in immunological diseases. We report here that GL (Iα, Iγ and Iε) mRNA are expressed differentially during ontogeny of B cells and in various organs. GL mRNA of all three isotypes could be detected in PBMC from healthy donors, whereas, there was a decrease of specific GL transcript synthesis in Ig deficiency diseases. Furthermore, in a parasitic disease we could demonstrate an induction of GL Iε mRNA as well as productive ε transcripts during *in vivo* immune response. These findings implicate a potential role of GL transcription during *in vivo* Ig class switching.

F 315 supF AS A MUTABLE SUBSTRATE FOR SOMATIC HYPERMUATION OF IMMUNOGLOBULIN GENES, Emily L. Klotz, John R. Hackett, Jr., and Ursula Storb, Dept. of Molecular Genetics & Cell Biology and Committee on Immunology, Univ. Chicago, Chicago, IL 60637.

Currently, to determine the presence or absence of somatic mutations in either endogenous or transgenic rearranged immunoglobulin (Ig) genes, the genes must be cloned and sequenenced. This limitation makes investigations into the sequences necessary for targeting somatic hypermutation very difficult. In an attempt to circumvent this problem, a mutatable substrate (supF) has been inserted into the variable region of a rearranged Ig kappa (κ) light chain transgene. This light chain construct includes ~4kb upstream of the leader through ~9.5kb downstream of the C region. Previous studies in our lab have demonstrated that the light chain construct is targeted for somatic hypermutation (1). The supF gene has been shown to be a mutagenic target in shuttle-vector plasmids in mammalian cells (2). Mice carrying a low number of copies of the supF/ κ light chain construct were hyperimmunized and hybridomas made form splenic B cells. DNA was extracted from IgG+ hybridomas. The design of the construct allows for plasmid rescue of the supF/ κ light chain transgene. When a bacterial strain that lacks β-gal activity, due to an amber mutation, is transformed with an unmutated supF gene, the colony appears blue on an x-gal plate. About 25% of mutations in the supF gene result in a light blue or white phenotype. Mutations can be confirmed by sequencing. We are investigating if supF is a mutable substrate for somatic hypermutation.

Supported by NIH grants GM38649 and HD23089. E.K. is supported by NIH predoctoral training grant GM07183.

1. Hackett, J., B.J. Rogerson, R.L. O'Brien and U. Storb. Analysis of somatic mutations in κ transgenes. J. Exp. Med. 172:131-137, 1990.
2. Kraemer, K.H. and M.M. Seidman. Use of *supF*, An *E.coli* tryrosine suppressor tRNA gene, as a mutagenic target in shuttle vecotr plasmids. Mut. Res. 220:61-72, 1989.

F 316 TIMING OF SOMATIC GENE CONVERSION OF RABBIT VDJ GENES, Mary A. Crane and Katherine L. Knight, Department of Microbiology and Immunology, Loyola University Chicago, Chicago, IL, 60153

The mammalian genome contains multiple V_H, D and J_H gene segments that are rearranged during B cell differentiation. Normally, various V_H gene segments combine with various D and J_H gene segments to create a vast repertoire of antibody VDJ genes. We found that rabbits utilize one V_H gene segment (V_H1) in 70-90% of their VDJ gene rearrangements. We now know that rabbits diversify this V_H1 gene segment by somatic gene conversion in which upstream V_H gene segments serve as donor genes to "convert" the utilized V_H1 gene segment. Chickens also diversify their V_H and V_L genes by somatic gene conversion, and this process occurs in the bursa during fetal development. We do not know where or when somatic gene conversion occurs in rabbit. We sought to address the question of when gene conversion occurs by analyzing RNA isolated from peripheral blood lymphocytes of rabbits varying in age from 3 to 7 weeks. We cloned multiple VDJ gene rearrangements after amplification by PCR of cDNA made from the RNA. We sequenced the cloned VDJ genes and analyzed them for similarity to germline V_H1. At 3 weeks of age, rabbit VDJ genes were very similar to germline V_H1. By 7 weeks, however, the VDJ genes were highly diversified. The diversification included base pair changes as well as codon insertions or deletions. We searched our gene bank of known rabbit V_H gene segments for potential donor genes containing sequences that were identical to the diversified regions of the VDJ genes. We found potential donor genes for each of several clusters of nucleotide differences. Taken together, our studies indicate that heavy chain immunoglobulin genes in the rabbit begin to diversify at approximately 3 to 4 weeks of age and by 7 weeks of age are indistinguishable from the diversified VDJ genes of adult rabbits. We suggest that somatic gene conversion is involved in the development of the antibody repertoire during a narrow window of time beginning at approximately one month of age.

F 317 MOLECULAR CONFIRMATION OF ISOTYPE SWITCHING IN HUMAN B LYMPHOCYTES CULTURED WITH IL-4 IN THE CD40 SYSTEM, Serge Lebecque°, Laurent Galibert°, Joël van Dooren*, Roy Jefferis+, Hector Martinez-Valdez°, Françoise Rousset° and Jacques Banchereau°, °Schering-Plough, Laboratory for Immunological Research, Dardilly, France. *Ludwig Institute for Cancer Research, Brussels, Belgium. +School of Medicine, Birmingham, U.K.

Human B lymphocytes undergo long term proliferation when cultured in the CD40 system in the presence of IL-4. Such cultures contain low levels of IgM, IgG and most notably IgE. G8 idiotype positive, sIgD+ B cells have been sorted and single cells have been cultured for ten days. B cell clones have been PCR amplified using a VH1 specific primer and μ, γ, α and ε specific primers. Amplification products of a size corresponding to μ, γ, α and ε heavy chains have been obtained. Sequencing of the amplified products originating from three different cell clones has demonstrated isotype switching with the same VDJ genes coupled to different constant region genes. Sequencing indicate an error frequency of 1/800 bp both in VDJ and C regions. These errors are likely to result from the amplification step. Thus, this culture system appears to induce isotype switching but probably not somatic mutations.

F 318 **Locus Control of V(D)J Recombination: The Role of CpG Methylation.** Michael R. Lieber and Chih-Lin Hsieh, Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305-5324.

The physical parameters controlling the accessibility of antigen receptor loci to the V(D)J recombination activity are unknown. We have used minichromosome substrates to study the role that CpG methylation might play in controlling V(D)J recombination site accessibility. We find that CpG methylation decreases the V(D)J recombination of these substrates more than 100-fold. The decrease correlates with a considerable increase in resistance to endonuclease digestion of the methylated minichromosome DNA. The minichromosomes acquire resistance to both the intracellular V(D)J recombinase and exogenous endonuclease only after DNA replication. Therefore, CpG methylation specifies a chromatin structure that, upon DNA replication, is resistant to eukaryotic site-specific recombination. More recently, these studies have been extended using stable minichromosomes. These findings are important to V(D)J recombination as well as to the chromatin assembly of methylated DNA during replication.

F 319 SEQUENCING MUTATED VARIABLE REGION GENES FROM SINGLE B LYMPHOCYTES. Andrew H. Liu and Lawrence J. Wysocki, Division of Basic Sciences, Department of Pediatrics, National Jewish Center for Immunology and Respiratory Medicine and University of Colorado Health Sciences Center, Denver, CO 80206.

To better understand the cellular selection and hypermutation processes that shape humoral immunity, we isolated and sequenced the antibody variable region (V) genes of single B cells participating in a defined anti-hapten immune response. Splenic B cells from A/J mice immunized with para-azophenylarsonate (Ars) were stained with an Ars-associated anti-idiotype and isolated by flow cytometry 13 days after immunization. Single sorted cells were identified in microdrops by phase-contrast microscopy. The chromosomal copies of their Heavy and Light chain V (V_H and V_K) genes were individually amplified by a nested Polymerase Chain Reaction (PCR) and directly sequenced without cloning under conditions that reveal essentially no Taq polymerase errors.

We successfully isolated single B cells participating in the anti-Ars primary humoral immune response. From two Ars-immunized mice, 26/100 stained single cells yielded a V_H or V_K gene product; both V_H and V_K genes amplified in 13/26 of these cells. V gene segment usage in all 26 isolated cells was restricted to a specific combination (V_{HidCR}, $D_{f116.1}$, J_{H2}; and V_{KidCR}, J_{K1}) known through previous hybridoma studies to encode antibodies of the dominant anti-Ars associated idiotype. Furthermore, all V genes from these cells encoded specific junctional amino acids in both V_H and V_K genes known to be critical for Ars binding (V_K: Arg at position 96; and V_H: Ser at position 99). Finally, several cells had acquired somatic mutations in the H chain CDR2 region known through previous site-directed mutagenesis studies to increase affinity for Ars. In one experiment, 8 single cells represented at least 5 different clones based upon junctional differences not critical for Ars binding, and each cell was unique based on these junctional differences and the different somatic mutations each had acquired.

We conclude that both hypermutation and intense selection occur by Day 13 after immunization, as single idiotype-bearing B cells, by this time, use specific V gene segments, junctional residues, and somatic mutations critical for or that enhance antigen binding.

F 320 **GENETIC LINKAGE ANALYSIS FOR X-LINKED AGAMMAGLOBULINEMIA (XLA) AND X-LINKED HYPER IgM (XHM),** Ruth Lovering, Munoree Padayachee, Marie-Anne O'Reilly, Helen Middleton-Price, Sally Genet, Mo hammed Parkar, Lesley Alterman, Angela Sweatman, Linda Bradley, Sue Malcolm, Roland Levinsky and Christine Kinnon, Institute of Child Health, London WC1N 1EH, UK.

XLA is characterized by agammaglobulinemia involving all immunoglobulin subclasses and is the direct result of a lack of mature B cells. As pre-B cells are present in the bone marrow of affected boys at normal levels the XLA gene product is thought likely to be involved in B cell maturation.

The consensus order of loci in the Xq22 region is established as: cen-DXS3-(DXS178, XLA)-DXS94-DXS17-tel. Using genetic linkage analysis we have now identified two new flanking loci for the XLA locus and extended the genetic map to: cen-DXS3-DXS366-DXS442-(DXS178, DXS265, XLA)-DXS101-DXS94-DXS17-tel. We have found no recombination between XLA and DXS178 in over thirty informative meioses (maximum lod score $Z=10.48$, $\Theta=0$). To improve the genetic map in this region a further three loci DXS327, DXS454 and DXS458 have been mapped with respect to DXS178 and the XLA loci.

The previously reported flanking markers, DXS3 and DXS94, are 10-12 centiMorgans apart. With DXS442 and DXS101 as the new proximal and distal flanking markers the region of DNA which contains the XLA locus has been narrowed down to 2 centiMorgans. As we have a YAC contig of the region this should facilitate the isolation of this gene (see abstract Kinnon *et al.*).

XHM is also characterized by agammaglobulinemia, however, it is associated with enhanced levels of IgM and an absence of IgG. The gene responsible for this disorder maps to Xq26 which is therefore distinct to that which causes XLA. Multipoint linkage data in 6 families with XHM show that the most likely position of the gene is just distal to HPRT. At HPRT the maximum lod score (Z max) is 4.9 with $\Theta=0.05$. We will present a genetic map of the locus and detail the progress we have made so far.

F 321 SOMATIC REARRANGEMENT OF V_H-D-J_H TRANSGENES WITH ENDOGENOUS IMMUNOGLOBULIN HEAVY CHAIN DNA RENDERS THEM SUSCEPTIBLE TO HYPERMUTATION. Tim Manser and Angela M. Giusti, Department of Microbiology and Immunology, Jefferson Cancer Institute, Thomas Jefferson Medical College, Philadelphia, PA, 19107

To investigate the cis-acting elements involved in the hypermutation of mouse immunoglobulin variable (V) region genes, transgenic lines of mice were derived using plasmid constructs containing a V_H-D-J_H gene and varying amounts of 5' and 3' flanking DNA, sometimes including the intronic IgH enhancer but always lacking any isotype "switch" or constant region (C) DNA. Hybridomas were generated from immunized transgenic mice and the V_H genes encoding their expressed antibodies characterized. Unexpectedly, many of these V_H genes were found to be of transgenic origin. Somatic events had generated hybrid genomic loci containing the transgenic V_H-D-J_H gene and its 5' flanking natural Ig and plasmid vector DNA upstream of endogenous heavy chain C region genes. Thus, V_H-D-J_H transgenes lacking "switch" and C region DNA can recombine with endogenous IgH DNA, leading to the expression of transgene-encoded antibody (1). Transgenic V_H-D-J_H genes in the hybrid loci present in antigen-induced hybridomas display the same frequency, distribution and type of somatic mutation characteristic of conventional Ig loci. Some of the hybrid loci contain only 150 bp of natural Ig, and several kb of plasmid 5' flanking DNA. In contrast, in these same hybridomas V_H genes resident in transgenes that have not recombined with endogenous IgH DNA are never observed to be hypermutated. Some of these unrearranged transgenes contain 2.8 kb of natural DNA flanking the 5' side of V_H, and 2.5 kb of natural DNA flanking the 3' side of V_H which includes the intronic enhancer and a MAR element. Collectively, our findings show that DNA 5' of the V_H promoter is not required, the combination of a V_H promoter, enhancer and MAR are not sufficient, and regions 3' of the intronic IgH enhancer appear indispensable, for hypermutation of V_H transgenes.

1) Giusti, A.M., R. Coffee and T. Manser. 1992. Proc. Natl. Acad. Sci. USA, in press.

F 322 THE GERMINAL CENTER MICROENVIRONMENT DOES NOT ENSURE SOMATIC HYPERMUTATION, Carla Miller, Jaroslav Stedra, Joshy Jacob, Jan Cerny and Garnett Kelsoe, Department of Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, Maryland 21201

The hypermutation and selection of immunoglobulin (Ig) variable (V)- regions that typically accompany the response to thymus (T)-dependent antigens take place in the germinal centers (GC) of secondary lymphoid tissues. Using a novel method of microdissection and PCR amplification, we have studied GC formation, Ig mutation, and the selection of mutants in C57BL/6 and BALB/c mice immunized with the haptens NP [(4-hydroxy-3-nitrophenyl)acetyl] and PC (phosphocholine) conjugated to the immunogenic protein carrier molecules KLH (keyhole limpet hemocyanin) or CG (chicken γ globulin). Interestingly, while all combinations of hapten and carrier induce vigorous GC reactions, the responses to NP fundamentally differ from those to PC. NP-conjugates induce GC formation by 4 days postimmunization and somatic mutants appear on day 8. Ig H chain mutations accumulate at a constant rate until day 14 of the response, thereafter mutation frequencies either remain constant or decline slightly. Initially, mutations are distributed proportionally among the framework and complementarity-determining regions of V_H. However, with time, strong phenotypic selection may be inferred from skewed distributions of mutations and biased R:S ratios. In contrast, neither PC-conjugate elicits somatic mutants even though extensive B cell proliferation occurs in abundant and phenotypically normal GC. The mechanism(s) responsible for this difference are unknown. Presumably, the CG- and KLH-specific T_H cells are competent to support hypermutation for they do so in the anti-NP response. To determine if the GC microenvironment is capable of supporting Ig hypermutation we have doubly immunized mice with NP- and PC-conjugates; preliminary data suggest that in single GC that contain both NP- and PC- specific populations of B cells mutants in the NP-responsive population do arise. Thus we hypothesize that the majority of B cells that respond to (T-dependent forms of) the PC hapten are unable to initiate somatic hypermutation, perhaps because they belong to the $CD5^+$ class of B lymphocytes. This notion is supported by the transfer studies of Rajewsky that identified the B cells responding to challenge with PC-KLH as belonging to the $CD5^+$ compartment.

F 323 GENETIC LOCALIZATION OF THE OF THE MOUSE SCID MUTATION, Robert D. Miller[1,2], Jennifer H. Ozaki[1], David McElligott[3], and Roy Riblet[1], The Medical Biology Institute[1], The Scripps Research Institute[2] and The Salk Institute[3], La Jolla, CA 92037

Mice homozygous for the *scid* mutation lack mature, functional lymphocytes due to an impairment of normal antigen receptor gene rearrangement. This phenotype is a consequence of defective repair of double strand DNA breaks. The *scid* gene was previously mapped to mouse chromosome 16 (MMU16) by linkage to the immunoglobulin light chain lambda genes and the coat color mutation, *mahoganoid*. We set out to localize the *scid* gene further by analyzing the progeny of four, reciprocal interspecific backcrosses between C.B-17 *scid* and MOLF/Ei or CAST/Ei. These mice were typed for the *scid* phenotype and molecular markers from the centromeric end of MMU16. One marker was found which did not recombine with *scid* in 290 meioses. This marker has been used to isolate 5 Yeast Artificial Chromosome (YAC) clones from two YAC libraries containing large inserts of mouse DNA. In addition 2 YACs from a library of human DNA inserts have also been isolated using a homologous marker from the human genome. These YACs are currently being characterized for size and DNA content in an effort to identify the *scid* gene by positional cloning.

F 324 ROLE OF IL-6 IN IgE PRODUCTION BY LPS AND IL-4, Kenji Nakanishi[1], Kiyoshi Yasukawa[2], Nobuhiko Nagai[1], Hatsuhiko Matsumoto[1], Kazunobu Hase[1], Tomohiro Yoshimoto[1], Tadamitsu Kishimoto[3] and Sohei Shinka[1]
[1]Department of Immunology and Medical Zoology, Hyogo College of Medicine, Hyogo, [2]Biotechnology Research Laboratory, Tosoh Corporation, Kanagawa, [3]Third Department of Internal Medicine, Osaka University, Osaka, Japan

We investigated role of IL-6 in LPS and IL-4 driven IgE production by B cells. B cells were purified from 7-9 week old BALB/c mice by Leibson's method. B cells ($3X10^5$ per 0.2 ml per well) were cultured alone or with LPS (20 μg/ml) and/or IL-4 (1,0000 U/ml) for 7 days. IgE and IgG1 productions were obtained when B cells were stimulated with LPS and IL-4. Although IL-4 caused B cells to produce a relatively small amount of IL-6 when measured at 24 h or 48 h after initiation of culture, a larger amount of IL-6 was produced when B cells were stimulated with LPS. Flow cytometrical analysis indicated that resting B cells expressed IL-6R and LPS stimulation enhanced this expression modestly when such expression was analyzed at 24 or 48 h after LPS stimulation. Addition of anti-IL-6R antibody (guinea pig IgG; 20 μg/ml) but not of control IgG antibody almost completely inhibited IgE production and markedly inhibited IgG1 production. Northern blot analysis of B cells at 96 h after stimulation indicated that anti-IL-6R antibody inhibited LPS plus IL-4-dependent accumulation of productive ε transcripts without affecting accumulation of germline ε transcripts. These results indicate that LPS and IL-4 driven IgE production was mediated by endogenous IL-6 which was responsible for causing a change in the ratio of εs/εm mRNA and an increase in the level of εs mRNA which occurs as the cells ininiate IgE production.

F 325 SOMATIC HYPERMUTATION OF A TRUNCATED IMMUNOGLOBULIN TRANSGENE. Andrew Peters#,John Hackett@, Ursula Storb*.# Biochemistry and Molecular Biology,University of Chicago,@Abbott Laboratories,*Molecular Genetics and Cell Biology, University of Chicago.

We have previously demonstrated that an immunoglobulin kappa light chain gene derived from the myeloma MOPC167 is capable of undergoing the somatic hypermutation process as a transgene in mice. In order to locate the cis targeting sequences which direct the process of somatic hypermutation to the kappa gene, the transgene has been truncated. Three kilobases upstream of the promoter have been removed leaving one kilobase upstream, and six kilobases between the C region and the 3' kappa enhancer were also removed. The effects of these deletions upon the transgene's ability to undergo somatic hypermutation will be presented.

Also to be presented are the data from fusions of spleen B-cells from immunized mice to a fusing cell line Ag8, which was transfected with a mutable substrate. The fusion should trap B cells in the process of mutating which then mutate the marker gene in Ag8. Two different substrates were transfected into Ag8, prJ/Rlac which is the kappa 167 gene with the alpha portion of the beta-gal gene as the marker, and the prJ/Vs5 gene which is the kappa 167 gene which contains the supF gene as the marker gene in the variable region. After fusion the genes were plasmid rescued into E. coli and screened visually for mutations. Supported by NIH grants GM38649 and HD 23089. Andrew Peters is supported by NIH training grant GM 07183.

F 326 YAC CLONING THE IGH LOCUS AND FLANKING REGIONS OF DISTAL MOUSE CHROMOSOME 12,
Roy Riblet, Robert Miller, Ruth Epstein-Baak, Jennifer Ozaki, and Condie Carmack, Medical Biology Institute, La Jolla, CA 92037

In the mouse the immunoglobulin heavy chain (Igh) complex locus is composed of at least 200 gene segments encoding the variable, diversity, joining and constant portions of the heavy chain protein. This locus is at or near the distal end of mouse chromosome 12 and is spread over 1-2 centiMorgans of recombination distance. Estimates made from phage and cosmid cloning suggest that the locus covers 1-2 megabases of DNA, a segment of genome suitable for YAC (Yeast Artificial Chromosome) *analysis*. We have been screening the YAC libraries made at Princeton and St. Mary's Hospital Medical School, London, and have identified about 20 clones containing parts of the Igh locus. The largest contig is at least 500 kb and contains all Ch, Jh, Dh and some Vh gene segments. Our goal is to completely clone and define the Igh locus and identify all Vh gene segments. We are also examining the adjacent chromosomal regions to identify closely linked genes that regulate certain immune responses.

F 327 THE EFFECT OF RANDOM MUTATIONS IN THE VH CDR2 ON ANTIGEN BINDING. Marvin B. Rittenberg, Ching Chen and Susan Stevens, Department of Microbiol. and Immunol., Oregon Health Sciences University, Portland, OR 97201.
We previously reported the impact of somatic mutations on the binding functions of the phosphocholine (PC)-specific T15 antibody in the absence of antigen selection (J. Exp. Med. 176: 855, 1992). The VH CDR2 was saturated with random point mutations by in vitro random mutagenesis. and a mutant library prepared which was then placed in the VHS107 of T15. We reported 26/46 mutant antibodies either lost or had reduced ability to bind to PC-protein or the pneumococcus, R36a. This suggested that somatic mutation may cause extensive wastage among B cells during clonal expansion after antigen selection. We have now placed the same mutant CDR2 library in the VH of an antibody with specificity for phenyl-PC (D16) which also uses the VHS107 gene used by T15 but in the context of different DJ and Vκ. 31/43 D16 mutants either lost or had decreased binding for PC-protein. Five D16 mutants displayed increased binding for antigen, a feature not observed in the T15 molecules bearing the same mutations. In five cases mutations that caused loss of antigen binding in T15 did not cause a similar loss in D16 and in one case a mutation (58glu->gln) that did not affect T15 caused a loss of antigen binding in D16. The mutant antibodies were tested for reactivity with the monoclonal TC54 which recognizes an epitope on VHS107 independent of the L chain (Molec. Immunol. 21: 961, 1984). Thirty seven D16 and T15 antibodies bearing the same CDR2 mutations reacted comparably with TC54. Interestingly, although TC54 does not block antigen binding several sets of mutations in CDR2 that resulted in loss of antigen binding also resulted in loss of binding by TC54. That the sensitivity of the S107 CDR2 to deleterious somatic mutations was maintained in two antibodies differing in fine specificity and L chain association supports the hypothesis that B cell wastage may be extensive in cells undergoing mutation during somatic diversification. Supported by NIH grants AI 14985 and AI 26827.

F 328 **NUCLEIC ACID SUBSTRATE FOR BOTH SOMATIC AND GERM-LINE EVOLUTION OF IgV GENES ?** *H.S. Rothenfluh,[1] G.W. Both [2] and E.J. Steele.[1,3] [1]Department of Biology, University of Wollongong, NSW; [2]CSIRO Division of Biomolecular Engineering, North Ryde, NSW; [3]Division of Cell Biology, John Curtin School of Medical Research, ANU, Canberra, ACT, Australia.*
A challenge to understanding the phylogenetic evolution of immunoglobulin variable [IgV] genes is to explain first how they arose and second how both multiple germ-line homologies *and* hypervariabilities can be maintained. Meiotic duplication and segmented gene conversion [1,2] may explain the former but it is problematical whether such processes can fully explain V gene hypervariation. To investigate the mechanism of somatic hypermutation we are defining the nucleic acidsubstrate for this process in murine VDJ genes [3,4]. Data from >20 somatically mutated VDJ genes show that >96% of mutations occur downstream of the transcription start site. However, we and others have also considered the possibility that related mechanisms generate both somatic and germ-line hypervariability [5,6]. Therefore we are also comparing the pattern of mutations in the coding and flanking regions of the VDJ genes with the substitutions occurring in the same regions of related sets of germ-line VH segments. VH genes from the murine J558 family (related to the VH186.2 and VH205.12 genes) have been harvested by PCR from C57BL/6j liver and sequenced. From the 5' flanking region sequences it is clear that duplication-recombination events have occurred during phylogeny. However, the greatest number of nucleotide differences accrued in complementarity-determining [CDR] regions [7].These 'non-random' changes imply that strong positive selection pressure caused amino acid replacements and confirmed previously reported germ-line CDR patterns in mouse [8,9] and human V loci [10,11]. This pattern of substitutions in the germline CDR regions is strongly reminiscent of the pattern observed during IgV somatic evolution. As presently envisaged, the latter process is thought to involve antigen-directed selection via epitopes presented on antigen-antibody complexes at the surface of follicular dendritic cells in germinal centers. It is difficult to visualize these complex mutation-selection events at work in tissues harbouring germ cells. This conceptual difficulty in rationalising the two mechanisms will be discussed **1.** Edelman, G.M. & Gally,J.A [1967] PNAS 57:353.**2.**Baltimore,D.[1981] Cell 24:592.**3.**Steele,E.J.,Rothenfluh,H.S & Both, G. W. [1992] Immunol.Cell Biol 70:129.**4.**Rothenfluh,H.S,Taylor,L et al [1992] In prep. **5.**Bothwell, A.L.M.[1984] In: *Biology of Idiotypes* Ed. M.I.Greene & A. Nisonoff , Plenum, NY. p.19 .**6.** David,V,Folk,N.L.& Maizels,N [1992] Genetics In press. **7.**Wu,T.T & Kabat,E.A. J.Exp.Med [1970] 132:211.**8.** Bothwell ,A.L.M. et al [1981] Cell 24:625. **9.**Givol,D et al [1981] Nature 292:426. **10.**Bentley,D.L& Rabbitts, T.H.[1983] Cell 32:181. **11.**Lautner,-Rieske,A et al [1992] Eur.J. Immunol 22:1023.

F 329 GERMLINE COMLEXITY, RESTRICTION FRAGMENT POLYMORPHISM, AND CODING REGION SEQUENCES OF THE HUMAN VH7 GENE FAMILY, D.B. Rubinstein, R.S. Schwartz, T. Guillaume, A.K. Stewart, Division of Hematology/Oncology, New England Medical Center, Boston, MA 02111, and the University of Louvain, Brussels, Belgium
In studies of expressed gene libraries, a new human VH gene family, VH7, with only 78% overall homology to any previously described VH family has been described in B cell derived from cord blood, as encoding the heavy chain of rheumatoid factor, and in the normal adult. While nearly 90% homologous to the VH1 family through the CDR1 and Framework 2 (FR2) segments, VH7 genes differ substantially (65% homology) from VH1 in the CDR2 and FR3. Because of this, full-length coding region probes are unable to distinguish between the two groups. We have used an oligonucleotide probe to the most family-specific gene segment, the 5' end of FR3 of VH7, (nucleotides 200-227), to dissect VH7 germline complexity, and to isolate genomic cDNA library clones for sequencing. We conclude that. (1) the VH7 family is a small one, with three monomorphic and one polymorphic fragment on EcoRI-digested genomic DNA. (2) the four VH7 fragments also hybridize with the VH1 coding region probe. Although co-migrating VH1 and VH7 genes cannot be ruled out, the VH1 family is likely to be somewhat less complex than previously estimated (3) Direct PCR-amplified genomic sequencing from four individuals identified 7 sequences: Gene 7A.4, identical with the previously-identified VH7 gene V1-4.1b, 2 sequences differing from 7A.4 in single, non-repetitive differences, possibly PCR-induced, and 4 pseudogenes. (4) The expressed VH7 genes described to date are >96% homologous to their germline counterpart, the single 7A.4 gene or the small group of highly (>99.7%) homologous genes; the differences seen are the result of somatic mutation.(5) VH7 gene(s) is among the very limited number of VH genes encoding the heavy chain of polyreactive antibody with both anti-self and exo-antigen activity, and selectively expressed both in the pre-immune fetal repertoire and in the adult repertoire. This overlap in gene use suggests that any distinction between unique fetal and adult VH gene repertoires may be arbitrary.

F 330 STUDIES OF RAG PROTEINS GENERATED IN A TISSUE CULTURE SYSTEM.

Moshe Sadofsky and Martin Gellert, Laboratory of Molecular Biology, NIDDK, Bethesda Md. 20897

The mechanism of V(D)J recombination is essentially unknown at the protein level. The proteins RAG-1 and RAG-2 are involved in this process, but their role, direct or indirect, remains to be established. Toward this end we have been attempting the biochemical characterization of these proteins. One approach has yielded preliminary results. Modified versions of these genes bearing an epitope tag at the carboxy terminus can be introduced into tissue culture cells. These modified genes are still functional in the fibroblast transfection assay. The proteins that are subsequently produced are uniquely identified and can be isolated by immunoprecipitation. The RAG-1 product is soluble and appears to have a short half-life. It is hoped that this approach will illuminate the biochemical activities of these proteins.

F 331 TRANSMEMBRANE SIGNALING DEFECTS IN WISKOTT-ALDRICH SYNDROME PATIENT B CELLS REVEAL A DICHOTOMY IN THE SIGNALING PATHWAYS WHEREBY PLATELET-ACTIVATING FACTOR AND SURFACE IG RECEPTOR ENGAGEMENT ELICIT INCREASES IN CYTOSOLIC CALCIUM, Katherine A. Siminovitch, Hans-Uwe Simon and Gordon B. Mills, Departments of Medicine, Immunology and Obstetrics, University of Toronto, Toronto, Ontario M5S 1A8.

To investigate the basis for impaired responses to polysaccharide antigens in Wiskott-Aldrich syndrome (WAS) patients, we have examined Epstein-Barr virus (EBV)-transformed B cells derived from these patients with regards to their capacity to transduce early activation signals via the surface immunoglobulin (sIg) and platelet-activating factor (PAF) receptors. Ligation of sIg was found to induce markedly less increases in proliferation, intracellular calcium concentration ($[Ca^{2+}]_i$), inositol phosphates and tyrosine phosphorylation in WAS compared to control EBV-B cells. In contrast, while PAF also induced much less tyrosine phosphorylation in the WAS compared to control cells, PAF-induced increases in $[Ca^{2+}]_i$ were essentially normal in the WAS cells. Also in contrast to anti-Ig antibody, PAF did not induce tyrosine phosphorylation of PLC-γ1 in normal or WAS EBV-B cells and PAF-mediated increases in $[Ca^{2+}]_i$ were not affected by pretreatment with tyrphostin tyrosine kinase inhibitor. These results provide evidence for a defect at, or proximal to, both PAF and anti-Ig-induced tyrosine phosphorylation in WAS EBV-B cells and suggest that tyrosine phosphorylation is not required as an intermediary in transduction of PAF-initiated calcium mobilizing signals. These findings also suggest that the pathways linking engagement of the sIg and PAF receptors to increases in B cell $[Ca^{2+}]_i$ are distinct and that further characterization of signaling defects in WAS EBV-B cells may be of value in delineating the basis for lymphocyte dysfunction in WAS as well as the molecular mechanisms mediating the early stages of normal B cell activation.

F 332 A SEARCH FOR THE HUMAN XLA (*X-LINKED AGAMMAGLOBULINEMIA*) GENE USING YACs AND COSMIDS TO ISOLATE cDNA CLONES MAPPING AROUND Xq22.

C.I. Edvard Smith, Igor Vorechovsky, Paschalis Sideras, Lennart Hammarström, J Holland, David R Bentley and David Vetrie.
Center for BioTechnology, Karolinska Institute, NOVUM, S-14157 Sweden and Division of Medical and Molecular Genetics, Guy's Hospital, Guy's Tower, London SE1 9RT, UK.

X-linked agammaglobulinemia (XLA) was previously mapped using genetic linkage analysis to Xq22. No recombinants have to date been reported between the XLA locus and the marker DXS178 in over 30 informative meioses. Two overlapping yeast artificial chromosomes (YACs), which cover a region of approximately 1000 kb around DXS 178, and form part of a larger YAC contig, were hybridised to an ordered cosmid library constructed from a human fibroblast cell line with the karyotype 49,XXXXX. Positive cosmid clones were gridded in high density arrays onto nylon filters and rescreened with a series of YACs, YAC end probes and additional markers from the region. A 640 kb YAC hybridising to DXS178 (p212) was used for direct selection of PCR-amplified cDNA from two cDNA libraries. Enriched clones were hybridised back to cosmids and positive clones were hybridised to the subcloned cDNA libraries. Candidate cDNA clones have been isolated, sequenced and are currently being used for the analysis of the corresponding genes in patients with XLA.

F 333 DISTINCT ONGOING REARRANGEMENT PROCESSES IN CHILDHOOD PRECURSOR-B ACUTE LYMPHOBLASTIC LEUKEMIA. E.J.Steenbergen, O.J.H.M.Verhagen, E.N. van Leeuwen, A.E.G.Kr. von dem Borne, C.E.van der Schoot. Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam The Netherlands.

IgH-gene junctional regions have found widespread application as clonal marker for the monitoring of residual disease in B-cell malignancies. Recent reports have shown the relative instability of these rearrangements in Precursor-B ALL, although exact rearrangement processes within single patients remain obscure. The combined use of V_H-family specific FR1-primers and a consensus FR3-primer for VDJ-PCR, allowed us to perform a very detailed analysis of leukemic VDJ-rearrangements in 3 cALL patients (diagnosis) and 1 Ph^+ CML patient in blast crisis (diagnosis and relapse) who presented with multiple rearranged bands on IgH southern blot analysis. cALL patient A revealed 3 related clones which were probably the result of V to DJ joinings. In cALL patients B and C, V_H-gene replacement events generated exclusively 2 relatively large groups of related clones suggesting that the transformational event took place in a single VDJ-rearranged progenitor. In patient B all subclones probably originated from the predominant initial clones in a selection independent way, whereas in patient C there had been a selection for exclusively in frame rearrangements. Ph^+ CML patient D, revealed a whole set of unrelated and related (V to DJ-joinings) clones at both diagnosis and relapse, probably originating from an unrearranged progenitor cell. Our data suggest that multiple distinct selection dependent or independent rearrangement processes exist in childhood Precursor-B ALL, involving a single rearrangement mechanism in every patient.

F 334. DIFFERENT PATTERNS OF V_H GENE EXPANSION IN HUMAN SPLEEN AND PERIPHERAL BLOOD B LYMPHOCYTES, William Varade, Ann Kittelberger, Elides Marin, Richard Insel, Piotr Kraj, and Leslie Silberstein, Dept of Pediatrics, U Rochester Sch of Medicine, Rochester, NY 14642; Dept of Pathology, U Pennsylvania, Phila, PA 19104.
Cloning and sequencing of human rearranged immunoglobulin heavy chain variable region (V_H) gene transcripts of the small V_{H6} family from human spleen showed a high frequency of relatedness between clones. In 72 unique V_{H6} clones sequenced, there were only 43 rearrangements. Multiple unique clones arose from the same rearrangement and were expressed with IgM, IgG, and IgA isotypes, with 2 of the 3 isotypes, or with only one isotype. These findings suggest that isotype switching and divergence by somatic hypermutation of proliferating precursors, presumably in germinal centers, occurred in the spleen. In contrast, studies of $V_HDJC\gamma$ gene expression in peripheral blood lymphocytes (PBL) by anchored PCR showed a distribution of V_H families close to that expected in one instance (16%V_{H1}, 12%V_{H2}, 48%V_{H3}, 22%V_{H4}, 2%V_{H5}, and 0%V_{H6}) while a relative expansion of the V_{H4} family was seen in 2 others (74% and 65% of clones). The $V_HDJC\mu$ library from the last individual also showed skewed V_{H4} usage (44%). Sequencing of $V_{H4}DJC\gamma$ clones of the first case showed all had unique rearrangements with unbiased expression of V_{H4} gene family members. Nine clones were randomly selected from 101 $V_{H4}DJC\gamma$ clones from one of the latter two individuals and sequenced. All had an identical nucleotide sequence that appeared to be derived from the $V_{H4.11}$ germline gene. Screening of 52 more of the $V_{H4}DJC\gamma$ clones by CDR3 size suggested a minimum of 3 different rearrangements present, but 88% appeared to express the sequenced predominant rearrangement. Thus, it appears that in the spleen, expansion of V_H gene families is often associated with divergence via somatic hypermutation of a few rearrangements during proliferation, while in the peripheral blood, expansion of individual previously mutated clones can be found that gives rise to skewed V_H family expression in response to environmental stimuli.

F 335 A CLONED MURINE B LYMPHOMA HAS GENERATED VARIANTS WITH SOMATIC MUTATIONS IN H AND L CHAIN V REGIONS, Carol Victor-Kobrin and Michael Kuehl, NCI-Navy Medical Oncology Branch, NIH, Bethesda, MD 20889-5105
We have previously shown that a cloned murine B lymphoma (K46.056) co-expresses μ and γ_{2a} H chains as well as κ and λ_1 L chains. The K04.2 cell line was isolated from K46.056 by syngeneic passage through Balb/c mice, and subcloned. All 12 subclones continued to express μ, γ_{2a}, κ and λ_1 mRNAs. Sequences of clones derived by PCR from the K04.2 line demonstrated that the μ and γ_{2a} V_H regions are comprised of different V, D, and J segments, indicating separate VDJ rearrangements for the μ and γ_{2a} H chains. This analysis also demonstrated 3 different V_H μ sequences, with each subclone expressing only one of these 3 sequences. To determine whether there were differences in $V_H\gamma_{2a}$, V_κ and $V_{\lambda1}$ sequences in the different subclones as well, each of these regions was amplified by PCR, analyzed by SSCP and sequenced using PCR cycle sequencing analysis. Different subclones expressed one of two $V_{\lambda1}$ sequences and one subclone expressed two different V_κ sequences. All subclones expressed the same $V_H\gamma_{2a}$ sequence. In summary, this unusual B lymphoma, which co-expresses κ and λ_1 L chains as well as unrelated μ and γ_{2a} H chains has generated somatically mutated variants at three of the four expressed Ig loci.

F 336 INDUCTION OF RESPONSIVENESS IN LYMPHOCYTES OF IMMUNODEFICIENT PATIENTS' CELLS IMPLANTED WITH FOREIGN PLASMA MEMBRANES. Israel Zan-Bar[1], Dalia Levy[1], Yael Porat[1], Israel Pick[2], Michael Schlesinger[3], Amir Tanay[4], and Zeev T. Handzel[5]. [1]Sackler Faculty of Medicine, Tel Aviv University, [2]Beilinson Medical Center, [3]Barzilai Hospital, [4]Wolfson Hospital, and [5]Kaplan Hospital, Israel.
Selected immunodeficiency and common variable immunodeficiency are acquired syndromes associated with inability to produce a normal quantity of one or all the Ig isotypes. The induction of Ig secretion by ID patients' cells treated with retinoic acid indicates that the defect/s are probably connected with the membranal receptors or with the enzymes located in the plasma membranes (PM) which operate in the signaling cascades. In order to test this hypothesis, B cells obtained from ID patients were implanted with foreign PM and were stimulated with "pusher" specific to the foreign implanted receptors. The insertion was carried out by fusion of PM to the cells via intact non infectious Sendai virus. In this paper we report the successful in vitro repair of Ig and proliferation responses in ID cells. ID and normal PBL cells were implanted with murine splenic cells derived PM. The modified cells were then stimulated with LPS, SAC, PHA, PWM and with murine IL-2,4,5,6, and their Ig and proliferative respones were examined. The results demonstrate that insertion of PM reconstituted the IgM and IgG response to SAC, PWM and LPS in 3/6 and the IgA response only in 1/6 modified ID patients. Upon stimulation of the modified cells almost the same amount of IgM and IgG secreted by normal cells was measured. The IgA response in the treated immunoimpaired cells reached only half of the magnitude obtained in normal donor cells. In conclusion, insertion of foreign receptors to cells taken from ID patients, and stimulation of the modified cells with reagents specific to the inserted receptor endows immediate activation and proliferation and even leads to the repair of Ig secretion. Efforts are now being made to examine whether this system can also be used for the induction of differentiation and long lasting memory cells.

F 337 THE ISOLATION OF IgG SWITCH-VARIANTS FROM A HUMAN IgM-BEARING HYBRIDOMA LINE

David F.H. Zhou, Tang Shao, MinYi Gu, and Marc Nasoff, OncQuest, Division of Specialty Laboratories Inc., San Diego, CA 92121

Following in vitro or in vivo immunization, spontaneous IgG secretory switch variants can be isolated from murine IgM-bearing hybridomas at a frequency of 10^{-6} - 10^{-7}. Analysis of these switch variants revealed that the antigen-binding specificity is conserved despite the fact that point mutations could be detected in the DNA sequences within the VH region. The ability to change the isotype of antibodies from IgM to IgG in vitro without altering the antigen-binding specificity could prove to be of great clinical value.

Here, we report the development of a sensitive screening method which has enabled us to isolate several IgG1/IgG4 switch variants from a human IgM bearing hybridoma line, SAL-1 (rheumatoid factor, lambda/mu). IgG secreted by these switch variants competes with IgM produced by the parental line for binding to human immunoglobulin FC fragment, suggesting that the switch variants have identical or very similar antigen-binding properties. In addition, the switch variant and parental lines appear to be identical in that they all secrete lambda light chains. The spontaneous switch rate of the human SLA-1 hybridoma occured at a frequency of 10^{-7}- 10^{-8} which is lower than that reported for murine hybridomas.

To determine whether the VH region of these switch-variants is identical to their parental IgM, or if further affinity maturation has occured in the absence of antigen presentation, we have also isolated and sequenced the VH region gene from the parental IgM and several IgG switch variants. The results of these experiments will be presented.

F 338 THE RATES OF V AND C REGION MUTATIONS IN HYBRIDOMA CELL LINES, Minghua Zhu, Jennifer Rabinowitz, Nancy Green, Barry Kobrin and Matthew D. Scharff, Department of Cell Biology, Albert Einstein College of Medicine, Bronx, NY10461

We have examined the rates of V and C region mutations in hybridoma cell lines. pSV2neo plasmids containing an IgM heavy chain gene with nonsense mutations in either V or C were transfected into hybridoma cell lines. Reversions of the nonsense mutations were analyzed using the ELISA spot assay and fluctuation analysis. The rates of V and C region mutations varied in different cell lines and amongst clones in each cell line. In any one cell line, the rates of V and C region mutations were roughly comparable. For example, in the 2C3 hybridoma, the rate of V region mutation was 10^{-6}/bp/generation while the rate of C region mutation was 10^{-5}/bp/generation. It was possible to identify subclones undergoing higher rate of C region mutation. A few revertants were sequenced and it was determined that those reversions were due to point mutations.

B Cells in Marrow Transplantation and Disease

F 400 EVIDENCE THAT THE CLONOGENIC CELL IN MULTIPLE MYELOMA ORIGINATES FROM A SOMATICALLY MUTATED AND HEAVY CHAIN SWITCHED B CELL, Marleen H.C. Bakkus, Ivan Van Riet, Ben Van Camp and Kris Thielemans, Department of Hematology-Immunology, Medical School of the Vrije Universiteit Brussel, Brussels, Belgium.

There is still much controversy about the precursor cell type in multiple myeloma. Some authors claim that it is a pre-B cell, others state that it is a B cell or plasmablast. By analyzing the expressed Ig heavy chain VDJ sequences in multiple myeloma plasma cells, we have shown that these myeloma Ig genes contain non-random somatic mutations, like in memory B cells, and that there is little or no intraclonal variation (Bakkus M *et al*, Blood, *in press*, 1992). In order to find out whether precursor myeloma cells are already present in the B cell compartment we have looked for expression of these myeloma Ig sequences in purified B cells from bone marrow or peripheral blood in 5 myeloma patients.

B lymphocytes were separated from plasma cells based on the expression of CD19 and HLA class II using immunomagnetic beads. The expressed Ig sequences were amplified by RT-PCR using VH family specific leader primers and isotype specific primers (for Cμ, Cγ or Cα). Myeloma-specific Ig sequences were detected by a myeloma-specific CDR3 probe, based on the Ig-sequence derived from the plasma cells. With this RT-PCR technique using patient specific CDR3 probes we can usually detect one tumor cell in 10,000-100,000 normal blood or bone marrow cells.

The VH-nucleotide sequences, expressed by the myeloma plasma cells, were also found in the purified B cell fractions but only in those B cells that expressed the same isotype as the myeloma plasma cells. Even after enrichment of the IgM-expressing peripheral blood B cells by the use of imunomagnetic beads coated with anti-IgM we weren't able to detect the myeloma specific Ig sequence in this fraction, using this very sensitive technique.

These findings, together with the fact that myeloma-Ig genes contain somatic mutations without intraclonal variation, implicate that in multiple myeloma clonal proliferation takes place in a B cell that has already switched its Ig isotype and has already gone through the phase of somatic hypermutation.

F 401 HUMAN HIGH AFFINITY ANTIBODIES ELICITED BY A FOREIGN ANTIGEN DISPLAY A "FETAL-LIKE" RESTRICTED V_HIII GENE UTILIZATION AND ARE SOMATICALLY HYPERMUTATED, Paolo Casali, Yuji Ueki, Nagaradona Harindranath, and Hideyuki Hikematsu, Department of Pathology and Kaplan Comprehensive Cancer Center, New York University School of Medicine, New York, New York 10016.

The analysis of the genetic composition of human antibody responses has been hampered by the difficulty in generating human mAbs of predetermined class and specificity. Using B lymphocytes from 3 healthy subjects vaccinated with inactivated rabies virus vaccine, we generated 9 human mAbs binding to rabies virus and analyzed the genes encoding their V_H regions. Six mAbs (5 IgG1 and 1 IgA1) were monoreactive and displayed high affinities for rabies virus Ags. The remaining 3 mAbs (IgM) were polyreactive and low affinity. Seven mAbs (3 IgG1, the IgA1 and the 3 IgM) utilized V_H genes of the V_HIII family. The remaining 2 IgG1 mAbs utilized genes of the V_HI and V_HIV families. Of the 7 expressed V_HIII family genes, 3 were similar to the germline V_H26c gene, 2 to the germline 22-2B gene, 1 to the germline H11 gene, and 1 to the germline 8-1B gene. The expressed V_HI and V_HIV genes displayed sequences similar to those of the germline hv1263 and V71-4 genes, respectively. The V_H genes of all but one mAb (mAb55) resembled those that are predominantly expressed by $C\mu^+$ clones in human fetal liver libraries. When compared with known germline sequences, the V_H genes of the rabies virus-binding mAbs displayed variable numbers of nucleotide differences. That such differences resulted from a process of somatic hypermutation was formally demonstrated (by analyzing DNA from PMN of the same subject whose B lymphocytes were used for the mAb generation) in the case of the V_H gene of the high affinity (anti-rabies virus glycoprotein) IgG1 mAb57 that has been shown to efficiently neutralize the virus *in vitro* and *in vivo*. The distribution, mainly within the CDRs, and the high R/S ratio of the mutations were consistent with the hypothesis that the mAb57-producing cell clone underwent a process of Ag-driven affinity maturation through clonal selection. The D gene segments of the rabies virus-selected mAbs were heterogeneous and, in most cases, flanked by significant N segment additions. The J_H segment utilization was unbalanced and reminiscent of those of the adult and fetus. Four mAb utilized J_H4, 2 J_H6, 2 J_H3, and 1 J_H5; no mAb utilized J_H1 or J_H2 genes. The present data suggest that the adult human Ig V gene assortment expressed as the result of selection by a proteinic mosaic Ag is more restricted than previously assumed and resembles that of the putatively unselected adult B cell repertoire and the unselected $C\mu^+$ cell repertoire of the fetus. They also document somatic Ig V gene hypermutation in human B cells producing high affinity antibodies.

F 402 ALLOGENEIC BONE MARROW TRANSPLANTATION (alloBMT) for Lymphoproliferative Disorders (Lymphoma and Chronic Lymphocytic Leukemia), Fyles G, Smith A, Kolbasnik J, Selbach J, Lowsky R, Messner H, Lipton J, Minden M, Atkins H, Meharchand J, Ontario Cancer Institute, Toronto, and University Hospital, London, Ontario, Canada.

Seventeen patients with Non-Hodgkin's lymphoma (predominantly low grade transformed and intermediate grade) and 2 with advanced chronic lymphocytic leukemia (CLL) underwent alloBMT at one of 2 BMT centres. The median age was 44 years (range 30-48). All patients with lymphoma had been extensively pretreated prior to BMT (average no. of previous regimens = 3) and all had received anthracyclines. All lymphoma patients were ineligible for autologous BMT because of marrow involvement but otherwise satisfied the criteria for autoBMT. The 2 patients with CLL were resistant to chlorambucil. One was transforming to prolymphocytic leukemia at BMT. All received marrow from an HLA identical relative except for 1 patient who received a syngeneic BMT.

All patients were prepared with busulfan 16 mg per kilo over 4 days and cyclosphamide 120 mg per kilo over 2 days and all received a short course (3 doses) of methotrexate and cyclosporin A as graft-versus-host disease prophylaxis.

In this group of 19 patients there were 4 transplant related deaths (3 early and 1 late). The longest follow up is 3 years. At a median follow up of 2 years no relapses have occurred in the lymphoma group. The 2 patients with CLL have normal blood counts and no lymphadenopathy. These patients are currently being assessed for minimal residual disease using molecular probes.

F 403 INHIBITION OF MURINE B CELL DEVELOPMENT AS A RESULT OF SELECTIVE EXPANSION OF DONOR T CELLS DURING THE GRAFT-VERSUS-HOST REACTION, Beth A. Garvy, Robert B. Levy, Brian L. Hamilton*, Jeanne M. Elia, and Richard L. Riley, Department of Microbiology & Immunology, University of Miami School of Medicine, Miami, FL 33101 & *Children's Hospital, Oakland Research Institute, Oakland, CA 94609

B cell regeneration was examined over 6 weeks using a model of bone marrow (BM) transplantation in which donor (B10.D2) and recipient (BALB/c) are MHC matched ($H\text{-}2^d$), but non-MHC minor antigen (Mls-2,3) mismatched. Mice transplanted with allogeneic (T cell depleted) BM reconstituted pro-B cells ($S7^+B220^+$) within 7 days followed by pre-B cells ($S7^-B220^+$) by day 14 and B cells at day 21. In contrast, mice receiving allogeneic BM + T cells (GvH mice) did not develop significant numbers of B-lineage cells until 21 days after transplant. This corresponded to the inability of BM from GvH mice to respond to IL-7 (IL-7 CFU) at the early time points. The delay in B cell development correlated with the expansion of Mls 2^a,3^a reactive $V\beta3^+$ T cells in the spleen and BM of GvH mice which represented 40-70% of T cells present in both tissues 7 days after transplant. By day 21 $V\beta3^+$ T cells were less than 20% of total T cells in the spleen or BM. Co-culture of GvH BM cells with equal numbers of normal BALB/c BM cells resulted in a 2-10 fold reduction in IL-7 CFU. This inhibition was consistently observed when day 7 GvH BM was used, but was not present when day 21 BM was used in the co-cultures. The inhibition could be reversed by removing T cells from the GvH BM or treating the co-cultures with anti-γ IFN monoclonal antibody. Additionally, $V\beta3^+$ T cells sorted by FACS also suppressed BALB/c IL-7 CFU formation by 2-4 fold. This inhibition was also reversed by anti-γ IFN antibody. These data suggest that the delay in B cell development observed during the GvH reaction is due to the secretion of γ IFN presumably from activated $V\beta3^+$ T cells. (Supported by AI 30997 to BLH and CHORI subcontract to RLR).

F 404 AGE-RELATED CHANGES IN V_H GENE USE, Edmond A. Goidl, X. Helen Chen, Dan H. Schulze, Department of Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, Maryland 21201

The heterogeneous immune responses to 2,4,6-trinitrophenylated (TNP) TI-2 and TD antigens are characterized by stochastic use of V_H genes in the young adult mouse (2-4 mo). We have studied the response to TNP conjugated *Brucella Abortus* in the aged C57B/6NNIA (18-24 mo) as compared to that of young adult mice. Data is presented supporting the concept that for the highly heterogeneous anti-TNP antibody system, the aged mount a pauciclonal response. In six different fusions, only V_{H10} and S107 were identified among monoclonal IgM anti-TNP hybridomas (Mabs). A majority of these Mabs are cross-reactive to other antigens including self-antigens. Other antibody systems that are pauciclonal in the young adult (*ie*: the anti-phosphoryl choline antibody in the BALB/C mouse) tend to become more diversified in their V_H gene use in the aged. The reverse phenomenon is seen in the age-associated changes of the anti-TNP antibody repertoire. We are presently investigating the role of T cell in the regulation of the B cell repertoire. [Supported in part by USPHS Grants R01-AG-10118 (to EAG) and P01-AG-10207 (to DHS).]

F 405 CLL IN THREE SISTERS: PREFERRED USAGE OF SPECIFIC IG GENE SEGMENTS, Hakim I., Brok-Simoni F., Grossman Z., Amariglio N., Mor O., Gokel E., Kneller A., Hulu N., Ramot B., Ben-Bassat I., Rechavi G., Institute of Hematology, The Chaim Sheba Medical Center, Tel Hashomer 52621, ISRAEL

Familial Chronic Lymphocytic Leukemia (CLL), although rare, is the most common familial leukemia. The study of such families may increase our understanding of CLL in general. We studied a family where three sisters, two of them identical twins as proven by microsatellite analysis, developed CLL. Immunophenotyping of the leukemic cells revealed surface IgM,k in all three cases. Subgroup specific PCR demonstrated that the rearranged Ig heavy chain genes of the malignant cells of all three contained V_HIII gene segments. PCR cloning enabled the detailed analysis of both light and heavy chain gene sequences. The heavy chain gene of all three contained highly homologous V_HIII gene segments. The D segments of the twins were identical and differed from that of the third sister. One of the twins shared J_H4 with the non-twin sister while the second twin used a J_H3 segment. The malignant cells of all three had a rearranged V_KIII with either J_K1 or J_K3 which are almost identical. It can be speculated that on a specific genetic background, the proliferation of CD5+ cells trigered by common antigens encountered in early development (possibly in utero), created an expanded cell population at risk for the development of additional genetic changes resulting in malignant transformation. Familial CLL may therefore represent the combination of a suitable genetic milieu with environmental trigers. We searched for relevant mutations in the p53 gene, which has recently been implicated in some CLL cases, but so far none have been identified.

F 406 THE DISEASE PROGRESSION IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) IS PARALLELED BY AN INCREASING DYSFUNCTION OF THE LYMPHOCYTIC β_2-ADRENERGIC SIGNAL TRANSDUCTION. Michael Hallek, Torsten Kamp, Bernhard Liebl, Uschi Göhly, Ekkehard Haen, and Bertold Emmerich, Medizinische Klinik, Klinikum Innenstadt, Universität München, W-8000 München, Germany.

The second messenger 3':5' cyclic adenosine monophosphate (cAMP) mediates considerable antiproliferative effects in lymphoid cells. We have shown previously that the β_2-adrenergic regulation of intracellular cAMP levels is severely impaired in CLL lymphocytes. In an attempt to further elucidate the biochemical mechanism of this signaling defect, we investigated the β_2-adrenergic signal transduction of mononuclear cells (MNC) in 27 CLL patients (pts; 10 female, 17 male; mean age ± S.D. 62 ± 9 yrs) as compared with 10 healthy adults (3 f, 7 m; 47 ± 9 yrs). Disease progression was assessed by clinical staging according to the Binet classification: 13 pts were in stage A, 7 in st. B, 5 in st. C, and 2 had complete remissions. Saturation binding experiments with [^{125}I]cyanopindolol revealed a stage-dependent decrease of the β_2-adrenergic receptor (β_2AR) density on MNC of CLL pts ($P<0.05$; ANOVA), without significant change of the receptor affinity (Kd; Table 1). Intact MNCs were then stimulated with different agents that induce the cellular cAMP production at different steps along the β_2-adrenergic signal transduction pathway: The effect of the β_2AR agonist isoproterenol (ISO; 10^{-4} mol/l) showed a significant, stage-dependent decrease ($P<0.05$, ANOVA; Table 1). Cholera toxin (CTX; 10^{-6} g/ml), an activator of the stimulatory G protein (G_s), tended to induce less cAMP accumulation in stage C than at lower stages or in controls (Table1). The response to forskolin (FK; 100 µmol/l), an activator of the adenylate cyclase (AC), was considerably impaired in CLL, without further decrease with disease progression (Table 1).

Table 1: β_2-adrenergic signaling in CLL

Parameter	controls	CLL			
	-	stage A	stage B	stage C	all
β_2AR/cell	1268.2	371.3	236.9	141.4†	312.8*
β_2AR affinity (Kd, pmol/l)	16.6	4.6	4.2	5.2	4.4
cAMP accumulation#					
basal	9.4	10.3	10.6	2.7†	8.8
ISO (10^{-4} mol/l)	15.9	14.0	13.7	3.1†	12.1
CTX (10^{-6} g/ml)	70.1	95.2	70.7	33.2	73.4
FK (10^{-4} mol/l)	188.6	18.6	21.0	14.3	17.9*

All results are mean values. †$P<0.05$ (ANOVA) for stage-dependent difference; *$P<0.05$ (Mann-Whitney) for differences between pts and controls. #pmol cAMP/10^6 cells.

These results suggest a complex and significant impairment of the β_2-adrenergic signaling in CLL cells which increases with disease progression. This impairment seems to be particularly prominent at the post-receptor level. Studies to characterize the molecular basis of these changes are currently underway.

F 407 A HERITABLE B LYMPHOCYTE DEFICIENCY IN A/WySnJ MICE: SEGREGATION ANALYSIS OF MASTOCYTOSIS AND B CELL DEFECTS. Colleen Hayes†, Jessica Gorski†, Daniel Malone‡, and Cory Teuscher#, Departments of †Biochemistry and ‡Rheumatology, University of Wisconsin, Madison, WI 53705, and #Department of Microbiology, Brigham Young University, Provo, UT 84602.

The A/WySnJ strain mice have a unique, heritable B cell defect. Compared to the very highly related A/J subline, the A/WySnJ mice have a striking peripheral B cell deficiency, and they make very poor IgG responses, but good IgM responses. The mechanism underlying the A/WySnJ B cell defect is unknown. The defect could interfere with a homeostatic mechanism that maintains peripheral B cells, or impair a mature B cell differentiation step, or impede a late-arising B cell lineage, or some combination of these mechanisms. We recently discovered that A/WySnJ mice have excessive numbers of splenic mast cells and basophils. Experiments are in progress to determine whether widespread mastocytosis is a characteristic trait of the A/WySnJ strain, and to test the hypothesis that a single variant locus controls B cell deficiency and excess splenic mast cells and basophils in (A/J X A/WySnJ)F2 mice. Moreover, we are studying defined genetic polymorphisms in strain A sublines to determine whether one of the polymorphisms could be linked to the hypothesized variant B cell deficiency locus. The results of our mast cell analysis and segregation and linkage studies will be reported.

F 408 GROWTH REGULATION OF IgG SECRETING MYELOMA CELLS BY CD16$^+$ HOST LYMPHOCYTES. Richard Hoover, Cynthia Lary, Robert Page, Jacki Kornbluth, Joshua Epstein, Jeffrey Woodliff and Bart Barlogie. Arkansas Cancer Research Center, University of Arkansas for Medical Sciences, Little Rock, AR.

Previous studies in murine myeloma have shown that mice with plasmacytomas develop an expansion of circulating, CD8$^+$ lymphocytes bearing Fc Receptors (FcR). The isotype-specificity of the FcR is concordant with the isotype of the Ig produced by the myeloma tumor cells. Soluble FcR (sFcR) are produced by the FcR$^+$ T cells and can bind to sIg on plasmacytoma cells. Subsequent to binding of sFcR, tumor growth, as well as Ig secretion, is inhibited. We examined 28 newly diagnosed patients with multiple myeloma (MM) for the expression of CD16$^+$ cells. 18/20 newly diagnosed patients with IgG myeloma showed an increase in CD16$^+$ cells in the peripheral blood (mean fold increase ± SEM: 7.0 ± 1.1). 0/4 newly diagnosed patients with IgA myeloma, 0/1 with IgD myeloma and 0/3 with light chain disease showed expansion of CD16$^+$ cells. Soluble CD16 (sCD16) was isolated from patient lymphocytes and was shown to be structurally identical to sCD16 isolated from control cells. sCD16 was shown to suppress growth and Ig secretion by the human IgG myeloma cell line, ARH-77. In addition, sCD16 was shown to suppress the transcription of the c-myc gene as well as the IgH and IgL genes. Incubation of IgG-producing myeloma cell lines with sCD16 for >48 hours resulted in apoptotic cell death. These results suggest that patients with newly diagnosed IgG MM produce a marked expansion of IgG-FcR$^+$ suppressor T cells and sFcR produced by the suppressor cells can inhibit the growth of myeloma tumor cells.

F 409 ROLE OF CD9 IN B CELL SUSCEPTIBILITY TO LYSIS BY LYMPHOKINE ACTIVATED KILLER (LAK) CELLS. Jacki Kornbluth, Suhair Shallal and Priscilla Gray, Departments of Medicine and Microbiology and Immunology, University of Arkansas for Medical Sciences, Little Rock, AR 72205.

In studying the anti-tumor cytolytic activity generated from the bone marrow and peripheral blood of myeloma patients, it was observed that myeloma tumor cells are relatively resistant to cell-mediated lysis. Susceptibility to lysis could be increased five to 20 fold by culturing myeloma cells with either pokeweed mitogen (PWM), all-trans retinoic acid (RA) or IL-4. This increase in susceptibility was maximal after 48 hours of culture and associated with alterations in cell surface phenotype. A consistent phenotypic change induced by these agents was induction of CD9 on the myeloma cells. CD9 is normally expressed on pre-B cells and is lost upon B cell differentiation. Anti-CD9 antibody treatment of PWM-treated myeloma cells resulted in modulation of CD9 from the cell surface and a concomitant decrease in cell susceptibility to LAK-mediated lysis. Exposure of PWM-treated myeloma cells to CD9 antisense oligonucleotides completely abrogated the PWM-induced sensitivity to lysis. These results strongly suggest a role for CD9 in LAK-B cell interactions. Clinical trials in which RA and IL-4 are being examined for efficacy in treatment of myeloma are currently being performed at our institution. The effects of in vivo administration of IL-4 and RA on altering myeloma cell susceptibility to lysis are under investigation.

F 410 ANALYSIS OF THE EXPRESSED V_H-GENE REPERTOIRE IN RESTING AND NATURALLY ACTIVATED B LYMPHOCYTES IN HUMAN ADULTS, Inger Lundkvist[1], Valter Hillörn[2], Dan Holmberg[2] and Genoveva Davidkova[1], [1]Dept. of Clin. Immunology, Karolinska Institute at Huddinge Hospital, 141 86 Huddinge, and [2]Dept. of Applied Cell and Molecular Biology, Umeå University, 901 87 Umeå, Sweden

We are interested in the mechanisms that select and regulate B cell repertoires in humans, in particular in relation to physiological and pathological autoreactivity. Autoimmune responses depend on the expressed repertoires, and it is therefore important to establish whether these constitute a random representation of the potential repertoire or are the result of selection of certain specificities. We have addressed this question by comparing the V_H-gene utilization patterns in the available repertoire (i.e. the different V-regions expressed by small resting immunocompetent B cells) and the actual repertoire (which includes the V-domains that are used by large activated B lymphocytes). For this purpose we have used a non-radioactive *in situ* hybridization technique that allows the detection of V_H-gene expression at a single cell level. Mononuclear cells from peripheral blood and spleen of adult human individuals were hybridized to digoxigenin(DIG)-labeled probes specific for the human V_H families known to date. Except for V_H3, the V_H-gene family utilization patterns did not correlate with the genomic complexity of the human V_H-genes. The patterns of V_H-gene expression varies between the available and actual repertoires, and also between peripheral blood and spleen cells. Our data indicate that selective forces regulate the shaping of the B-cell repertoire in humans.

F 411 DECREASED SECRETION OF CD23 BY B CELLS FROM RHEUMATOID ARTHRITIS PATIENTS, Rebeca Rezonzew[1], Marie Sarfati[2], Guy Delespesse[2] and Marianna Newkirk[1], [1]Department of Medicine, McGill University and [2]Department of Immunology, University of Montreal, Montreal, Quebec.

CD23 the low affinity receptor for IgE is present on the surface of a broad spectrum of cells, but the fact that one of the two isoforms is restricted to B cells shows that this molecule has a unique role in B cell physiology. Its secreted form, a polypeptide of 25 Kd has been found in elevated levels in serum and synovial fluid of Rheumatoid Arthritis (RA) patients. We wanted to determine if its secretion by B cells was different in RA patients when compared to healthy individuals, and to correlate it with the Ig secretion. B cells from 10 patients (6 positive and 4 negative for Rheumatoid Factor) and 7 controls were obtained and cultured after stimulation with an anti-CD3 activated $CD4^+$ T cell line. The supernatant was analyzed at day 3 and 5 for the presence of sCD23, IgG and IgM. Levels were elevated only when the activation system was used, both in patients and in controls. The B cells from patients and controls had a different response to the activation system, as demonstrated by a higher increase in IgG and IgM secretion in the RA patients compared to control individuals (34% Vs 20% and 46% Vs 30% respectively). We found that the B cells from the RA patients secreted 65% less sCD23 than the healthy individuals ($p=0.0123$). A possible explanation for the higher levels of sCD23 in the joints of patients could be a result of a unique population of B cells or secretion by other types of cells as increased expression of CD23 receptors in monocytes and macrophages from RA patients has been reported. Our study demonstrates that peripheral blood B cells from RA patients respond differently to activated T cells and they secrete a lower amount of sCD23 when compared to normal individuals.

F 412 ISOLATION AND CHARACTERIZATION OF DORMANT LYMPHOMA CELLS, Richard H. Scheuermann, Eitan Yefenof†, Louis J. Picker, Thomas F. Tucker† and Jonathan W. Uhr†, Department of Pathology, and †Department of Microbiology, University of Texas, Southwestern Medical Center, Dallas, TX 75235-9072.

Tumor dormancy is an operational term used to describe a prolonged quiescent state in which tumor cells are present, but tumor progression is not clinically apparent. Although clinical examples of tumor dormancy abound, little is known regarding the mechanisms underlying this state. We have utilized a murine B cell lymphoma, BCL_1, as a model system for the analysis of tumor dormancy experimentally. These cells express a clonal immunoglobulin, $IgM\lambda_3$, on their cell surface; since the expression of the λ_3 light chain in mouse B cells is exceedingly rare, both λ_3 and the Ig idiotype (Id) functionally serve as tumor-specific antigens. If BCL_1 cells are transferred to a syngeneic recipients (Balb/c), tumor cells home to the spleen, and splenomegaly is detected by palpation within 30 days. However, if recipient mice are immunized with purified BCL_1 Ig prior to transfer, ~70% of the mice will survive beyond 60 days with no evidence of tumor growth. The absence of tumor growth is not simply due to the cytotoxic effect of the immune response since tumor cells can be detected in the spleen of these animals by FACS analysis, and these splenocytes are able to transfer tumor to naive recipients. Thus, the BCL_1 lymphoma in Id-immune mice serves as a model for tumor dormancy induction.

Dormant lymphoma cells (DLC) have been isolated from Id-immune mice by FACS, and compared with growing lymphoma cells (GLC) in terms of morphology, cell cycle status, and pattern of oncogene expression. The results can be summarized as follows: 1) While GLC have an active, immunoblastic morphology, DLC are small and compact, and appear inactive. 2) In contrast to GLC, the vast majority of DLC are in cell cycle arrest, as judged by Hoecsht staining for DNA content. 3) Dormancy of BCL_1 cells can be induced in SCID mice by the addition of anti-Id antibodies. 4) Growth arrest by anti-Id treatment can also be demonstrated *in vitro*. 5) Even though DLC are predominantly quiescent, they express unusually high levels of both *c-fos* and *c-jun*. These findings demonstrate that the expression of *c-fos* and *c-jun* has been uncoupled from cell cycle progression in these tumor cells, and that cell cycle arrest might be actively induced by an AP-1 transcription factor.

F 413 ETIOLOGIC MECHANISMS OF IMMUNODEFICIENCY ASSOCIATED B-CELL LYMPHOMA, L.E. Silberstein and T.J. Kipps, Univ. of Pennsylvania, Philadelphia, PA and Univ. of California-San Diego, La Jolla, CA

The Epstein-Barr virus associated (EBV) human B-cell tumors arising in mice with severe combined immunodeficiency (SCID) engrafted with human peripheral blood lymphocytes (SCID/hu-PBL) resemble human B-cell tumors, that may occur in a large proportion of immunosuppressed organ transplant recipients or patients with AIDS. It is unclear whether lymphomagenesis involves the direct outgrowth of latently infected B-cells present in the donor PBL at the time of engraftment or, a process of viral replication and infection of new B-cells *following* engraftment. In addition, the role of clonal selection by antigen in the pathogenesis of these tumors also has not been addressed. We examined these issues in the SCID mouse by immunoglobulin (Ig) gene analysis of SCID/hu tumors to determine whether: (1) tumor-related clonotypes show evidence of ongoing somatic mutations, (2) tumor-related clonotypes can be isolated from donor PBL-DNA, and (3) tumor related clonotypes undergo further somatic diversification following transfer into the SCID mouse. To this end, we analyzed an EBV^+, SCID/hu tumor arising 42 days after transfer of PBL. Southern blot analysis using a JH probe showed an oligoclonal pattern of 4 rearranged Ig gene restriction fragments. Gel separated DNA corresponding to one of these restriction fragments was amplified using FR1 and JH consensus primers and then cloned in E. Coli. Six bacterial isolates were sequenced and all were identical, confirming the homogeneity of rearranged Ig genes in the restriction fragment. The sequences were 90% homologous to the VH3 gene, 9.1. The subsequent isolation of this tumor related clonotype from the original donor PBL was achieved. However, this required a total of 60 cycles of amplification with two different anti sense primers corresponding to overlapping CDR3 regions. Five bacterial isolates were sequenced and all were identical to the SCID/hu tumor sequence except for one base pair difference in one isolate. Importantly, the donor PBL DNA used as template, was prepared from the donor three months prior to when PBL were harvested to generate the SCID/hu -PBL mouse. The presence of the tumor-related clonotype in donor PBL three months prior to transfer into the SCID mouse suggests that the SCID/hu tumors are the direct outgrowth of latently infected EBV^+cells, that belong to a long-lived subset of circulating B-cells. Moreover, the absence of base pair differences between the donor-PBL and SCID/hu tumor clonotypes indicates that selection by antigen may not be required for lymphomagenesis in the SCID/hu tumor model.

F 414 THE BINDINGCHARACTERISTICS OF AN IN VITRO GENERATED PRECURSOR OF A PATHOGENIC HUMAN AUTOANTIBODY.

Johan H. van Es, Henk Aanstoot, Frits H.J. Gmelig Meyling and Ton Logtenberg. Dept. of Immunology, University Hospital Utrecht, The Netherlands

The molecular analysis of human IgG anti-DNA autoantibodies derived from patients with SLE strongly suggests that at least part of the autoimmune response in SLE is (auto)antigen driven. This results in the generation of high affinity anti-DNA autoantibodies. In addition, these analysis have shown that these antibodies utilize V_L, V_H and D_H elements that frequently encode low affinity IgM autoantibodies. Conclusive evidence for the suggested relationship between natural and pathogenic autoantibodies may be obtained by returning the gene elements encoding these pathogenic autoantibodies to their germline configuration by site directed mutagenesis approach. Upon transfection and expression in eukaryotic cells, the binding specificity and affinity of the precursor antibody can be determined and may unveil the putative natural, low affinity autoantibody activity. The results of these studies, which are currently in progress, will be discussed in the context of the development of autoimmune disease in patients with SLE.

F 415 IS STAGE I AND II FOLLICULAR LYMPHOMA A LOCALISED DISEASE ? A PCR-STUDY IN t(14;18)-POSITIVE LYMPHOMA.

Mars.B. van 't Veer *, Caro Lambrechts **, Pauline E. Hupkes **, Lambert.C.J. Dorssers **, Department of * Hematology and ** Molecular Biology, Dr. Daniel den Hoed Cancer Center, Rotterdam, The Netherlands.

Most patients with follicular lymphoma, the most common type of Non-Hodgkin's Lymphoma and in the majority of the cases characterised by the chromosomal translocation (t)(14;18), present with disseminated disease (stage III and IV). After clinical staging, including CT scan and morphological and immunological examination of blood and bone marrow, 10 - 20% of the patients appear to have localised disease (stage I or II). These patients are usually treated with involved field radiotherapy only. Longstanding complete remissions and even cure has been described, suggesting that these patients have really localised disease. We found in 11 patients with follicular lymphoma stage I or II t(14;18)+ cells in the blood and/or bone marrow with the polymerase chain reaction (PCR) at initial staging, during clinical complete remission or both. Out of 8 patient, of whom a lymph node biopsy was available and t(14;18)+, 7 showed t(14;18)+ cells in the blood or bone marrow. At initial staging in 6 patients (4 proven to be t(14;18)+ on the biopsy) t(14;18)+ cells were found in the blood and/or bone marrow. Nine patients (6 proven to be t(14;18)+ on biopsy) in complete remission showed t(14;18)+ cells in the blood or bone marrow at one or more occasions. Of these 9 patients 3 relapsed at 55, 60 and 100 months after therapy, respectively. Six patients are in clinical complete remission with a mean follow-up time of 32 months (range 3-148 months). From these data we conclude: 1. stage I and II t(14;18)+ follicular lymphoma is usually not a localised disease and 2. the presence of t(14;18)+ cells, demonstrated by PCR, in the blood or bone marrow in these patients appears not to be predictive for an impending relapse.

F 416 BONE MARROW CELLS OF MULTIPLE MYELOMA PATIENTS EXPRESS MULTIPLE IgH ISOTYPES THAT ARE CLONALLY RELATED TO THE MALIGNANT POPULATION,

Brian Van Ness and Daniel Billadeau, Institute of Human Genetics, University of Minnesota, Minneapolis, MN 55455

Multiple myeloma is characterized by the clonal expansion of the plasma cells in the bone marrow (BM) and production of monoclonal immunoglobulin (Ig). A number of reports have indirectly suggested that various populations of pre-B and B cells are clonally related to the plasma cell malignancy. The implication from these findings is that various populations of a common B-lineage may result from an oncogenic process affecting the B cell early in differentiation. If B cells at various stages of differentiation are clonally related, it would be expected they share common Ig gene rearrangements. We have developed a PCR approach that utilizes consensus primers to amplify the CDR3 region of a rearranged IgH allele from the tumor population, followed by sequence determination of the PCR product. This sequence is clonally unique due to novel combinations of V-D-J gene segments, random nucleotide insertions and deletions, and somatic mutations. Allele specific oligonucleotide (ASO) primers were synthesized and used to detect the tumor specific allele. Because this approach is specific for the tumor rearranged heavy chain allele, it can provide a useful tool to identify expression of clonally related transcripts. RNA isolated from the BM of patients with IgG or IgA myeloma was reverse transcribed with μ, δ, γ, or α constant region primers, and the cDNA amplified using the tumor specific ASO. Based on the sequence of the PCR products obtained, we confirm the presence of transcripts that are identical to the tumor IgH V-D-J CDR3 region, but associated with different isotypes, including μ. These results suggest the presence of B cells clonally related to the tumor at earlier stages of differentiation. Moreover, sequence comparisons between μ–δ expressing transcripts and the clonally related, more differentiated γ or α transcripts show a remarkable lack of accumulating somatic mutations. Several models to explain these results will be presented.

F 417 *BCL-2* PREVENTS PROGRAMMED CELL DEATH IN *CAENORHABDITIS ELEGANS*.

David Vaux, Irv Weissman and Stuart Kim, Department of Developmental Biology, Stanford, CA 94305.

Cell death is a normal part of development and homeostasis in both vertebrates and invertebrates. For example, in vertebrates cell death is used to eliminate neurons and auto-reactive immune cells, and during development of the nematode *C. elegans*, 131 cells undergo programmed cell death out of the 1090 somatic cells that are formed.

The *bcl-2* gene encodes a protein that has been shown to regulate programmed cell death in mammalian haemopoietic cells, but the molecular mechanism by which it works is not known.

We wished to know whether programmed cell deaths in nematodes and humans can occur via the same molecular pathway. To do this, we constructed a vector that directs expression of the human *bcl-2* gene from a nematode heat shock promoter, and used antibody staining to show that the transgenic embryos can express the human *bcl-2* protein. We next showed that *bcl-2* expression during embyogenesis significantly reduces the number of cell corpses in the nematode, as embryos that expressed the *hsbcl-2* transgene had a significantly lower number of cell corpses.

These experiments indicate that the programmed cell death pathway mediated by *bcl-2* in humans is similar at the molecular level to the programmed cell death pathway in *C. elegans*. This conservation of function implies that the mechanisms that execute programmed cell death are basic and are used in diverse organisms. Furthermore, the fact that activated forms of the *bcl-2* gene are found in follicular lymphomas suggests that human homologues of nematode cell death genes may play a role in oncogenesis.

F 418 STRUCTURE AND SPECIFICITIES OF ANTI-GANGLIOSIDE AUTOANTIBODIES ASSOCIATED WITH MOTOR NEUROPATHIES, Nan-ping Weng[*], Li-yuan Yu-Lee[*+#], Inaki Sanz[O], Bernard M. Patten[**] and Donald M. Marcus[#+**], [*]Departments of Microbiology & Immunology, [+]Medicine, [#]Cell Biology and [**]Neurology, Baylor College of Medicine, Houston, TX 77030 and [O]Department of Medicine, University of Texas Health Science Center at San Antonio, San Antonio, TX 78284

Autoantibodies that bind to GM1 ganglioside and asialo GM1 (GA1) have been implicated in the pathogenesis of motor neuropathies. To investigate the structure and specificity of these autoantibodies, peripheral blood B cells from patients with motor neuron diseases and from normal individuals were immortalized by Epstein-Barr virus (EBV), and B cells secreting anti-GM1 or GA1 antibodies were cloned. We report an analysis of the structure and specificities of 8 autoantibodies from patients with motor neuropathy, and 2 from normal individuals. Four antibodies were IgM, 6 were IgG, and all bound predominantly to GA1. The sequences of V domains of H and L chains were determined by a reverse transcription-PCR procedure. A variety of V genes were used to encode these Ab: 4 V_H1, 2 V_H3, 3 V_H4, 1 V_H5, 2 VjI, 2 VjII, 3 VjIII and 2 VkII. Most V genes (13/19) exhibited less than 95% similarity to known germline genes, which suggests that somatic mutation was required to generate these autoantibodies, or that the relevant germline genes have not been identified. The average length of the H chain CDR3 was 16 amino acids, and in three antibodies this segment contained more than twenty amino acids. It was not possible to identify amino acid sequences that were encoded by germline D segments by conventional alignment of sequences. Partial analogies could be identified by introducing gaps, allowing mismatches and searching for D-D fusions and inversions. These results indicate that anti-GA1 antibodies can be encoded by a variety of VH-VL pairs, that the antibodies exhibit extensive somatic mutation, and that the CDR3 segments are generated by a number of non-conventional mechanisms.

Cytokine Interactions with B Cells/B Cell Responses in Vivo

F 419 REGULATION OF CD23 EXPRESSION AND IGE SECRETION BY GM-CSF AND IL-3, Mark Alderson, Teresa Tough and Kenneth Grabstein, Department of Immunology, Immunex Research and Development Corporation, 51 University St. Seattle WA 98101.

We recently demonstrated that GM-CSF and IL-3 are potent stimulators of monocyte cell surface and soluble CD23 (FcεRII), particularly when acting in the presence of IL-4. Additionally, IL-3, but not GM-CSF enhances CD23 expression by B cells. Since soluble CD23 has been reported to play a role in IgE production by B cells, we assessed whether GM-CSF or IL-3 may play a secondary role in IgE production via the stimulation of release of soluble CD23 by monocytes. Somewhat surprisingly, GM-CSF and IL-3 were found to inhibit IgE secretion by human PBMC that were stimulated with IL-4, either alone or in combination with CD40 antibody (G28.5). Interestingly, neither IFN-α nor IFN-γ, which have been demonstrated to inhibit IgE production by PBMC stimulated with IL-4 alone, were able to inhibit IgE production in cultures stimulated with CD40 antibody plus IL-4. Addition of GM-CSF or IL-3 as late as day 5 of a 10 day culture significantly inhibited IgE production, suggesting a late mechanism of action. Thus our data suggest that GM-CSF and IL-3 may be important regulators of IgE production.

F 420 REGULATION OF DIFFERENTIATION IN $CD5^+$ AND CONVENTIONAL B CELLS BY LPS AND IFN-γ. Jacqueline H. Chace, Nashwa S. Abed, Gina L. Adel and John S. Cowdery. Department of Internal Medicine, University of Iowa College of Medicine, and Department of Veterans Affairs Medical Center, Iowa City, IA 52242

Cultured $CD5^+$ B cells secrete more IgM than conventional B cells in response to low dose LPS stimulation. This increase in LPS-induced IgM production is due to an increased precursor frequency of IgM secreting cells in the $CD5^+$ population. The IgM secreting clones derived from $CD5^+$ peritoneal or conventional splenic B cells exhibit similar kinetics of their IgM secretion in response to LPS, but conventional B cells have a somewhat greater burst size of IgM secreting cells. Interferon-γ (IFN-γ) inhibits LPS driven IgM secretion in both conventional and $CD5^+$ B cells. IFN-γ inhibits LPS-induced IgM secretion by decreasing the precursor frequency of IgM secreting cells by three-fold. IFN-γ also decreases the anti-ssDNA precursor frequency of LPS-driven splenic B cells from autoimmune NZB mice. This study documents an important role for IFN-γ in the inhibition of polyclonal B cell activation and suggests that loss of IFN-γ activity may permit or promote excessive B cell activation.

F 421 MECHANISM OF INTERFERON-γ-MEDIATED INHIBITION OF B CELL DIFFERENTIATION. John S. Cowdery, Jacqueline H. Chace, and Nashwa S. Abed. Department of Internal Medicine, University of Iowa College of Medicine, and Department of Veterans Affairs Medical Center, Iowa City, Iowa 52242

Interferon-γ (IFN-γ) inhibits lipopolysaccharide (LPS) induced differentiation of B cells. We have shown that this is due in part to the decrease in precursor frequency and to a reduction in the number of immunoglobulin secreting cells (burst size). IFN-γ significantly reduced IgM secreting precursor frequency, burst size, and total secreted IgM even when added to cultures as late as 72 hours post-LPS stimulation. The inhibitory affect of IFN-γ was dominant even when B cells were cultured with LPS and IL-5. When resting B cells were pulsed with IFN-γ for one hour and washed, there was no inhibition of subsequent LPS-driven differentiation. However, when B cells were activated with LPS for 24 hours first, a one hour pulse with IFN-γ inhibited subsequent differentiation to IgM production.

These results demonstrate that IFN-γ is a late acting inhibitor of B cell differentiation. Prior activation of B cells is a prerequisite for this observed inhibitory effect of IFN-γ. Our studies also suggest that activation induces the B cell receptor for IFN-γ.

F 422 IN VIVO PRODUCTION OF INTERLEUKIN-10 BY MALIGNANT CELLS IN AIDS LYMPHOMAS
Dominique Emilie, Martine Raphael, Irène Joab, Michel Peuchmaur, Odile Devergne and Pierre Galanaud.
INSERM U131, Clamart, France.

Expression of the IL-10/BCRF1 gene was studied by *in situ* hybridization in tissue samples from AIDS lymphomas using a BCRF1 probe which also recognizes the human IL-10 sequence. Hybridization was detected in 8 out of 15 lymphomas. In contrast, IL-10/BCRF1 gene expression was detected in only 1 out of 11 lymphomas from HIV-seronegative patients (p=0.05).

In AIDS lymphomas, the number of cells labelled with a BCRF1 specific probe was dramatically lower than that of cells labelled with the IL-10/BCRF1 probe. Thus, the IL-10 rather than the BCRF1 gene was expressed.

Production of IL-10 was associated with that of IL-10 mRNA, as shown by immunodetection of the protein in numerous cells. In contrast, BCRF1-producing cells were rarely detected.

Both *in situ* hybridization and immunochemical experiments indicated that malignant cells were involved in this IL-10 synthesis. Moreover, IL-10 production in AIDS lymphomas was associated with the presence of EBV in lymphomatous cells (p=0.02).

As IL-10 is a potent growth factor for human B lymphocytes, these results suggest that IL-10 may stimulate the proliferation of malignant cells in an autocrine pathway in a number of AIDS lymphomas, and that EBV and HIV may synergistically trigger its production.

F 423 B CELL HYPERREACTIVITY IN IL-4 TRANSGENIC MICE, Klaus Erb, Annelise Schimpl, Kathrin Bothe, Thomas Holtschke and Ivan Horak, Institut für Virologie und Immunbiologie der Universität Würzburg, Germany

The in vivo effect of IL-4 was investigated in IL-4 transgenic mice in which IL-4 is expressed under the regulatory control of the MHC class I promotor/ enhancer. Following the pattern of class I expression, transgene derived mRNA could be detected in all nonlymphoid organs tested, in addition to thymus, spleen and lymph nodes. In the thymus, a hypoplasia is observed, the number of $CD4^+CD8^+$ cells is decreased, and the $CD4^+CD8^-$ population is increased. Spleen and, to a lesser extent, lymph nodes exibit greatly reduced numbers of T lymphocytes, particularly of $CD8^+$ cells. In the spleen the number of nonT/nonB cells is drastically enhanced. Splenic and lymph node B cells are enlarged and show increased levels of class II and CD23 expression, furthermore, they show an increased proliverative response to anti μF(ab)2. Serum levels of IgG1, IgE and IgA are elevated as compared to age matched controls. A high proportion of the IL-4 tg animals develop autoantibodies of various specifities, rising the question whether negative selection/ anergy of T and/or B cells may be overcome by IL-4.

F 424 DISRUPTION OF THE IL4 GENE IN MICE BLOCKS DEVELOPMENT OF TH2 IMMUNE RESPONSES, Graham S. Le Gros, Manfred Kopf, Martin Bachman, Marinus Lamers, Horst Bluthman, and George Kohler. Ciba, CH 4002 Basel, Switzerland.

To elucidate the central role of interleukin 4 (IL-4) in regulating B and T cell responses after immunisation, we generated IL-4-deficient (IL-40/0) mice by gene targeting. Serum IgE was undetectable even after helminth infection or immunisation with anti-IgD antibody. This confirms the absolute requirement of IL-4 for IgE responses in vivo. In vitro stimulated CD4+ T cells from naive IL-40/0 mice fail to produce the Th2-derived cytokines IL-5 and IL-10. This shows that IL-4 is a necessary factor for the development of Th2 cells in vitro. Th2 cytokines from CD4+ T cells measured ex vivo after Nippostrongylus brasiliensis infection were significantly reduced. However, helminth-induced eosinophilia, which has been shown to be dependent on IL-5 in vivo, was observed in the IL-40/0 mice, albeit two- to three-fold reduced. A non-CD4+ T cell type was found to produce IL-5 in these N. brasiliensis infected mice. We conclude that IL-4 is required in generating a Th2 response, but that a Th2 independent source of IL-5 production exists in vivo.

F 425 RECOMBINANT HUMAN NATURAL KILLER CELL STIMULATORY FACTOR STIMULATES B CELL GROWTH BY INDUCING IFN-γ IN B CELLS. Li Li, Deborah Young*, Stanley F. Wolf* and Yong S. Choi, Laboratory of Cellular Immunology, Alton Ochsner Medical Foundation, New Orleans, LA 70121 and *Genetics Institute, Inc., Cambridge, MA 02140

Human natural killer cell stimulatory factor (NKSF) is a 70 kDa polypeptide that activates human natural killer cells. It has recently been purified from the culture supernatant of a human Epstein Barr-Virus transformed B cell line and cloned. Our laboratory has been investigating the functions of various recombinant cytokines involved in proliferation and differentiation of human B cells. We found that native as well as recombinant NKSF (rNKSF) promoted growth of SAC or anti-μ antibody-activated B cells in a dose-dependent manner. NKSF also synergized with IL-2 in growth and differentiation of SAC-activated B cells. Since anti-IFN-γ antibody completely abrogates BCGF activity of NKSF, the BCGF activity is mediated by IFN-γ. This conclusion is clearly supported by the results that NKSF indeed induced IFN-γ production by activated B cells. Taken together, these results suggest that the B cell proliferative effect of NKSF is mediated by autocrine IFN-γ.

F 426 INHIBITION OF IgE PRODUCTION BY IL-2, Nobuhiko Nagai[1], Kenji Nakanishi[1], Hatsuhiko Matsumoto[1], Kazunobu Hase[1], Tomohiro Yoshimoto[1], Toshiyuki Tanaka[2], Masayuki Miyasaka[2] and Sohei Shinka[1]
[1]Department of Immunology and Medical Zoology, Hyogo College of Medicine, Hyogo, [2]Department of Immunology, Metropolitan Institute of Medical Science, Tokyo, Japan

Freshly purified B cells are composed of IL-2Rα(-)β(-), α(+)β(-), α(-)β(+) and α(+)β(+) B cells, determined based on the expression of IL-2Rα and/or β chains. Expression of these chains is differentially regulated by LPS and IL-4: LPS induces IL-2Rα chain and IL-4 induces IL-2Rβ chain. This combination also caused B cells to develop into IgE and IgG1 producing cells. Therefore B cells ($3x10^5$/0.2ml/well) stimulated with LPS (20 μg/ml) and IL-4 (10,000 U/ml) produced IgE and IgG1. However, costimulation of B cells with IL-2 completely suppressed the production of IgE and markedly diminished the production of IgG1. These results indicate that precursor cells which develop into IgE or IgG1 producing cells express IL-2Rβ chain. Northern blot analysis of B cells at 96 h after initiation of culture demonstrated that IL-2 inhibited LPS and IL-4 dependent accumulation of germline and productive ε transcripts as well as γ1 transcripts. We also investigated whether IgE-inducing stimuli, characterized by their capacity to induce IL-4, caused an increase in the proportion of B cells positive for IL-2Rβ in vivo and also whether exogenous IL-2 inhibited this IgE production. Inoculation of mice with Nippostrongylus brasiliensis (Nb) caused an increase in the proportion of B cells positive for IL-2Rβ(+) and injection of IL-2 into the mice inoculated with Nb inhibited IgE production strongly and IgG1 production moderately, possibly providing an intriguing therapeutic means for the treatment of allergic disorder.

Late Abstracts

ON THE INTRINSIC SPECIFICITY OF THE ANTIBODY HYPERMUTATION MECHANISM Alexander G. Betz, Cristina Rada, Richard Pannell, César Milstein and Michael S. Neuberger, MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH

The maturation in affinity of antibodies during a immune response is largely attributable to somatic hypermutation, in which nucleotide substitutions are introduced into the rearranged variable region gene segments, followed by antigenic selection. To identify the intrinsic specificity of the hypermutation mechanism and remove the strong biases caused by antigenic selection, we have analysed mutations in an immunoglobulin κ transgene in B cells either selected by different antigens, or with the transgene as a passenger. In the first case the transgene is contributing the light chain of the expressed antibody. Therefore the pattern of mutations shows the influence of different antigenic selections superimposed on hypermutation. In the second case the transgene is not contributing to the antibody, revealing the intrinsic specificity of the mutation mechanism liberated from selection by the antigen.

We conclude that the intrinsic specificity solely yields base substitutions and leads to hotspots occurring in individual positions as well as mutations preferentially accumulating around the transgene's first complementarity determining region (CDR1). Other intrinsic features of the hypermutation mechanism are that transitions are favoured over transversions and the process distinguishes between the coding and non-coding strands since pyrimidines (particularly T) mutate less frequently than purines.

BETTER IMMUNE RESPONSE IN ANTIGEN-FREE MICE, COMPARED TO GERMFREE AND CONVENTIONAL MICE. Nicolaas A. Bos and Vicky Ploplis[1], Dept. of Histology and Cell Biology, Immunology Section, University of Groningen, Groningen, The Netherlands and [1] American Biogenetic Sciences, Notre Dame, IN.

The B cell immune response to DNP-KLH was compared in antigen-free, germfree and conventional BALB/c mice. The total numbers and the numbers of DNP-specific IgM-, IgG- and IgA-secreting cells in the spleen were determined by ELISA-plaque assays after primary, secondary and hyper-immunization. After primary immunization the absolute number of DNP-specific IgG-secreting cells was about the same in all three groups of mice. However, next to the specific response, a rise in the total number of IgG-secreting cells was seen in conventional, but not in germfree and antigen-free mice, resulting in a much better frequency of DNP-specific IgG-secreting in antigen-free and germfree mice, compared to conventional mice.
After secondary immunization, again a great bystander IgG response was seen only in conventional mice. At the end of the secondary response (day 7) 90% of all IgG-secreting cells were DNP-specific in antigen-free mice, while this was 63% and 14% in germfree and conventional mice, respectively. After hyperimmunization, the absolute number of DNP-specific IgG-secreting cells in the spleen was five-fold and eleven-fold higher in antigen-free mice then in germfree and conventional mice, respectively. Approximately 54% of all IgG-secreting cells were DNP-specific in antigen-free mice, while this was 25% and 3% in germfree and conventional mice, respectively. The higher absolute and relative numbers of antigen-specific IgG-secreting cells after hyperimmunization in antigen-free mice compared to germfree and conventional mice provide a better source for antigen-specific B cells that can be used for hybridoma production.

HEAVY CHAIN VARIABLE REGION SEQUENCES OF SEVEN HYBRIDS DISPLAYING AUTOANTIBODY ACTIVITY, DERIVED FROM CD5+ B CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) LYMPHOCYTES. **Guillaume Dighiero, Christian Magnac, Gérard Dumas and Coumaran Egile.Institut Pasteur, Paris, France.**

Natural autoantibodies (NAAB) constitute an important part of the normal B cell repertoire. They frequently display a widespread pattern of binding and are the products of clones expressing germline Ig variable region genes. In a previous work, we derived heterohybridomas from CD5+ CLL B lymphocytes, and demonstrated that these were frequently committed to NAAB production (Blood: 76,562,1990). We report here the heavy chain variable region sequences from seven of these heterohybridomas displaying NAAB activity. The three hybrids displaying a polyreactive pattern of binding used VH4 family members. Two of them employed the same VH4 2-1 gene in germinal configuration, whereas the third one used a VH4 gene with 90% homology with VH4-21. The clone expressing anti-Sm activity employed a VH3 family member, with 95.26% homology to the 30P1 gene. The three hybrids exclusively displaying rheumatoid factor activity expressed VH1 family genes. Two of them encoded the previously reported 51P1 gene (in germinal configuration in one, and with 93.2% homology in the other), whereas the third one used a different VH1 gene, with 98.8 homology to the 21/28 anti-DNA autoantibody. Definitive homology with known germ-line D segments could not be found for five of the seven hybrids and JH gene utilization appeared to be random. With the exception of the VH4 2-1 gene, all these VH genes have been reported to be encoded by different clones secreting NAAB, and some of them, like the 51P1 and 30P1, have been found in the early human fetal repertoire. These results demonstrate that CLL B lymphocytes committed to production of NAAB use a restricted set of V genes. It is presently unknown whether continuous challenge of these autoreactive B by self antigens could favor malignant transformation, or whether the fetal repertoire expressing developmentally regulated genes has a selective advantage to undergo malignant transformation.