NORMAL AND NEOPLASTIC HEMATOPOIESIS

David W. Golde and Paul A. Marks, Organizers

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Plenary Sessions

Poster Sessions

Gene Expression in Normal and Abnormal Hematopoiesis I

0476 ONCOGENES OF AVIAN LEUKEMIA VIRUSES, J. Michael Bishop, Department of Microbiology and Immunology, University of California, San Francisco, CA 94143

A number of retroviruses possess genetic loci (viral oncogenes) whose actions can induce leukemia in specific hemopoietic lineages. Retroviral oncogenes arose by transduction of cellular genes, known as proto-oncogenes or cellular oncogenes. Study of viral and cellular oncogenes offers direct access to the genetic anomalies and biochemical mechanisms of leukemogenesis, and insight into molecular aspects of normal hemopoiesis. I will illustrate these principles by reviewing recent findings with three leukemogenic oncogenes, myc, erb-B and myb.

Mechanisms of leukemogenesis by viral oncogenes. The products of myc, erb-B and myb differ in structure, sub-cellular location and - apparently - mechanism of action. The nucleotide sequence of myc displays homology with known DNA-binding proteins, a finding that is consonant with previous reports that the product of myc binds to DNA and is located in the nucleus. Erb-B encodes a 61kd protein that becomes glycosylated into heterogeneous forms soon after its synthesis and localizes primarily in intracellular membranes; only a small fraction of the erb-B product apparently reaches the plasma membrane. The newly identified myb product is a 45kd phosphoprotein of presently unknown function. The nucleotide sequence of myb raises the possibility that it too encodes a DNA-binding protein.

Implicating cellular oncogenes in tumorigenesis. Several independent lines of evidence have raised the possibility that the cellular oncogenes first identified thorough the study of retroviral oncogenes may participate in the genesis of human neoplasms. I will add a further provocative finding describing studies of cells derived from a human neuroendocrine tumor. The tumor cells contain two widely recognized but previously mysterious chromosomal anomalies: double minute chromosomes, and homogeneously staining regions in what is reputed to be the X chromosome. The anomalies reflect amplification of the human myc gene to approximately 30 copies. The amplified unit takes two forms: one in which the myc locus appears normal; and one in which DNA immediately upstream from c-myc has been rearranged. As a consequence of the amplification, the expression of cellular myc is enhanced proportionately. It appears that amplification of c-myc has accompanied - and perhaps anticipated - the genesis of the tumor, and that the amplified gene has been translocated from its normal position on chromosome 8 to multiple positions on the X chromosome.

Structure and function of cellular oncogenes. Cellular oncogenes can differ appreciably from their viral homologues, and may participate in normal differentiation. I will illustrate these possibilities with a comparison of viral and cellular myb. The transduction that gave rise to viral myb seized only a portion of cellular myb. As a consequence, the protein encoded by cellular myb is appreciably larger than the viral myb protein - 75kd as opposed to 45kd. The two proteins may therefore differ in their functions. Cellular myb is expressed with great specificity: expression can be detected readily only in hemopoietic tissues, particularly in cells of the lymphoid and erythroid series rather than myelomonocytic cells.

0477 RETROVIRAL ONC GENES AND RETROVIRUSES IN HUMAN HEMATOPOIETIC CELLS, R.C. Gallo, R. Dalla Favera, V. Manzari, G. Franchini, B. Hahn, and F. Wong-Staal, Laboratory of Tumor Cell Biology, NCI, NIH, Bethesda, MD 20205

Retroviruses are involved in leukemias/lymphomas in many animals. Acutely transforming viruses carry onc genes derived from conserved normal cell genes (c-onc). Chronic leukemia viruses may cause leukemia by activating c-onc genes. We molecularly cloned four human c-onc genes: sis (SSV)[1], fes (FeSV)[2], myc (MC29)[3], and myb (AMV). All four contain introns and have been chromosomally mapped[4-6]. Of interest, myc is on chromosome 8 at the point of translocation in Burkitt's lymphoma[6]. We examined human cells for expression of six c-onc genes[7,8]. Some are univerally transcribed; others show more cell lineage or differentiation specificity. In fresh and cultured cells of an APL patient (HL60), abundant transcription of c-myc correlated with amplification of this gene[9].

A human retrovirus (HTLV) isolated from T-cell leukemia and lymphoma cells of patients from the U.S., Caribbean, Japan, Israel, and S. America is a novel exogenous type-C virus which has been linked to adult T-cell neoplasias. HTLV infection can acutely transform normal cord blood human T-cells. Probes from cloned HTLV provirus and flanking cell sequences showed that cells from patients with adult T-cell leukemia were clonally derived cells containing a highly conserved HTLV genome. Several samples contain HTLV integrated at the same immediate cellular locus, suggesting that the integration site is important in disease induction. For references on HTLV see recent reviews[10-11].

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0478 CONSTITUTIVE GENE EXPRESSION AND THE UNCOUPLING OF CONTROLS IN LEUKEMIA: REGULATORY PROTEINS THAT CONTROL GROWTH AND DIFFERENTIATION IN NORMAL AND LEUKEMIC MYELOID CELLS, Leo Sachs, Department of Genetics, Weizmann Institute of Science, Rehovot 76100, Israel

Experiments with hematopoietic cells have identified a family of macrophage and granulocyte inducing (MGI) proteins. Some of these induce cell growth (MGI-1); others induce differentiation (MGI-2). In normal myeloid cells MGI-1 induces growth and induces MGI-2. This induction of MGI-2 by MGI-1 ensures the coupling of growth and differentiation that occurs in normal development. MGI-1 has also been called mashran gm, CSF, CSA and MGI. Normal myeloid precursors require an external source of MGI-1 for growth. However there are myeloid leukemic cells that no longer require MGI-1 for growth or constitutively produce their own MGI-1. In both types MGI-1 does not induce MGI-2, but the leukemic cells can be induced to differentiate to mature macrophages and granulocytes either in culture or in vivo by adding an external source of MGI-2. These mature cells are then no longer malignant. The results indicate that the origin of myeloid leukemia is due to the uncoupling of growth and differentiation. Once this uncoupling occurs there can be further evolution of leukemia, due to uncoupling of specific pathways of gene expression within the differentiation program which produce blocks in the ability of the cells to be induced to differentiate by MGI-2. But even these leukemic cells can still be induced to differentiate by various compounds, either singly or in combination, that induce the differentiation program by other pathways of gene expression than those used by MGI-2. Changes from inducible to constitutive expression of specific genes, due to differences in gene dosage or integration of appropriate genes near growth regulatory sites, can change the requirement for growth and produce asynchrony in the co-ordination required for the normal developmental program. This can then result in the uncoupling of pathways of gene expression that occurs in malignancy (1-5).

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Gene Expression In Normal and Abnormal Hematopoiesis II

0479 INTERGENIC DNA AND GENE REGULATION IN THE HUMAN GLOBIN SYSTEM, S.M. Weissman[1], F.L. Collins[1], R. Kole[1], C. Stoeckert[1], P. Jagadeeswaran[2], Y. Yasukochi[1], Y. Fukumaki[1], J. Pan[1], B.G. Forget[3]. [1]Department of Human Genetics, Yale University School of Medicine, New Haven, CT 06510; [2]Center for Genetics, University of Illinois Medical Center, Chicago, IL 60612; [3]Department of Internal Medicine, Yale University School of Medicine, New Haven, CT 06510, USA.

The non-alpha globin gene cluster of man extends over more than 60 kilobases of DNA. A large portion of this DNA has been sequenced from normal subjects. We have prepared cosmid vectors with a unique Kpn 1 cleavage site and used these vectors to clone a 36 kb Kpn fragment from chromosomes bearing normal non-alpha globin gene cluster and from chromosomes carrying the mutation resulting in hereditary persistence of fetal hemoglobin of the nondeletion type, either from black subjects who produce mainly G-gamma globin in adults or from Meditteranean subjects who produce predominantly A-gamma globin chains. Restriction analysis of these cloned clusters failed to reveal any smaller deletions. The results of sequence analysis of these cosmids will be presented. In other work we have used the normal and mutant globin genes as templates for transcription and for in vitro transcription and processing systems. Recombinant genes have been produced in which the globin transcript containing embedded poly-deoxy(T) sequences so that the transcript is already polyadenylated. The results of analysis of RNA produced in these templates in vitro will also be presented.

Hematopoietic Stem Cells In Vivo and In Vitro

0480 REGULATION OF SELF-REPLICATION IN NORMAL AND LEUKEMIC STEM CELLS, Donald Metcalf, Walter and Eliza Hall Institute, P.O. Royal Melbourne Hospital, 3050, Victoria, Australia.

Dividing stem cells have the choice of forming progeny that retain stem cell properties (self-replication) or progeny that differentiate ultimately to form non-dividing end cells. An excess of self-replicative versus differentiative divisions is the essential abnormality in the behavior of a leukemic stem cell.

These processes can be analyzed in clonal semisolid cultures because of the ability of both normal and leukemic myeloid stem cells to generate colonies of progeny cells available for clonal reanalysis.

Three major families of glycoprotein regulators, the granulocyte-macrophage colony stimulating factors, control cell division, differentiation commitment and expression of functional activity in mouse granulocyte-macrophage populations. Three have been purified to homogeneity - M-CSF, MW 70,000; GM-CSF, MW 23,000 and G-CSF, MW 23,000. Interactions between these three regulators modulate the production of maturing granulocytes and macrophages from progenitor cells that are mainly bipotential. Commitment to one or other differentiation pathway is irreversible, requires 1 - 2 full cell cycles in the presence of the regulator and is a markedly asymmetrical event. A fourth type of CSF exhibits the ability to modulate self-generation in the stem cells generating granulocyte-macrophage progenitor cells.

Studies with clonogenic mouse myelomonocytic leukemia cells (WEHI-3B) have shown that G-CSF is highly active (at the same molar concentrations of 10^{-10} - 10^{-12} required for actions on normal cells) in enforcing differentiation to maturing granulocytes. This process is accompanied by suppression of stem cell self-replication. Studies on micromanipulated individual progeny cells of leukemic stem cells have shown that G-CSF action is irreversible, requires at least one full cell cycle and is also an asymmetrical event.

Both differentiation-responsive and differentiation-unresponsive WEHI-3B cells synthesise CSF constitutively and under some conditions can be induced to exhibit autoinduced differentiation. However the biology of CSF production in vivo indicates that leukemic cells are relatively unimportant as a source of CSF's.

The CSF's appear likely to control self-generative and differentiative divisions by modifying the newly-synthesized daughter chromatid during chromosome duplication.

0481 FACTOR-DEPENDENT PERMANENT MULTI-POTENTIAL HEMATOPOIETIC STEM CELL LINES, Joel S. Greenberger, Mary Ann Sakakenny, Donna Reid, Keith C. Humphries, and Robert J. Eckner, Joint Center for Radiation Therapy, Harvard Medical School and Sidney Farber Cancer Institute, and Department of Microbiology, Boston University School of Medicine, Boston, Massachusetts and Clinical Hematology Branch, National Heart, Lung and Blood Institute, Bethesda, Maryland.

Multipotential hematopoietic stem cell lines have been established from non-adherent cell populations harvested from continuous mouse bone marrow cultures. Clonal sub lines of B6SUTa or B6JUT formed mixed colonies containing erythroid cells, neutrophilic granulocytes, and basophil/mast cells in semi-solid medium in pokeweed mitogen spleen cell conditioned medium and erythropoietin. Each of several sub-clones of line ROCL3-1 formed mixed colonies containing eosinophils, neutrophil granulocytes, and basophil/mast cells in semi-solid medium. Multipotentiality was maintained in vitro for over 2 1/2 years. In contrast, numerous other cell lines including line 32D formed purely basophil/mast cell colonies with no detectable differentiation to other pathways. Multipotential lines did not produce dectectable spleen colonies (CFUs) in vivo nor did intravenous inoculation of up to $5x10^7$ cells protect lethally-irradiated syngeneic mice from bone marrow failure. Newborn and adult mice inoculated with $5x10^7$ syngeneic cells from clonal lines showed no detectable leukemia or solid tumors after one year. Both multipotential and committed factor-dependent basophil/mast cell lines deomostrated absolute dependence on a source of a 41,000 molecular weight glycoprotein growth factor termed interleukin-3. These cell lines should be of value in studies of the regulation of hematopoietic stem cell differentiation in vitro.

0482 HIERARCHY OF HEMOPOIETIC STEM CELLS ASSAYABLE IN CULTURE: STATISTICAL ANALYSIS OF THEIR SELF-RENEWAL AND DIFFERENTIATION, Makio Ogawa, Pamela N. Porter and Alan J. Gross, VA Medical Center and Departments of Medicine and Biometry, Medical University of South Carolina, Charleston, SC 29403.

Recent progress in clonal cell culture methods provided the means for quantitating multipotential hemopoietic progenitors in culture. One such progenitor forms a macroscopic colony consisting of granulocytes (G), erythrocytes (E), macrophages (M) and megakaryocytes (M) and is termed CFU-GEMM. We recently identified a class of hemopoietic progenitors in cultures of mouse and human cells which appears to be more primitive than CFU-GEMM. The latter progenitor produces a small colony consisting of undifferentiated blast cells after long periods of incubation in the presence of conditioned medium prepared with lectin-stimulated leukocytes (1). In replating studies, the blast (stem) cell colonies reproduced themselves and yielded large numbers of secondary GEMM colonies, while GEMM colonies did not produce blast cell colonies and produced only a small number of GEMM colonies. We then analyzed the frequencies of progenitors for stem cell and GEMM colonies in 68 primary stem cell colonies. The distribution of both colonies was extremely heterogeneous and could be approximated by variates of the gamma distribution but not by a Poisson distribution (2). Thus, our observations in culture corroborated the observations by Till et al in vivo and suggested that the renewal and differentiation of hemopoietic stem cells is governed by a stochastic rule.
We also identified the murine mixed hemopoietic colonies expressing only granulocyte, macrophage and megakaryocyte (GMM) differentiation and yet lacking erythroid elements (3). Cytological examination of GMM colonies revealed non-synchronous differentiation and varying numbers of undifferentiated blast cells. While some CFU-GMM appeared to be terminally differentiated to GMM lineages, others proved to be pluripotential in nature by replating studies. We thus concluded that a replating study is required to uncover the unexpressed lineage potentials when a mixed colony contained blast cells. In order to assist the development of a model for the progressive hemopoietic differentiation, we analyzed the correlation between multi- and monopotent progenitors derived from the 68 stem cell colonies described above. The highest correlation was observed between BFU-E and CFU-EM and between CFU-GEMM and CFU-GMM. Analysis of partial correlation of the multipotent progenitors derived from single colonies may be important in determination of the differentiation pathway of hemopoietic progenitors.

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Factors Regulating Proliferation and Differentiation I

0483 HUMAN LYMPHOKINES AND HEMATOPOIESIS, Irvin S.Y. Chen[1], Heinz G. Remold[2], Carol A. Westbrook[1], Richard H. Weisbart[3], Jerome E. Groopman[1], Aldons J. Lusis[1], and David W. Golde[1], [1]Division of Hematology-Oncology, Department of Medicine, UCLA School of Medicine, Los Angeles, CA 90024, [2]Harvard Medical School, Boston, MA 02115, [3]Veterans Administration Medical Center, Sepulveda, CA 91343

Lymphokines are glycosylated polypeptide hormones produced by lymphocytes undergoing a proliferative response to antigen or lectin. It is believed that these hormones are normally elaborated by T lymphocytes participating in cellular immune reactions. We have studied two human lymphokines in detail which affect the proliferation and differentiation of hematopoietic stem cells. We partially purified colony-stimulating factor (CSF) from T lymphocytes and characterized its messenger RNA by translation in Xenopus oocytes. We have also performed biochemical and biological studies on erythroid-potentiating activity (EPA), a glycoprotein which regulates the earliest steps in hematopoiesis. EPA is assayed by its effect on erythroid colony formation in vitro. In addition to regulators of hematopoietic cell proliferation, there are a number of lymphokines which affect the function of mature progeny. These include neutrophil migration-inhibitory factor (NIF-T) which specifically inhibits the movement of neutrophils and causes an enhancement in cellular function. The equivalent of NIF-T in the mononuclear phagocyte series is macrophage migration-inhibitory factor (MIF) which appears to analogously inhibit the movement of macrophages and heighten cellular functions. In addition, activated T lymphocytes produce fibroblast growth factor (FGF) which may be important in pathologic states involving fibrotic reactions in myelo- and lymphoproliferative disorders. We have used a human T-lymphoblast cell line which constitutively produces these lymphokines in order to dissect the relationship between activated T cells and other hematopoietic cells and to define the biochemical characteristics of the hormones. A synthesis of the data available at present suggests that activated T lymphocytes produce hormones which increase hematopoiesis and thereby increase the number of cells available for host defense. They also produce hormones which activate terminal cells to allow them to function more effectively in host defense. The development of some of these hormones as therapeutic agents holds great promise.

0484 HEMOPOIETIC GROWTH FACTORS INVOLVED IN MONONUCLEAR PHAGOCYTE PRODUCTION, E.R. Stanley, L.J. Guilbert, R.J. Tushinski & S.H. Bartelmez, Departments of Microbiology & Immunology, and Cell Biology, Albert Einstein College of Medicine, Bronx, N.Y. 10461

Several hemopoietic growth factors appear to be involved in regulating the production of mononuclear phagocytes from pluripotent hemopoietic stem cells. Central to this regulation is the colony stimulating factor, CSF-1, which specifically regulates the survival, proliferation and differentiation of cells of the mononuclear phagocytic lineage (precursor cell→monoblast→promonocyte→monocyte→macrophage) (1,2). CSF-1 is delineated from other colony stimulating factors by its detection in specific radioimmuno- and radioreceptor assays (3). It is a glycoprotein (M_r ∿45,000-∿76,000), consisting of 2 similar, disulphide bonded subunits with N-glycosidically linked acidic "complex" carbohydrate moieties that contribute as much as 50% to the M_r of the molecule (4). Specific cell surface receptors for CSF-1 occur only on mononuclear phagocytic cells. They mediate the biological effects as well as the degradation of the growth factor (5). These receptors are excellent markers of mononuclear phagocytes irrespective of their state of differentiation or tissue of origin (6). Studies of the mechanism of CSF-1 action have been carried out on homogeneous bone marrow-derived macrophage populations (2). CSF-1 causes very rapid changes in macrophage morphology and protein synthetic rates. The mechanisms by which the CSF-1 receptor mediates these changes are being studied. Rapid assays for other hemopoietic growth factors, which regulate stages of differentiation between the stem and mononuclear phagocytic precursor cells, have recently been developed. These assays involve measurement of the increase in total ^{125}I-CSF-1 binding exhibited by primitive (non-adherent) bone marrow cells following a 3 day incubation. Three hemopoietic growth factors possessing this activity have been resolved: They are CSF-1 itself, a factor (M_r ∿30,000) which acts independently of CSF-1 and a third factor (M_r ∿20,000), the action of which requires CSF-1. The specificity of the new growth factors and the mechanism of their synergism with the lineage specific growth factor CSF-1 are currently under investigation.

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0485 REGULATION OF PROLIFERATION AND DIFFERENTIATION OF NORMAL AND LEUKEMIC CFU-GM, Malcolm A.S. Moore and Louis M. Pelus, Department of Developmental Hematopoiesis, Sloan Kettering Institute for Cancer Research, 1275 York Avenue, New York, NY 10021

Human CFU-GM proliferation is regulated by a family of colony stimulating factors and we have proposed that this stimulation is counteracted by at least two negative regulators, prostaglandin E (PGE) and acidic isoferritin. The inhibitory effects of both negative regulators on CFU-GM proliferation are selective for a subpopulation of progenitors expressing Ia-like HLA-DR antigen on their surface. Selective removal of Ia-antigen positive CFU-GM by cytolytic treatment with anti-Ia monoclonal antibodies results in virtual complete loss of CFU-GM responsiveness to inhibition. Expression of Ia antigen is also lost following 3 - 6 hrs of culture of CFU-GM and this is associated with simultaneous loss of responsiveness to inhibition. Suspension culture of CFU-GM with as little as 1 pM of PGE for 24 hrs is associated with an absolute increase in total and S phase CFU-GM, their Ia-antigen expression and restoration of sensitivity to acidic isoferritin inhibition. In patients with acute and chronic myeloid leukemia, CFU-GM Ia-antigen expression is absent or greatly diminished and this correlates with hyporesponsiveness of leukemic colony-forming cells to PGE or acidic-isoferritin inhibition. Reinduction of CFU-GM Ia-antigen expression and/or stabilization, with return of normal responsiveness to stimulatory and inhibitory growth regulators is a potential approach to the therapy of myeloid leukemia and data will be presented to suggest that this may be achieved using biological response modification with vitamin analogs, prostaglandin E or endotoxin induced differentiation protein.

Factors Regulating Proliferation and Differentiation II

0486 HUMAN MEGAKARYOCYTE COLONY STIMULATING ACTIVITY: PRELIMINARY FUNCTIONAL AND PHYSIOCHEMICAL CHARACTERIZATION, R. Hoffman, E. Mazur, A. Gewirtz, E. Bruno, Indiana University School of Medicine, Indianapolis, IN 46223, and Mary Imogene Bassett Hospital, Cooperstown, NY 13326

The proliferation and differentiation of the human megakaryocyte progenitor cell (CFU-M) is at least partially dependent upon megakaryocyte colony stimulating activity (Meg-CSA). We have attempted to characterize the physiochemical nature of Meg-CSA and define the requirements of the CFU-M for Meg-CSA. Aplastic anemia serum (AAS) containing Meg-CSA was fractionated by passage through ultrafiltration membrane cones with molecular weight cut-offs of 25,000 and 50,000 daltons. These fractions were then assayed for their ability to promote megakaryocyte colony (MC) formation by normal human marrow cells (NHMC). Meg-CSA was detectable only in the retentates while ultrafiltrates were free of Meg-CSA. Ultracentrifugation of AAS at 160,000xg for 6 hours failed to diminish Meg-CSA. In order to define requirements of the CFU-M for Meg-CSA, NHMC were cloned in the absence of AAS and then subsequently AAS was added at varying time intervals and MC were enumerated after 12 days of incubation. Compared to assays containing AAS at time 0, MC numbers were stable during the first 48 hours of AAS deprivation, declined by 50% after 144 hours and at 192 hours the effect of added AAS was no longer detectable. In addition peripheral blood and NHMC were placed in AAS containing liquid suspension culture for 1-24 hours and subsequently cloned in Meg-CSA containing and Meg-CSA free plasma clot cultures. No Meg-CSA effect was demonstrable after these short term exposures alone. These data suggest that Meg-CSA is not membrane bound but circulates freely solubilized and that its molecular weight is over 50,000 daltons. In addition, the first 48 hours of CFU-M development appears to be independent of Meg-CSA, yet the CFU-M remains responsive and sensitive to Meg-CSA for at least 142 hours. A prolonged exposure of the CFU-M to Meg-CSA seems necessary since short pulse exposures do not result in augmentation of MC formation.

Lymphopoiesis

0487 LYMPHOCYTE RECEPTORS IN LYMPHOMA GROWTH. Irving L. Weissman, Thomas St. John, Michael S. McGrath, Glen Tamura, W. Michael Gallatin, Roger Reichert, Georg Kraal and Eugene C. Butcher. Laboratory of Experimental Oncology, Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305.

Lymphocytes are the central cells in immunity, and as such must carry on their surface receptors allowing recognition of antigen, of other hematolymphoid cells with which they must interact, of target cells bearing foreign antigens, and of the blood vessels lining the lymphoid organs through which they traffic. We here discuss the characterization of two classes of receptors present on T and B lymphocytes: Those involved in retrovirus recognition during retrovirus-induced lymphomagenesis; and those present on normal and neoplastic lymphocytes responsible for their homing from the blood stream into defined lymphoid organs. We shall present evidence that the virus binding sites on B cell lymphomas are in the variable regions of immunoglobulins present on these lymphomas, and that antiidiotypic antibodies raised against these virus-complementary shapes detect similar classes of molecules on T cell lymphomas. The potential significance of this for slowly transforming virus-induced lymphomas in mice, chickens, and man shall be discussed. The second class of receptors involved in lymphocyte traffic will be analyzed as a function of lymphocyte differentiation; monoclonal antibodies to these structures have allowed precise mapping of their expression during lymphocyte development at the single cell level, and have provided a marker for the distribution of homing lymphocytes in tissue sections. Using such monoclonal antibodies as a probe, we shall discuss means by which one can isolate cDNA copies of the messenger RNA's encoding these important receptor proteins.

0488 B CELL MALIGNANCIES AND B-CELL DIFFERENTIATION, Max Cooper, Hiromi Kubagawa, William Crist and Alan Landay, University of Alabama in Birmingham, Birmingham, AL 35294

Cells of B lineage are unique in their expression of the immunoglobulin genes, and progression along this differentiation pathway can be discerned by determining which immunoglobulin genes are expressed. The initial step involves rearrangement of a set of V_H, D and J_H genes and their transcription along with the C_μ gene. Cells expressing μRNA are known as pre-B cells, and at this stage few of the μ chains reach the cell surface. Next, a set of V_L and J_L genes is productively rearranged leading to expression of a complete IgM molecule on the cell surface. Antigens may begin to influence the B cell's behavior at this point in differentiation.

The foregoing stages, stem cell→ pre-B→ immature B cell, occur initially within the fetal liver and thereafter in bone marrow. Subsequent stages in B cell differentiation entail changes in the expression of the C_H genes. Immature B cells first express surface IgM and later co-express IgD molecules with the same V_H-D-J_H and light chain (κ or λ). Some members within each B cell clone undergo a further heavy chain switch to express an IgG, IgA or IgE isotype with the same antibody specificity and idiotype.

We have used monoclonal antibodies to human Ig heavy and light chain isotypes, V_H subgroups and idiotypes to identify and trace the extent of clonal involvement in malignancies featuring pre-B, B or plasma cell phenotypes. These diverse B lineage malignancies share a common feature: clonal involvement prior to the heavy chain switch regardless of the immunoglobulin isotype expressed by the prevalent cell type. Pre-B leukemias frequently exhibit isotype switching that is non-random in order and consistently linked to κ light chain expression. Our results suggest that the roots of both B cell and plasma cell malignancies can be traced to the bone marrow pre-B cell, and that secondary events may differentially affect growth patterns of isotype committed members of an affected B cell clone.

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Cytogenetics and Hematopoietic Tissues

0489 ASSOCIATION OF CHROMOSOME REARRANGEMENTS WITH SPECIFIC MALIGNANT HEMATOPOIETIC CELL TYPES, Janet D. Rowley, Dept. of Medicine, University of Chicago, Chicago, IL 60637

Nonrandom chromosome changes have been identified in a number of malignant human tumors. The leukemias are among the best studied malignant cells and they provide the largest body of relevant cytogenetic data. In chronic myeloid leukemia, a reasonably consistent translocation [t(9;22)(q34;q11)] is observed in 93% of all Ph^1 positive patients (1). In the other patients, translocations are either two-way, involving No. 22 with some other chromosome or complex translocations involving Nos. 9 and 22 and another chromosome. In acute nonlymphocytic leukemia, two translocations are each specifically associated with leukemic cells arrested at two different stages of maturation. One of these, t(8;21)(q22;q22), is found almost exclusively in patients with acute myeloblastic leukemia with maturation (AML-M2) (1). The other, t(15;17)(q22;q21), is seen only in patients with acute promyelocytic leukemia (APL-M3) (1). An inversion of chromosome 16(p13q22) has recently been described in patients with AMMoL and abnormal marrow eosinophils (2); other patients with marrow eosinophilia have had a deletion of 16 at q22 (3). Translocations or deletions involving 11q23 have recently been described in patients with AMoL, particularly of the monoblastic type (4).

Various translocations have been observed in B-cell acute lymphoblastic leukemia or in Burkitt lymphoma. The most common is t(8;14)(q24;q32), but variants of this, namely t(2;8)(p13?;q24) and t(8;22)(q24;q11), have also been observed; in all of these, the consistent change involved 8q24. The various immunoglobulin loci are located on chromosomes 2, 14, and 22 in the same chromosome band affected by the translocations in B-cell leukemia (5). More recently it has been shown that the myc gene is located at 8q24 and that the translocation results in the movement of the myc gene adjacent to the Ig locus on No. 14 (6,7).

These translocations may occur randomly. If a specific translocation provides a particular cell type with a growth advantage, possibly through alteration in control of an oncogene, then selection could act to cause the proliferation of this aneuploid cell line vis-a-vis cells with a normal karyotype. In this view, the chromosome change could be the fundamental event leading to the leukemic transformation of an otherwise normal cell. The challenge for the future is to define the genes located at the sites of consistent translocations in myeloid leukemias, and to determine the alterations in gene function that are associated with the translocation.

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0490 CHROMOSOMAL MARKERS : TOOLS FOR THE STUDY OF NORMAL AND ABNORMAL DIFFERENTIATION IN HUMAN LEUKEMIA, Roland E. BERGER, Hôpital Saint-Louis, Paris, France.

Clonal chromosome abnormalities have been clearly shown to be nonrandom in blood malignant diseases. Some are however not related to a specific type of proliferation such as trisomy 8 whereas others are correlated with specific cellular types of proliferation such as t(15;17) and acute promyelocytic leukemia (APL) and its variant form, t(8;21) and a particular form of acute myeloblastic leukemia (AML), and t(8;14), t(8;22) or t(2;8) and Burkitt type of malignancy (Bm). No detectable clonal chromosome abnormality was found in 50 % of acute leukemia. A concommittant study of mitoses with cytological and cytogenetic techniques from the same bone marrow or blood samples was undertaken in the view of ascertaining the nature of dividing cells. It was observed that in APL erythroblasts do not have the specific chromosome translocation as the "promyelocytes" do, and in t(8;21) AML erythroblasts do not have the chromosome rearrangement as granulocytic and eosinophilic cell lines do. In Bm granulocyte precursors have normal karyotype whereas blast cells have a specific translocation. These results suggest that chromosome abnormalities occurred in a precursor cell committed into a particular differentiation, granulocytic, granulocytic and eosinophilic, or lymphocytic B, depending on the type of disease. In addition it suggests also that erythroblastic precursors diverge earlier than eosinophilic from the rest of committed precursors in hematopoiesis. On the contrary in some cases (with monosomy 7 and 5q-) as in chronic myeloid leukemia the chromosomal abnormality occurred in a pluripotent precursor cell. Chromosome translocations are obviously related to DNA rearrangements, some of which being possibly related to oncogene sequences as it was suggested by animal models. In Bm a correlation between the type of variant translocation and the type of surface light chain immunoglobulin expressed was found giving the opportunity to study the DNA rearrangements in the vicinity of chromosome breakpoints. Other specific translocation may be studied in a similar way, and some lights may be given on the changes occurred in leukemic cells without any detectable chromosome abnormality. The existence of non specific acquired chromosome in leukemias suggests a subdivision of cytogenetic changes : they may be secondary to a primary event whereas specific translocations are closer from the initiating event leading to leukemic process. If the relations between these specific translocations and the type of abnormal cell differentiation are clear, its molecular basis remains to be understood.

Cell Lines and Cytodifferentiation

0491 SURFACE ANTIGENS OF NORMAL AND LEUKEMIC MYELOID CELLS DEFINED BY MONOCLONAL ANTIBODIES, Giovanni Rovera, Silvana Pessano, Dario Ferrero and Beverly Lange, The Wistar Institute of Anatomy and Biology and Childrens Hospital of Philadelphia, PA 19104

Fourteen different monoclonal antibodies directed against differentiation antigens of human hemopoietic cells were tested for their reactivity with leukemic cells from patients with acute myelogenous leukemia. The results of these analysis indicate that: 1) In each case of acute myelogenous leukemia, the cell population is phenotypically heterogeneous. The relatively small subpopulations expressing a given surface antigen may represent cells in which partial and imbalanced differentiation occurs. 2) The surface phenotype of leukemic cells most commonly reflects deficiencies in the expression of differentiation antigens and does not correspond to the surface phenotype of normal cells at an equivalent stage of differentiation. This suggests that severely disturbed differentiation accompanying the differentiation block is the norm in these cells. 3) Acute myelogenous leukemia cells quite often lack surface markers that are present on bone marrow CFU-GM and are retained throughout the maturation process to the level of terminally differentiated cells. This suggests that the target cell for leukemia transformation is, in general, a cell more immature than the bone marrow myelomonocytic stem cell.

0492 THE STUDY OF PROLIFERATION AND DIFFERENTIATION USING MYELOID LEUKEMIA CELL LINES. H. Phillip Koeffler, Department of Medicine, University of California, Los Angeles, CA 90024

Study of myeloid differentiation and proliferation is greatly aided by the use of human acute myelogenous leukemia cell lines blocked at different stages of differentiation. The cell lines include the myeloblastic KG-1 and ML-3, the promyelocytic HL-60 and the erythroleukemic K562. The HL-60 promyelocyte line can differentiate to granulocytes after exposure to a variety of agents. Nonphysiological inducers of HL-60 differentiation include polar-planar drugs (optimal activity 10^{-1}-10^{-2}M), purine and pyrimidine analogs (optimal activity 10^{-3}-10^{-6}M) and several chemotherapeutic agents (optimal activity 10^{-4}-10^{-6}M). Physiological inducers include retinoids, $1,25(OH)_2$ vitamin D_3, and differentiation inducing factor. Trans retinoic acid stimulates proliferation (200%) of normal myeloid colony forming cells (10^{-7}M) and inhibit (50%) proliferation of the KG-1 and HL-60 (10^{-6}M) and fresh patient leukemia cells (10^{-7}-10^{-6}M). Also at 10^{-6}M retinoic acid, 90% of the HL-60 cells differentiate to granulocytes. Induction of differentiation and inhibition of proliferation is not mediated through cytoplasmic retinoic acid binding protein. The $1,25(OH)_2$ vitamin D_3 (10^{-9}-10^{-8}M) stimulates clonal growth of normal myeloid and HL-60 cells, but at 10^{-7}-10^{-6}M the $1,25(OH)_2D_3$ is a potent inducer of HL-60 differentiation. The study of variant HL-60 sublines suggests that the ability of the $1,25(OH)_2D_3$ to induce differentiation is proportional to the number of $1,25(OH)_2D_3$ receptors present on the cells. Other studies show that induction of granulocyte differentiation of HL-60 does not require cell division.

The tumor promoting phorbol esters and teleocidins induce leukemic cell lines (KG-1, ML-3, HL-60) blocked at the myeloblast/promyelocyte stage of maturation to differentiate to macrophage-like cells including the development of adherence, nonspecific acid esterase activity, reduction of NBT, display of Fc receptors, and phagocytosis and killing of yeast. Differentiation is probably triggered through an interaction with high affinity phorbol ester receptors ($Kd \approx 1.4\times10^{-8}$M) which are present on the leukemic cell membrane. However, variant leukemia cell lines have been established that do not differentiate after exposure to phorbol diesters, but nevertheless have the same number of high affinity phorbol receptors ($Kd \approx 0.85\times10^{-8}$M). Induction of macrophage differentiation of the leukemic cells does not require cell division. Also, we have found that the KG-1 cells transcribe the Myb, Myc and fes oncogenes and with macrophage differentiation both Myb and fes, but not Myc, transcription markedly decreases. We have constructed a cDNA library from undifferentiated and differentiated HL-60 cells. The cDNA clones that represent genes that are transcriptionally up- or down-regulated with differentiation were identified. Using the cDNA clones we found that methylation changes in the genes examined may not be necessary for differentiation of promyelocytes to granulocytes.

0493 RELATIONSHIP OF CHROMOSOME STRUCTURE AND INDUCED GLOBIN GENE EXPRESSION, Paul A. Marks, Michael Sheffery, Zi-xing Chen, Judy Banks, and Richard A. Rifkind, DeWitt Wallace Research Laboratory, Memorial Sloan-Kettering Cancer Center, New York, New York 10021

Murine erythroid leukemia cells (MELC), virus transformed cells which appear blocked at the erythroid precursor stage analogous to CFU-e, can be induced to express characteristics of erythroid differentiation by a variety of agents. These studies examine hexamethylene bisacetamide (HMBA) mediated induction of MELC with emphasis on characterizing changes in chromatin associated with expression of α_1 and βmaj globin genes. Previous studies showed that different inducers cause different patterns of expression of α and β genes, e.g., HBMA increases the rate of accumulation of mRNA 10 to 12 hours before detectable increase in mRNA and commit cells to terminal divisions. Hemin induces α and βmRNA accumulation simultaneously, without causing a commitment to terminal cell division. Employing a "runoff" transcription assay, HMBA causes substantial increase in the rate of transcription of both α_1 and βmaj genes. Hemin causes little or no detectable increase in transcription of α_1 or βmaj genes. Thus, HMBA leads to increased expression of globin genes primarily by causing increased transcription: hemin acts primarily at a post transcriptional level. Alterations in chromatin structure was assayed by several techniques. Increased sensitivity to DNase I of globin gene domains is detected in uninduced MELC, and does not change with induction. A pattern of hypomethylation has been defined in the globin domains which also does not change with induction. By comparison, HMBA mediated induction causes the development of DNase I hypersensitive sites 5' proximal to the α_1 and βmaj genes. In addition, a second S nuclease sensitive site appears immediately 5' to α_1 and βmaj genes with induction. Thus, changes in chromatin structure occurring earlier in the differentiation lineage of these cells appear to be stably propagated. Additional changes in chromatin structure develop with inducer mediated expression of globin genes. This suggests a time sequence of chromosome alterations characterizes gene activation during differentiation.

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Clinical Leukemia

0494 RECENT ADVANCES IN THE TREATMENT OF ACUTE LEUKEMIA, Robert Peter Gale, M.D., Ph.D., UCLA School of Medicine, Los Angeles, CA 90024 U.S.A.

In the past 10 years there has been substantial progress in the treatment of acute myelogenous leukemia (AML) and acute lymphoblastic leukemia (ALL). In AML, intensive induction chemotherapy with cytosine arabinoside (ara-C) and daunorubicin with or without 6-thioguanine, have increased remission rates to 70-80%. This improvement combined with high-dose post-remission chemotherapy (consolidation, early intensification) has resulted in medians remission duration of 18-24 months with an increasing 3-5 year disease-free survival (10-20%). There are no convincing data that other therapeutic interactions such as maintenance or late intensification, or immunotherapy are effective in prolonging remission. In patients with resistant AML both high-dose ara-C and AMSA produce $\geq$20% remissions. These drugs are currently being evaluated in prolonging remissions. Trials of "differentiating" agents such as low-dose ara-C and retinoic acid have recently been initiated in resistant AML. Bone marrow transplantation (BMT) has also been evaluated in AML. In advanced AML 10-20% long-term leukemia-free survival has been achieved. In >300 patients transplanted in 1st remission the relapse rate is 15% and survival 50%. Controlled trials comparing chemotherapy and BMT are in progress; preliminary data indicate fewer relapses with improved survival in young patients. Autotransplants have not been critically evaluated.

Adults with ALL have a poor prognosis similar to AML. Intensive induction and consolidation chemotherapy produces remissions in 70-80% but these tend to be brief. Long-term leukemic free survival is 10-30% in most series. Some subsets of adults such as those with common ALL may have a better prognosis similar to children.

There have been few recent advances in the chemotherapy of adult ALL. BMT in $\geq$ 2nd remission is associated with a high relapse rate nd relatively poor survival. Recently some BMT have been performed in 1st remission in high-risk patients, these data are too preliminary for critical analysis.

0495 DRUG RESISTANCE IN LEUKEMIA, Joseph R. Bertino, Yale University School of Medicine, New Haven, CT 06510

Although the probable "cure" rates of acute lymphocytic and acute granulocytic leukemia are 50% and 15% respectively, development of drug resistance continues to be a major obstacle to further improvement over these figures. Resistance to methotrexate (MTX) in experimental systems has been shown to be due to (1) increased levels of dihydrofolate reductase (DHFR), (2) altered DHFR, such that it binds less avidly to MTX, and (3) impaired uptake of MTX. Highly resistant cell lines have also been isolated that are characterized by both an increase in DHFR and either an altered DHFR or an impaired transport to MTX. Gene amplification has been shown to be responsible in all mammalian lines tested thus far, as the cause for elevated DHFR in MTX resistant mutant cells, including human leukemic (K562) cells. MTX resistant K562 cells are also characterized by chromosomes (#5, 6 and 19) which have homogeneous staining regions. Knowledge of the mechanism of drug resistance allows alternate strategies to be developed that either prevents drug resistance, or allows selective therapeutic approaches. For example, resistant cells with an altered DHFR may be selectively killed by folate antagonists which inactivate the mutant enzyme, but not the normal enzyme.

Gene Expression

0496 H-2 HAPLOTYPE SPECIFIC CHANGES IN MuLV PROTEIN PROCESSING, John H. Wolfe, Elizabeth P. Blankenhorn and Kenneth J. Blank, Univ. Penna., Sch. Med., Philadelphia, Pa. 19104.

Genes which map to the mouse H-2 complex have been found to render an animal resistant to Gross virus-induced thymic lymphoma and Friend virus-induced splenic erythroleukemia. The $H\text{-}2^k$ haplotype is associated with both susceptibility to virus-induced disease in vivo and continuous infectious virus production by tumor cell lines in vitro; $H\text{-}2^b$ is associated with resistance and cessation of infectious virus production after several passages in culture. EM shows the "non-producer" cells to be making and budding immature viral particles. FACS analysis of cell surface expression shows that the $H\text{-}2^b$ derived lines have significantly fewer gp70 and p30 determinants per cell. Studies of gag-gene products have revealed a 47K protein in the BALB.B-gv ($H\text{-}2^b$)(BGV) line not seen in the BALB.K-gv ($H\text{-}2^k$)(KGV). This protein is labelled early and apparently is not processed in a 2hr pulse-chase experiment. However, peptide mapping reveals that this protein shares an extensive number of fragments with the KGV-derived Pr65. A small amount of 65K protein precipitated from BGV cells is not related to KGV-Pr65. The p30 molecules from both lines appear to be the same. Thus it appears that the $H\text{-}2^b$ non-producer cell line has a truncated gag-gene precursor polyprotein. The BALB.B-fv ($H\text{-}2^b$)(BFV) line also has a 47K band precipitated by anti-p30 antiserum which is not present in the BALB.K-fv ($H\text{-}2^k$)(KFV) cell line. These studies show that haplotype specific defects in the production of viral proteins occurs in these virus-induced cell lines. This defect in the $H\text{-}2^b$-derived lines apparently results in the production of defective virus particles.

0497 LACK OF REQUIREMENT FOR AKR-LIKE MULV RECOMBINATION IN X-RAY AND CHEMICALLY INDUCED LYMPHOMAS IN C57BL/6 MICE, James P. Allison, Rodney S. Nairn and Bradley W. McIntyre, U.T.S.C.C., Science Park - Research Division, Smithville, Texas 78957 USA

Polytropic murine leukemia viruses which arise by recombination between endogenous MuLV genomes have been implicated in the etiology of lymphoma in mouse strains with high spontaneous incidence. The role of MuLV in lymphoma induction in strains with low spontaneous incidence is less clear, since these tumors do not generally produce virus. We have carried out immunochemical analyses of MuLV antigen expression by a panel of induced lymphomas of C57BL/6 mice, and have used env-specific probes to examine the DNA of the tumors for the presence of recombinant MuLV sequences. A RADLV-induced lymphoma, C6VL, and two transplanted X-ray induced lymphomas, WEHI-74 and ERLD, were found to express, in addition to the xenotropic env protein found on normal lymphocytes, env proteins related to ecotropic MuLV. Two-dimensional peptide maps revealed that the novel env protein of each tumor possessed unique peptides, suggesting these proteins were the products of different, possibly recombinant, MuLV genes. DNAs of C6VL and WEHI-74 were found to contain recombinant MuLV env sequences with the XbaI/BclI linkage characteristic of leukemogenic AKR MuLV. On the other hand, two primary lymphomas, SPRD-1, induced by X-irradiation, and C57-282, induced by ethylnitrosourea, were found to express only xenotropic MuLV env proteins with tryptic and chymotryptic peptide maps identical to that of normal lymphocytes. The DNA of these tumors did not contain the AKR-like XbaI/BclI linkage. The results suggest that intragenic MuLV recombination and expression of novel MuLV env proteins may not be essential for lymphomagenesis in C57BL/6 mice.

0498 THYMIC LYMPHOMA INDUCED IN ADULT MICE INOCULATED WITH A VARIANT GROSS MULV. Kenneth J. Blank, Donna M. Murasko, and John H. Wolfe, University of Pennsylvania School of Medicine, Philadelphia, PA. 19104.

Previous studies have demonstrated that neonatal inoculation of Gross murine leukemia virus causes thymic lymphomas after a 8-10 week latent period. Mice older than 1 week at the time of virus inoculation were resistant to lymphomagenesis. We have isolated an apparent variant of Gross virus which induces thymic lymphomas after a latent period of 6-10 weeks when adult mice are inoculated. These lymphomas develop readily in BALB/c-H-2^k(BALB.K) and C57BL/10-H-2^k(B10.K) but only sporadically in their H-2 congenic partners BALB/c-H-2^b(BALB.B) and C57BL/10(H-2^b). These results demonstrate that a gene(s) closely linked to the H-2 complex controls the development of lymphoma induced by this virus. Similar results had been obtained by Lilly in animals inoculated with low doses of the original Gross virus (Passage A) as neonates; however, at the high titers of virus used in our experiments, Lilly found that H-2^k and H-2^b animals were equally susceptible to lymphoma development. Thus, the process of lymphomagenesis induced by this virus appears to be more stringently controlled by an H-2-linked gene(s) than that induced by conventional Gross Passage A virus.

Cultured tumor cell lines derived from the greatly enlarged thymuses of infected BALB.K and B10.K are highly tumorigenic in both normal and sublethally irradiated (500R) syngeneic mice.

Studies on the cell surface characteristics of the tumor cells and the relationship of this virus to Gross Passage A are currently underway.

0499 RETICULOENDOTHELIOSIS VIRUS - INDUCED B-LYMPHOMAS IN CHICKENS; CHARACTERIZATION OF A TUMOR CELL DNA CLONE CONTAINING PROVIRAL AND C-MYC SEQUENCES. Donald J. Fujita*, Robert A. Swift+, Anthony A. G. Ridgway*, and Hsing-Jien Kung+. *Cancer Research Laboratory and Department of Biochemistry, University of Western Ontario, London, Ontario, Canada, N6A 5B7; +Department of Biochemistry, Michigan State University, East Lansing, Michigan, USA.

We have previously reported (R.A.S. and H.J.K.) that DNA isolated from B cell lymphomas induced in chickens by chicken syncytial virus (CSV), a member of the reticuloendotheliosis family of retroviruses, contains viral DNA sequences integrated in the vicinity of the chicken c-myc gene (Noori-Daloii et al (1981), Nature 294, 574). A 12.5kb fragment containing CSV and c-myc sequences has been cloned from tumor tissue and mapped by restriction endonuclease mapping (Swift et al, in prep.). This fragment contains 1.3kb of viral genomic sequences, which are flanked by 2 viral LTRS, followed by two exons containing the entire c-myc coding sequence homologous to v-myc. The two exons are separated by an intervening sequence of 0.9kb. The viral LTR closest to the c-myc gene is located 0.7kb upstream from c-myc coding sequences and viral and cellular sequences are oriented with the same 5', 3' polarity. In vitro transcription experiments, utlizing the RNA polymerase II system of Manley et al, are in progress. Appropriate DNA restriction fragments are being used in run-off transcription experiments to map the site(s) of initiation of transcription of c-myc sequences.

0500 A CLONED cDNA PROBE FOR HUMAN TERMINAL DEOXYNUCLEOTIDYLTRANSFERASE (TdT), Susan C. Wolf and Allen E. Silverstone, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, New York 10021

The capacity of TdT to add deoxynucleotide "tails" to the 3'-OH end of DNA in the absence of a primer is commonly utilized in molecular techniques. However, its function in vivo is still not known. Because TdT is present only in immature lymphoid cells, its activity is linked to the molecular events of differentiation. An examination of the biosynthesis of TdT mRNA would not only contribute significantly to our understanding of the enzyme biology but would help clarify the process of cellular differentiation on a molecular level. This endeavor necessitates the construction of a cDNA probe to the TdT message. We have extracted mRNA from a human cell line with high terminal transferase activity. When the mRNA was fractionated over sucrose gradients, the mRNA in the 16-20s peak directed the synthesis of a 60,000 molecular weight peptide immunoprecipitable with anti-TdT. Complementary DNA was transcribed from the mRNA in this fraction and cloned in a plasmid vector. Clones were screened by colony hybridization and hybridization-translation. Preliminary data will be presented utilizing this cDNA probe to determine TdT mRNA levels in cells at various stages of differentiation.

0501 ONCOGENE ORGANIZATION AND AMPLIFICATION IN HUMAN LEUKEMIA, David S. Leibowitz, Carl W. Miller, Kapil Bhalla, Steven Grant, and Arthur Bank. Columbia University, 701 West 168th Street, New York, New York 10032

We have begun to characterize the organization and potential amplification of oncogenes in human leukemia cells. We have isolated high molecular weight DNA from the HL60 and K562 leukemic cell lines, as well as from the blood of patients with acute and chronic leukemia. The DNA is cleaved with Eco RI, Southern blotted to nitrocellulose, and hybridized to ^{32}P labelled viral probes(v-myc, v-src, and v-abl). In confirmation of previous reports, we find significant amplification of sequences hybridizing to the v-myc probe in HL60, and to the v-abl probe in K562 cells. In addition, we find significant amplification of a 3.6 kilobase (kb)band in K562 cells that hybridizes to a v-src probe. This band is present but not amplified in HL60 and human leukemic cell DNAs. This band persists at high stringency hybridization conditions. Several other bands hybridizing to the v-src probe are not amplified in K562 cells. This indicates that the v-src probe is either hybridizing to several src genes, only one of which is amplified, or that other sequences in human cellular DNA are hybridizing to the v-src probe. No amplification of the src, myc, or abl sequences are found in the cellular DNA of five patients with chronic myelogenous leukemia, one with acute myelogenous leukemia, or one patient with chronic lymphocytic leukemia, all with high white blood cell counts. No DNA rearrangements were found in either the cell lines or the patients' cellular DNA with Eco RI digestion and the probes used.

0502 INCREASED NUCLEOSOMAL REPEAT LENGTHS ASSOCIATED WITH THE CHROMATIN OF TRANSCRIBED GENES, R.D. Smith, R.L. Seale and J. Yu, Research Institute of Scripps Clinic, La Jolla, CA 92037

A nucleosomal repeat length represents the average center-to-center distance for nucleosomes along the chromatin fiber. We compared the repeat lengths for bulk chromatin and chromatin from inactive and active genes in the following tissues:

Chromatin in Friend cells	Mean repeat length
bulk -	175.4±1.9b.p.
bulk (HMBA induction)	175.3±2.0b.p.
ε ẏ3 -	175.4±1.8b.p.
ε y3 (HMBA induction)	175.4±3.3b.p.
βmaj -	185.9±3.3b.p.
βmaj (HMBA induction)	185.9±3.4 b.p.

Chromatin in nonerythroid spleen	Mean repeat length
bulk	190.0±2.4 b.p.
βmaj	190.0±2.6 b.p.

Chromatin in B cell lymphoma	Mean repeat length
bulk	195.4b.p.
βmaj	184.4b.p.
immunoglobulin	199.4b.p.

Therefore, we concluded that chromatins of transcribed and potentially transcribed genes have an increased nucleosomal repeat length relative to that of bulk chromatin and inactive genes within the same cell type. One consequence of an increased repeat length is the increased production of mononucleosomes by micrococcal digestion.

0503 INHIBITION OF TRANSCRIPTION AS A MECHANISM OF LYMPHOCYTOTOXICITY IN ADA DEFICIENCY, Alice L. Yu, Steven Matsumoto, David Levey* and John C. Yu,* Univ. Calif. San Diego and Research Institute of Scripps Clinics*, La Jolla, California, 92093.

Lymphopenia is a common feature in children with inherited deficiency of adenosine deaminase (ADA) and in leukemic patients treated with an inhibitor of ADA, deoxycoformycin (dcf). The mechanism of lymphopenia in ADA deficiency has been ascribed to decreased DNA replication. We now present evidence suggesting inhibition of transcription as a new mechanism. Incubation of peripheral blood lymphocytes with deoxyadenosine (dado) and dcf was found to be accompanied by accumulation of dATP and depletion of ATP, and more recently by decreased incorporation of ^{3}H-uridine. The time sequence of reduction in uridine incorporation coincided with that of accumulation of dATP, but preceded the depletion of ATP by several hours, suggesting that decreased uridine incorporation was not caused by ATP depletion, but rather, dATP accumulation. Since the observed reduction of uridine incorporation may result from reduced incorporation of uridine into RNA or DNA, or decreased phosphorylation of uridine, we studied the in vitro transcription using isolated nuclei of lymphoid cells. Incubation of resting lymphocytes with dAdo and dcf for 3 and 15 hours led to 12% and 59%, reduction, respectively, of transcriptional activities in lymphocyte nuclei. In addition, transcription was also inhibited by 27% and 47% in nuclei of a T-lymphoblast line incubated with dado and dcf for 3 and 18 hours, respectively. Preliminary data suggested that inhibition of elongation of RNA chain synthesis by dATP could not account for the observed reduction in transcription, since dGTP, dCTP and dTTP caused similar degree of inhibition. Possibilities of a block at the initiation of transcription or processing of RNA will be discussed.

0504 DIFFERENT MAMMALIAN RETROVIRUS ONC GENE FAMILIES TRANSFORM DISTINCT HEMATOPOIETIC TARGET CELLS IN VITRO. Jacalyn H. Pierce and Stuart A. Aaronson. National Cancer Institute, Bethesda, Maryland 20205

The array of hematopoietic cellular targets whose growth and differentiation can be altered by different retroviral onc genes might be expected to provide information concerning similarities in the function of such genes. BALB- and Harvey-MSVs are members of a common onc gene family, while another family of transforming viruses, arising from distinct cellular onc genes, includes avian Rous and Fujinami sarcoma viruses, and mammalian Abelson-murine leukemia virus Ab-(MuLV) and Snyder-Theilen feline sarcoma virus (ST-FeSV). An in vitro colony forming assay that detects transformation of lymphoid cells by Ab-MuLV was used to demonstrate that BALB- and Harvey-MSV transform a novel lymphoid progenitor cell that is distinct from the pre-B lymphoid cell transformed by Ab-MuLV under identical assay conditions. Cells from BALB- and Harvey-MSV induced colonies could be established as continuous cell lines that demonstrated unrestricted self-renewal capacity and leukemogenicity in vivo. These cells lacked Thy-1 antigens and did not synthesize immunoglobulin μ chain, but they did contain high levels of terminal deoxynucleotidyl transferase, an enzyme associated with lymphoid progenitor cells. In contrast, cell lines established from colonies induced by ST-FeSV in the in vitro transformation assay possessed low levels of TdT, Fc receptors, and many synthesized immunoglobulin μ chain in the absence of light chains. This phenotype is analogous to that of Abelson-MuLV lymphoid transformants. These results support the concept that retroviruses within the same onc gene family may utilize a common pathway by which they exert their oncogenic potential.

0505 NUCLEOTIDE SEQUENCE OF THE ENV GENE OF THE ERYTHROLEUKEMIA-INDUCING RETROVIRUS, FRIEND SFFV, L. Wolff, E. M. Scolnick and S. K. Ruscetti, NCI, Bethesda, MD

The Friend spleen-focus forming virus (SFFV) is a replication defective retrovirus which causes a rapid erythroleukemia in susceptible strains of mice. The env gene of this virus encodes a glycoprotein, gp52, and has been previously shown to contain (1) the genetic region responsible for pathogenicity and (2) unique sequences derived by an in vivo recombinational event between an ecotropic helper virus and endogenous cellular sequences. For the present study, the nucleotide sequence of this gene was determined and used to deduce the amino acid sequence of gp52. When these sequences were compared to those of published ecotropic virus env sequences, it was deduced that the N-terminus of gp52 is encoded by the endogenously acquired genetic information and the C-terminus by a region which is homologous to ecotropic viral DNA. A deletion in the gene occurred during the generation of the virus, removing sequences from regions encoding the two retroviral envelope proteins, gp70 and p15E. The data show that gp52 is hybrid protein with a covalent linkage between gp70- and p15E-related sequences. Implications of this finding will be presented. In addition, the endogenously acquired sequences of the erythroleukemia-inducing SFFV were compared with the published sequence of a lymphoma-inducing virus, Moloney mink cell focus-inducing virus. Although these viruses cause different types of hematopoietic diseases, they have closely related unique sequences within their env genes.

0506 A TIME COURSE STUDY OF MULV ENV GENE EXPRESSION IN AKR THYMUS: QUALITATIVE AND QUANTITATIVE ANALYSIS OF ECOTROPIC AND RECOMBINANT VIRUS GENE PRODUCTS, Nancy G. Famulari and Dawn Cieplensky, Memorial Sloan-Kettering Cancer Center, New York, NY 10021

We have identified low levels of synthesis of two species of MuLV env gene polyprotein (PrENV protein) in thymocytes of 3 mo old AKR mice. Synthesis of PrENV proteins which comigrate with those of ecotropic and recombinant, dualtropic MuLV represented, respectively, 0.03-0.05% and 0.01-0.03% of total cell protein synthesis in these animals. An increase in the rate of synthesis of both PrENV species was observed in animals of 5-6 mo of age; ecotropic virus gene products represented 0.2-0.6% of total protein synthesis and recombinant PrENV 1-2.5% of total protein synthesis in thymocytes of mice of this age. This same increase in env gene expression of both the ecotropic and recombinant virus was induced in 3 mo old animals by intrathymic injection of recombinant MuLV at 4-6 weeks of age. The level of recombinant virus env gene synthesis in thymomas was similar to that observed in thymocytes of 5-6 mo old animals and in experimentally injected animals; elevated synthesis of ecotropic virus PrENV protein was detected in 85% of these leukemias. Partial protease digest mapping of the recombinant virus PrENV protein isolated from 23 primary thymomas revealed that the predominant type of recombinant (18/23) expressed in these cells was that of the MCF 247 type. A notable finding was the identification of expression of variant ecotropic MuLV in these thymomas. Of nine leukemias studied, eight expressed ecotropic virus PrENV proteins which were distinguishable from that of Akv virus. Four unique ecotropic virus PrENV proteins were observed.

0507 COMPARISON OF PATHOGENIC AND NONPATHOGENIC ISOLATES OF AKR DUALTROPIC (MCF) MULV, Paul O'Donnell*, Nancy Famulari*, Maria Lung, and Nancy Hopkins. *Memorial Sloan-Kettering Cancer Center, NY 10021 and Center for Cancer Research, MIT, Cambridge,MA 02139

Expression of MuLV env gene-coded antigens and thymocyte differentiation alloantigens on the surface of thymocytes infected by either of two classes of recombinant viruses was studied by flow microfluorometry. Expression of MuLV gp70 was comparable for the two classes of virus and highest on cortical thymocytes. Pulse-labelling of infected thymocytes also showed that rates of synthesis of the env gene polyprotein were comparable for the two classes. At steady-state infection (30-40d postinjection intrathymically) no significant differences could be detected in the levels of expression of Thy 1.1, Lyt1.2, Lyt2.1, or H-2K antigens on thymocyte subpopulations compared to control mice. Also, there were no apparent changes in the percent of total cells represented by small (cortical and medullary), intermediate, and large thymocyte subpopulations in infected mice. However, in mice injected with a pathogenic recombinant virus a novel population of cells could be detected by 45d postinjection which displayed properties of both cortical and intermediate-sized thymocytes.

In order to define genetic differences related to leukemogenicity, T1 oligonucleotide maps of three nonpathogenic viruses were compared to those of several pathogenic viruses. The striking difference was that all of the pathogenic MuLV recombinants share an oligonucleotide (#18) with Akv ecotropic MuLV which maps 227-244 bases downstream from the start of p15(E) and which is missing from the nonpathogenic recombinants.

0508 ONC GENE AMPLIFICATIONS AND TRANSLOCATIONS IN LEUKEMIA AND LYMPHOMA CELLS, Riccardo Dalla Favera[1], Eric H. Westin[1] Carlo M. Croce[2] and Robert C. Gallo[1], [1]Laboratory of Tumor Cell Biology, N.C.I., Bethesda, MD 20205, [2]Wistar Institute of Anatomy and Biology, Philadelphia, PA 19104.

Studies on the expression of several cellular onc-genes in different human tissues have indicated that c-myb and c-myc may be involved in hematopoietic cell proliferation and/or differentiation. The sequence homology between these cellular onc genes and the corresponding hematopoietic cell-transforming viral onc genes (v-myb and v-myc) is consistent with the idea that leukemic transformation may be due to abnormal regulation of cellular onc genes. Among the several models proposed for this mechanism we found evidence that gene amplification and chromosomal rearrangements may play a role in altering the physiologic regulation of the c-myb and c-myc genes in different types of leukemias and lymphomas. We report that the c-myc gene has been found 20-30 fold amplified in the genome of promyelocytic leukemia cells HL-60. This amplification correlates with relatively high levels of myc mRNA and was detectable in the primary cells from the same individual before establishment of the cell line. By analogy the c-myc gene was found amplified in another leukemic cell line, ML-3, also correlating with increased transcription into mRNA. Furthermore, chromosomal mapping by the somatic cell hybrid technique has allowed us to determine that c-myc is located in the region of chromosome 8 that is translocated in Burkitt lymphoma cells. In some cases this translocation brings the c-myc gene in close proximity to the heavy chain immunoglobulin locus on chromosome 14. Other examples of chromosomal rearrangements involving the c-myc and c-myb genes in different types of leukemic cells will also be presented.

0509 FUNCTIONAL MAPPING OF THE EPSTEIN-BARR VIRUS(EBV) GENOME BY FUSION-MEDIATED MICROINJECTION, David J. Volsky, Faruk Sinangil, Ilya M. Shapiro, Walter King* and Elliot Kieff*, Department of Pathology, University of Nebraska Medical Center 42nd & Dewey Avenue, Omaha, NE 68104, and*Kovler Viral Oncology Lab, University of Chicago, Chicago, Ill.

There are presently no mutants of EBV which would allow correlating between viral functions and the responsible viral genes. An alternative approach is to study the function of isolated EBV-DNA and DNA fragments. We report here that EBV-DNA and cloned DNA fragments are expressed in fresh human B lymphocytes and lymphoblastoid cell lines following the fusion-mediated microinjection. We used Sendai virus(SV) envelope reconstitution system to entrap and microinject DNA. The overall efficiency of the process has been studied using nick-translated, (^{3}H)-labeled EcoR1-B fragment probe. Up to 2.5 µg of viral DNA were loaded into fusogenic vesicles in the standard reconstitution system (1mg of SV envelope protein). Maximally 20-50ng of DNA in reconstituted vesicles could be then applied on $1X10^{6}$ recipient cells. The microinjection efficiency was 10% when using fresh B lymphocytes and 20% with lymphoblastoid cell lines. Thus, the technique allows microinjection of 10-25 DNA molecules per cell. EcoR-1 A and B fragments induced early (EA) and viral capsid (VCA) antigens after microinjection into Raji and Lukes cells of Burkitt's lymphoma origin. Whole EBV-ENA induced the EBV nuclear antigen (EBNA) and cellular DNA synthesis after microinjection into human B-lymphocytes. This approach will allow to dissect and ultimately map the EBV-related functions in natural host cells.

0510 MOLECULAR ANALYSIS OF LYMPHOID TUMOR-ASSOCIATED CHROMOSOME TRANSLOCATIONS, Jerry M. Adams, S.D. Gerondakis, Elizabeth Webb, Lynn Corcoran, Ora Bernard and Suzanne Cory, The Walter and Eliza Hall Institute of Medical Research, Melbourne, Victoria 3050, Australia.

We have cloned the regions of DNA corresponding to the chromosome translocations most frequently associated with murine plasmacytomas (15;12) and human Burkitt's lymphomas (8;14). For both types of neoplasm, the rearrangement involves the cellular gene c-myc homologous to the oncogene (v-myc) carried by the acute tumorigenic retrovirus avian myelocytomatosis virus (MC29) and which derives from mouse chromosome 15 and human chromosome 8. In plasmacytomas, rearrangement fuses the c-myc gene back to back with the immunoglobulin α heavy chain (C_α) gene on chromosome 12. For C_α, the breakpoint lies 5', within the so-called switch recombination region (S_α) normally utilised to associate C_α with a functional immunoglobulin variable region gene and thereby permit α heavy chain expression. For c-myc, the breakpoint is almost invariably localised to an ∿1kb region lying 5' to the exon homologous to the 5' v-myc sequence. Rearrangement prevents transcription of this region into mRNA, and new types of myc mRNAs are detected. The data suggests an "altered gene" model for the development of these neoplasms. Nucleotide sequencing studies are underway to delineate more precisely the changes in c-myc structure and expression.

0511 IDENTIFICATION AND CHARACTERIZATION OF DNA REARRANGEMENTS CORRESPONDING TO LYMPHOID TUMOR-ASSOCIATED CHROMOSOME TRANSLOCATIONS, Suzanne Cory, S.D. Gerondakis, Elizabeth Webb, Lynn Corcoran, Ora Bernard and Jerry M. Adams, The Walter and Eliza Hall Institute of Medical Research, Melbourne, Victoria 3050, Australia.

We have shown by molecular cloning that the chromosome translocations most frequently associated with murine plasmacytomas (15;12) and human Burkitt's lymphomas (8;14) involves the cellular homologue c-myc of the oncogene v-myc carried by the acute tumorigenic retrovirus MC29. The c-myc gene is translocated to the immunoglobulin heavy chain locus. Detailed Southern blot analysis of 25 murine plasmacytomas and 12 Burkitt's lymphomas has been performed to define the regions involved in the chromosome breakpoints. Nucleotide sequence studies are underway to investigate in more detail the nature of the chromosome recombination process.

0512 ACTIVATION OF ONCOGENE BY CHROMOSOME TRANSLOCATION IN B-CELL NEOPLASIA, L. Harris*, L. Stanton*, J. Erikson†, R. Watt†, C. Croce†, R. Greenberg*, P. Fahrlander*, J.-Q. Yang* and K. Marcu*. *State University of New York at Stony Brook, NY 11794 and †Wistar Institute of Anatomy and Biology, Philadelphia, PA 19104.

NIARD (Non-Immunoglobulin Associated Rearranging DNA) resides on mouse chromosome 15 at the breakpoint of a translocation (T(12;15)) commonly observed in plasmacytomas. S_α (the switch region of the α heavy chain immunoglobulin gene) is one of the genes which may become fused with NIARD because of these translocations. NIARD is the only sequence in the mouse genome detected by a cDNA specific for the transcript of the human c-myc (denoted H c-myc) sequence (the human analog of the v-myc oncogene of avian myelocytomatosis virus, strain MC-29). NIARD contains the mouse c-myc gene and is most likely an oncogene. The H c-myc gene detects DNA rearrangements in Burkitt's Lymphoma and appears to be at the breakpoint of the rcpT(8;14) translocations seen in this disease. We have evidence that M c-myc expression is both quantitatively and qualitatively different in plasmacytomas as compared to non-neoplastic tissue: 1) the c-myc gene within NIARD is transcribed in plasmacytomas at a 10-20x greater level than in spleen and at a 50-100x greater level than in liver; and 2) mRNA of 1.8 to 2.1 kb is detected in plasmacytomas possessing NIARD associated translocations, while only a 2.3 kb species is detected in spleen; 3) these new transcripts may be due to activation (or derepression) of a new promoter.

0513 DNA REARRANGEMENTS IN HUMAN LEUKEMIAS, Bruno Calabretta, and Grady F. Saunders, The University of Texas System Cancer Center M.D. Anderson Hospital and Tumor Institute, Houston, TX 77030.

Subtle rearrangements of genetic material could be associated with initial events leading a normal cell to the neoplastic transformation. The occurrence of DNA rearrangements in human leukemias has been investigated using DNA probes of low reiteration frequency initially present in clusters of Alu repeat sequences. This approach is based on the hypothesis that repetitive DNA facilitates sequence rearrangements.

With this approach two types of genetic rearrangements have been detected: Variations in copy number and restriction fragment length polymorphism of sequences homologous to the "Inter-Alu sequences" probes that have been used in this study. Sequences hybridized to pλH15A, a recombinant probe containing a human insert 0.8 kb in length are present in approximately 20-50 copies in DNA derived from normal leukocytes. In seventeen cases of ALL and AML the copy number of those sequences is constantly less than 5 copies, in contrast of nine patients chronic milogenous leukemias examined, 5 had copy number comparable with that found in healthy individuals, and 4 comparable with that found in acute leukemias.

With pλH27A, a recombinant probe containing a human insert 1 kb in length, a distinct pattern of restriction fragment polymorphism have been detected in some cases of ALL. These results confirm the presence of specific loci associated with molecular genetic variations in human cancers.

0514 MURINE ERYTHROID PROGENITOR CELLS EXPRESS A UNIQUE MULV ENCODED GP70, William J. Britt and John Portis, Department of Pediatrics, University of Alabama Medical Center, Birmingham, Alabama 35294 and LPVD, Rocky Mountain Laboratories, Hamilton, Montana 59840.

Previous studies have shown the presence of a non-ecotropic, murine leukemia virus (MuLV) encoded 70,000 dalton glycoprotein (gp70) on the surface of Friend virus (FV) - induced erythroleukemia cells. Characterization of this gp70 with a panel of mink cell focus-inducing (MCF) and xenotropic-MuLV specific monoclonal antibodies indicated it was antigenically unique, unrelated to a large number of different MCF and xeno-MuLV. Further investigations into the origins of this gp70 revealed the expression of an antigenically identical gp70 on 12 day embryonic liver cells as well as on a number of in vitro maintained FV-induced erythroleukemia cell lines. Structual analysis of this protein by chymotryptic peptide mapping indicated the gp70 on embryonic liver cells and FV-induced erythroleukemia cells were identical. A small percentage of normal spleen and bone marrow cells expressed this gp70, however this protein was not expressed on thymocytes, B-lymphocytes or fibroblasts. Furthermore, induction of anemia with phenylhydrazine resulted in a five to ten fold increase in the frequency of spleen and bone marrow cells expressing this gp70. These results suggested this unique gp70 was expressed only on erythroid cells and, therefore, might be a differentiation associated gp70 much like previously described gp70 on thymocytes.

0515 AVIAN ERYTHROBLASTOSIS: PROMOTER-INSERTION ACTIVATION OF C-erb$_B$ BY ALV LTR AS STUDIED BY DIRECT SEQUENCING AND CLONING ANALYSIS OF THE ACTIVATED C-erb$_B$ GENE, Hsing-Jien Kung , Wynne Lewis , Maribeth Raines , Yuen-Kai Fung and Lyman B. Crittenden, Michigan State University and USDA Regional Poultry Research Lab, E. Lansing, MI 48824.

Avian leukosis virus (ALV) induces a spectrum of neoplasms (e.g., lymphomas, erythroblastomas, nephroblastomas) in chickens. The studies of the induction of B-lymphomas by this virus led to the conclusion that ALV activates host oncogene by insertion of its LTR near the target gene. Thus, if ALV LTR can integrate adjacent to and activate different oncogenes, different neoplasms would develop. This offers one explanation for the multipotency of ALV. To test this hypothesis, we have characterized erythroblastosis (Ery) induced by ALV. Previously, based on Southern blot analyses, we showed that a host oncogene c-erb is altered in at least 50% of the Ery samples. In most of them, c-erb$_B$ but not c-erb$_A$, is the locus where ALV provirus integrates and activates. This implicates that c-erb$_B$ is the target gene for Ery. We now have confirmed this notion by direct cloning and sequencing analyses of an activated c-erb$_B$ gene. The clone carries the first 5' exon related to v-erb$_B$ and the intron adjacent to it. The ALV LTR is attached to the c-erb$_B$ at a site about 500 to 700 nucleotides upstream from the first exon and points to a direction same as c-erb$_B$. The last two nucleotides of ALV LTR are not present - a phenomenon associated with provirus integration. All these features are in agreement with the promoter-insertion model proposed by Hayward et al. In the characterization of other altered c-erb$_B$ fragments, it was found that some appears to contain only exons but not introns. Based on these findings, the mechanisms of ALV induced Ery will be discussed.

0516 COMPARISON OF NUCLEOLAR DNA-TOPOISOMERASE II ACTIVITY IN FANCONI ANEMIA AND OTHER CHROMOSOME BREAKAGE SYNDROMES, George Studzinski, Zamir Brelvi and Cesar Fernandez, UNDMJ-New Jersey Medical School, Newark, NJ 07103.

The presence of an enzyme with properties of a DNA-topoisomerase II can be demonstrated in the nuclei of cultured human cells by means of electron-microscopic cytochemical methods. The enzyme activity is heavily concentrated in the areas of nucleolar matrix which border on the intranucleolar channels. Less marked activity is found intermittently along the heterochromatin of the chromosomes. The activity of this enzyme is not altered by the transformation of the cells by the SV-40 virus. Comparison of the enzyme activity in the cells derived from normal subjects with the cells of patients with genetic defects predisposing to defective DNA repair and neoplasia show that while Fanconi's Anemia and Ataxia Telangiectasia have undiminished activity, the enzyme activity is significantly reduced in nuclei of cells from Bloom's Syndrome and Xeroderma Pigmentosum (XP) complementation group A. Even more striking is the almost complete absence of the evidence of this enzyme's activity in XP cells complementation group D. These data suggest that a DNA-topoisomerase II, an enzyme shown in many systems to determine the degree of the negative supercoiled tension of DNA, may be one of the factors necessary for the maintenance of chromosome integrity. The implications of these findings for the development of hematologic and solid tumor malignancies are being explored. (Supported by a grant from the American Cancer Society #IN-92K).

0517 INDUCTION OF TISSUE TRANSGLUTAMINASE IN MONOCYTES AND MACROPHAGES, Peter J.A. Davies, Michael P. Murtaugh, Kapil Mehta and Rudolph L. Juliano, University of Texas Medical School at Houston, Houston, TX 77025.

Tissue transglutaminase is a cytoplasmic enzyme that catalyzes both the covalent crosslinking of membrane proteins and the covalent incorporation of polyamines into proteins. The enzyme may play a role in the receptor mediated endocytosis of ligands by fibroblasts and macrophages. Inflammatory macrophages show 10 times higher levels of transglutaminase activity than do quiescent cells. To study the regulation of transglutaminase in macrophages we have prepared a monospecific, polyclonal antibody to tissue transglutaminase and used it to quantitate the enzyme in control and stimulated macrophages and monocytes. In mouse peritoneal macrophages the enzyme is present at a concentration of 20ng per mg cell protein (0.002%). Following attachment and culture in mouse serum containing media, the levels of the enzyme show no change for 60 min and then between 80-100 min begin to increase. The enzyme can accumulate at least 100 fold, increasing progressively for 24-48 hours and reaching levels of 1500ng per mg cell protein (.15%). Studies with inhibitors suggest induction is due to increased synthesis of transglutaminase mRNA. In human blood monocytes, transglutaminase increases 20-60 fold in cells cultured for 7 days. Induction of transglutaminase in monocytes shows a relative lag of 4 days. Bacterial LPS shortens this lag phase to 48 hours. Induction of transglutaminase appears to be an early and prominent change assosciated with terminal differentiation of macrophages. Induction of this enzyme may be related to enhanced receptor mediated endocytosis in differentiated macrophages.

0518 TRANSLOCATION OF THE HUMAN AND MOUSE C-MYC GENE TO THE HEAVY CHAIN LOCUS IN B CELL NEOPLASIA, Carlo M. Croce, The Wistar Institute of Anatomy and Biology, Philadelphia, PA 19104

By using somatic cell hybridization and Southern blotting techniques we have shown that the c-myc oncogene, that is on chromosome 8 in human and on chromosome 15 in mouse, translocates to the Ig heavy chain locus in human and mouse B cell neoplasia. As a result of the translocation, activation of the transcription of the c-myc gene occurs in these malignancies.

0519 Three distinct patterns of <u>in vitro</u> erythroid transformation by Kirsten (KiSV), Abelson (AbLV), Friend Spleen Focus Forming Virus (SFFV). Judi Luna, Claudia Chen, and W. David Hankins. Natl. Cancer Inst. Bethesda, Md. 20205

We showed previously that many murine retroviruses induced, in vivo and in vitro, extensive proliferation of erythroid precursors. Active viruses included envelope recombinants (e.g. SFFV) and cellular "onc" recombinants (e.g. myeloproliferative, Harvey, Kirsten, and Balb Sarcoma viruses). The virus-infected precursors were hypersensitive to erythropoietin and gave rise to large clusters of hemoglobin positive cells (erythroid bursts) in methylcellulose. AbLV also induced erythroid bursts from fetal tissues (Waneck and Rosenberg, Cell 26:79, 1981.)

From an analysis of erythroid burst induction by SFFV, KiSV, and AbLV in adult (spleen and marrow) and fetal tissues (liver, placenta, and peripheral blood), reproducible differences in the erythroid effects of these viruses were noted. Therefore, a transformation profile for each virus was established with regard to the preferred target tissue and the hormone sensitivity, size, time of appearance, clonal morphology and cellular composition of the virus-induced erythroid bursts. These results suggest that SFFV, KiSV, and AbLV affect different target cells, evoke qualititively different transformations, or both.

We have also examined the type of hemoglobin in fetal tissues and virus-induced bursts. On days 10-11 of gestation (when liver is poorly developed) the red cells in fetal blood are large, nucleated and contain embryonic hemoglobin. Preliminary electrophoretic analysis indicates that virus-induced erythroid bursts, from this tissue, synthesize predominantly adult hemoglobin.

0520 TWO COMMON REGIONS FOR PROVIRAL DNA INTEGRATION IN MoMuLV INDUCED RAT THYMIC LYMPHOMAS, Philip N. Tsichlis, P. Günter Strauss and Li Fu Hu, National Cancer Institute, Bethesda, Md. 20205

Moloney murine leukemia virus (MoMuLV) causes no morphologic transformation in infected tissue culture cells, as is the case for other mammalian and avian retroviruses that lack a transforming gene. However, following injection into an appropriate animal host, MoMuLV induces mainly thymic lymphomas after a long latency period. A common characteristic of neoplasms induced by retroviruses lacking transforming genes is their clonal nature. In the present study we have generated MoMuLV induced rat thymic lymphomas and confirmed their clonal nature. Furthermore we took advantage of the clonality of these tumors to investigate the specificity of provirus integration in the tumor DNA. We reasoned that if several independently derived thymic lymphomas would contain the provirus integrated in the same region of the cellular DNA, this would be a strong indication that this integration event is a contributing factor in oncogenesis. The specificity of provirus integration in the thymomas was tested by cloning provirus-host junction fragments from two tumors. The cellular DNA specific sequences of these clones were used as probes to detect a common integration substrate in restriction endonuclease digested DNA from 16 independent thymomas. The results indicate that there are indeed two cellular DNA regions (termed MLVI-1 and MLVI-2 locus) that serveas common substrates for proviral DNA integration in several of the tumors we examined.

0521 ALTERATIONS OF GLOBIN GENE CHROMATIN DURING DIFFERENTIATION OF ERYTHROLEUKEMIA CELLS, John Yu, Ronald L. Seale and R.D. Smith, Research Institute of Scripps Clinic, La Jolla, CA 92037

During differentiation of murine erythroleukemia cells chromatin of β major globin gene was found to become increasingly sensitive to micrococcal nuclease and low levels of DNAse I, suggesting structural maturation of this gene accompanying this differentiation process. The β globin gene in induced cells is more sensitive to μg/ml concentration of DNAse than uninduced cells. Furthermore, the actively transcribed β globin gene in induced cells is most sensitive to micrococcal nuclease, followed by the potentially transcribed β globin gene in induced cells and finally by non-transcribed genes such as embryonic globin (ε γ3) and immunoglobin (μ7) genes. We found that dexamethasone can inhibit such structural maturation of β globin gene chromatin, as reflected by the decrease of sensitivity toward both nucleases to the degree found in uninduced cells. Moreover, two distinct DNAse I hypersensitive sites are found on the 5' side of the β major globin gene. One hypersensitive site, located near the cap site of β major globin gene, was present only in chemically induced cells. The other, which is located 3560 bp upstream of the cap site, was also more sensitive to DNAse I in induced cells compared to uninduced cells. We found that dexamethasone inhibits the formation of these two differentiation-related DNAse I hypersensitive sites. In addition, it is shown that alteration of chromatin structure for β major globin gene must accompany the process of commitment of these cells toward terminal differentiation so that it becomes insensitive to dexamethasone afterwards.

0522 NUCLEOTIDE SEQUENCE OF FELV-B ENVELOPE GENE REVEALS HOMOLOGIES WITH A MURINE MCF VIRUS, John H. Elder, Research Institute of Scripps Clinic, La Jolla, CA, 92037

We have determined the nucleotide sequence of the envelope gene of feline leukemia virus, subtype B (FeLV-B). Comparison of this sequence to the sequences of several murine retrovirus envelope genes revealed that the gp70 of FeLV-B had considerable homology with the substituted (presumably xenotropic) portion of Moloney-derived MCF virus gp70 (M-MCF). Whether the similarities between FeLV-B and MCF gp70's are ancestral or are related to host range characteristics of these two viruses is yet to be determined. Experiments are in progress using synthetic peptides in an attempt to determine if common regions correlate with targets of virus neutralization on heterologous species cells. The amino acid sequence of FeLV-B gp70 dictates 12 possible sites of glycosylation versus 7 or 8 previously reported murine retrovirus gp70's. Whether this marked difference in glycosylation has a functional role (i.e. in affecting host immune surveillance) or whether an increased number of glycans is simply tolerated is the subject of future studies.

0523 GAG-MOS PROTEINS OF MOLONEY MURINE SARCOMA VIRUS (MuSV) HAVE AN ASSOCIATED PROTEIN KINASE, R. Arlinghaus, W. Kloetzer, G. Gallick, S. Maxwell and L. Stanker, Dept. Tumor Virology, UT M.D.Anderson Hospital and Tumor Institute, Houston, Texas 77030.

Three variants of Moloney MuSV encode gag-mos proteins of either 85,000 or 100,000 d. These proteins contain p15, p12 and varying amounts of p30 fused to a nearly complete v-mos gene product. Each of these gag-mos proteins, in contrast to $P37^{mos}$, is made in amounts easily detected by pulse-labeling infected cells with ^{3}H-leucine. Each is phosphorylated in vivo. To date, two have been shown to have an associated protein kinase activity when anti-p30 immunoprecipitates are incubated with γ-^{32}P ATP. Non-producer NRK cells (6m2) infected with ts110 Moloney MuSV produce $P85^{gag-mos}$ and $P58^{gag}$ at 33^{o} but only $P58^{gag}$ at 39^{o}. Immune complex kinase assays derived from 6m2 cells grown at 33^{o} have shown that $P85^{gag-mos}$ and $P58^{gag}$ are phosphorylated. Regarding the coding origin of the kinase, temperature-shift experiments in vivo indicate that both the kinase and the transforming function associated with $P85^{gag-mos}$ are temperature-sensitive. Cells begin to revert to a normal morphology within 2 hr after $33^{o} \rightarrow 39^{o}$ shift. Kinase activity is sharply reduced within 30 min even though P85 levels remain unchanged during this 2 hr period. In vitro heat treatments at $39^{o}C$ also indicate that the kinase is specifically heat-labile. Neither $P85^{gag-mos}$ nor $P58^{gag}$ were phosphorylated in kinase assays derived from 6m2 cells maintained at 39^{o} for 24 hr. Using extracts from virus producing cultures, $P85^{gag-mos}$ is phosphorylated in anti-p30 complexes but $Pr65^{gag}$ is not. All of these findings support the interpretation that the kinase activity is closely associated with $P85^{gag-mos}$.

0524 DELETIONS IN THE HUMAN β-GLOBIN LOCUS. Roberto Taramelli, Paula Henthorn, Elio Vanin, Dimitris Kioussis and Frank G. Grosveld. National Institute for Medical Research, Mill Hill, London NW7 1AA. U.K.

We have recently analyzed a Dutch case of γβ-thalassaemia. In this patient a large part of the β globin region 5' of the β globin gene has been deleted (ϵ- $^{G}\gamma$-$^{A}\gamma$-δ). We have cloned this defective locus and have shown that the remaining β-globin gene is normal and can be expressed in transformation experiments. Preliminary data indicate that the β-globin gene from the defective locus is hypermethylated in vivo in erythroid tissue and we are presently analyzing the transposed DNA to determine whether this DNA is responsible for the methylation and inactivation of the β-globin on the mutant chromosome.
In addition, recent results indicate that a common mechanism underlies the generation of different γβ thalassaemias and the generation of different HPFH phenotypes, a condition in which the sequences 3' to the γ genes (δ and β genes) have been deleted. We are presently analyzing the exact extent of these deletions by chromosome walking to provide a basis for a study on the mechanism by which such large deletions are generated.

0525 Regulation of Globin Genes after DNA Transformation into Mouse Erythroleukemia Cells. Pamela Mellon*, Moses Chao#, Patrick Charnay*, Richard Axel# and Tom Maniatis*
Harvard University, Cambridge, MA 02138* and Columbia University, New York, NY 10032#

We have transferred cloned mouse and human beta globin genes into mouse erythroleukemia cells (MEL) to study the regulatory sequences involved in induction of globin expression during differentiation of these cells in culture. The genes were cotransferred with the hamster APRT gene into APRT deficient MEL cells and stable APRT positive clones were isolated. The transferred genes are present at 1 to 200 copies per cell. Upon induction the transferred gene RNA transcripts increase 5 to 50 fold in 90% of the cell lines examined. The amount of RNA loosely correlates with the DNA copy number and appears to be correctly initiated. The mRNA of the transferred mouse beta major gene can be distinguished by S1 nuclease analysis from the endogenous transcripts by virtue of the substitution of a human third exon in the cloned gene. Nuclear transcription experiments demonstrate that the transferred gene is activated at the transcriptional level.

In order to more precisely define the sequences required for induction, we have analysed a series of truncated plasmids which contain from 1.2 kb of flanking sequence to none. Thus far genes with as little as 78 bp of 5' flanking sequence remain fully inducible and genes with 53 bp are constituitive or fail to transcribe. Thus we have localized sequences required or induction to within 78 bp of the 5' end of the mouse beta major globin gene.

0526 PROTEIN SYNTHESIS IN DIFFERENTIATING NORMAL AND tsAEV TRANSFORMED ERYTHROID CELLS, Becky Adkins, Hartmut Beug and Thomas Graf, Deutsches Krebsforschungszentrum, 6900 Heidelberg, West Germany.

Avian erythroblastosis virus (AEV) induces erythroleukemia in chickens and transforms immature erythroid cells from chicken bone marrow in vitro. Erythroblasts transformed by temperature sensitive mutants of AEV (tsAEV) can be induced by a shift to the restrictive temperature (42^{0}C) to synthesize hemoglobin and to differentiate terminally in vitro. To examine whether the synthesis of non-globin proteins is altered during this differentiation process, tsAEV infected erythroblasts were placed at 42^{0}C for 3-4 days and subsequently fractionated by Percoll density gradient centrifugation into sub-populations highly enriched for cells at different stages of maturity (erythroblasts, reticulocytes, or erythrocytes). The pattern of protein synthesis in cells from each fraction was examined by two-dimensional gel electrophoresis of 35S-methionine labelled cell lysates. Changes in protein synthesis could be grouped into 3 categories: (1) major increases, (2) major decreases, and (3) the de novo appearance of polypeptides during in vitro differentiation. To determine whether the same changes take place during the maturation of erythroid precursors in vivo, normal erythroid cells were immunoselected from total bone marrow cells by a "panning" technique, fractionated by Percoll density gradient centrifugation and lysates were analyzed by 2-D gel electrophoresis. Approximately 40 % of those changes found in tsAEV cells were also shown to occur during normal erythroid differentiation. Furthermore, these alterations in protein synthesis were shown to be specifically associated with erythroid differentiation since they were not observed in wtAEV transformed erythroblasts or in ts or wt AEV transformed fibroblasts shifted to 42^{0}C. Some of these differentiation specific proteins are being characterized further as to subcellular location, possible post-translation modifications, and reactivity with a series of erythroid-specific monoclonal antibodies.

0527 GENETICS OF HLA CLASS II GENES AND THEIR EXPRESSION IN LYMPHOID CELLS. Janet Lee, John Trowsdale and Walter F. Bodmer, Imperial Cancer Research Fund, London.

Cosmid clones were isolated from human genomic DNA libraries using a cDNA-probe corresponding to HLA-DRα chain. In addition to the gene coding for HLA-DRα chain, two sets of overlapping cosmids related but not identical, clearly non-allelic and mapping to chromosome 6, were identified. One of the cosmids contains an α chain gene probably coding for a DC antigen α chain, and the other probably for SBα chain. Interestingly, both of these sets of cosmids also contain HLA-DRβ gene-like sequences

We have now begun studies on expression of both α and β like genes on the RNA of human lymphoid cell lines. In several cases, expression has been observed in cells previously thought to be HLA-DR negative. In addition, the complexities of the mRNAs present have been different from those found in the HLA-DR positive B lymphoblastoid lines suggesting different structures for their protein products. Moreover, these findings imply that the Class II genes may function at very early steps in differentiation which the lines are thought to represent. It is possible that the human equivalent of I-J may be found amongst these products.

0528 EXPRESSION OF CELLULAR ONCOGENES IN EMBRYOGENESIS AND MALIGNANCY, D. Slamon, R. Müller, I. Verma, and M. Cline, UCLA, Los Angeles, 90024 and Salk Inst., La Jolla, CA 92138.

The acutely transforming RNA tumor viruses are known to contain specific genetic information responsible for malignant transformation. This information resides in a portion of the viral genome termed the oncogene. The viral oncogenes (v-oncs) appear to have arisen from a cohort of normal cellular genes (c-oncs). C-oncs are highly conserved across a broad span of evolution ranging from Drosophila to man. Little is known about their function in normal tissue; however, the avidity with which they are conserved connotes possible important physiologic functions. These genes may play a role in normal cell proliferation and/or differentiation. Using v-oncs as hybridization probes to detect homologous c-onc messenger RNA, we examined mouse embryos from day 6 to 18 of gestation. We observed significant levels of expression of 7 different c-oncs during development. Many of these were expressed in a time-related and/or tissue-specific pattern. Given both the close homology between the v-oncs and c-oncs and the role of all of the v-oncs and at least 3 of the c-oncs in malignant transformation in various animal models and cells in culture, we examined pathologic c-onc gene expression in human malignancies. Using the presence of mRNA as a parameter of expression, we screened a wide variety of fresh human tumors and corresponding normal tissues obtained from surgical specimens. Of the 15 known c-oncs, 6 were expressed at significantly elevated levels in malignant as compared to normal tissues. These included c-myc, c-fos, c-ras^{Ha}, c-ras^{Ki}, c-myb and c-fes. Based on these observations, we postulate that c-oncs have a critical role in physiologic functions in normal cell proliferation and/or differentiation, e.g., during development. Their untimely or inappropriate expression may result in neoplasia.

0529 INTERACTION OF DNA METHYLTRANSFERASE (DMase) WITH CARCINOGEN-MODIFIED DNA TEMPLATES.
Jean-Numa Lapeyre, David Finkel and Mathuros Ruchirawat, M.D. Anderson Hospital and Tumor Institute, Houston, TX 77030.

Several lines of evidence implicate DNA-cytosine methylation by the enzyme DMase as one level of gene regulation in eukaryotes. Demethylation of DNA during specific gene activation events and during development has been recently reported. We are presently studying the properties of carcinogen-modified DNA templates on the enzymatic activity of purified DMase and crude DNA demethylase isolated from Friend erythroleukemia cells using anti-benzo(a) pyrene (BPDE), N-acetoxy aminofluorene (N-OH AF), N-methylnitrosourea (NMU), and dimethylsulfate (DMN) as model carcinogens. DMase is purified over 1500-fold by phosphocellulose, hydroxylapatite, DEAE-Sephacel, and AcA34 chromatography. BPDE and N-OH AF adducts on poly(dC-dG) did not alter the circular dichroism spectra from that of the unreacted copolymer in normal B conformation under the conditions of salt and temperature employed in the assay. However, dose dependent modifications by BPDE or N-OH AF of poly(dC-dG) or M. lysodecticus DNA produced a progressive inhibition in DMase ability to methylate such modified templates. Competition experiments designed to test the selectivity of DMase binding to carcinogen modified or unmodified DNAs show almost irreversible binding of the enzyme to BPDE modified DNA. Results on the effect of other carcinogen modifications of DNA templates will be presented. Supported by NCI grants CA 31487 and CA 20657.

0530 MOUSE GENES CONTROL THE TYPE OF HEMATOPOIETIC NEOPLASM INDUCED BY FRIEND HELPER VIRUS
Jonathan E. Silver, Torgny N. Fredrickson and Wallace P. Rowe, National Institutes of Health, Bethesda, MD 20205

Biologically cloned NB-tropic Friend helper virus (F-MuLV) causes erythroleukemia in BALB/c mice inoculated as newborns. The latent period for development of this disease is 2-3 months (median 85 days, range 45-220 days, incidence >90%, N = 53 mice). C57BL/6 mice are completely resistant to erythroleukemia but develop lymphoma and/or myelogenous leukemia 5-6 months after inoculation as newborns (median 160 days, range 54-274 days, incidence >80%, N = 26 mice). This strain difference in type of leukemia induced by F-MuLV is not due to differences in XC-positive virus replication in the spleen or thymus of BALB/c versus C57BL/6 mice. Studies of F1 and first and second backcross mice suggest that one dominant gene confers resistance to erythroleukemia. This gene is distinct from H-2, Fv-1 and Fv-2. Studies of BALB/c x C57BL/6 recombinant inbred mice show that genes controlling the type of leukemia induced by F-MuLV are distinct from genes controlling the duration of the latent period: two recombinant inbred strains have the same incidence curve for development of disease, but one strain develops erythroleukemia (incidence >75%, N = 42 mice) while the other strain develops lymphoma and myelogenous leukemia (incidence >75%, N = 39 mice). Analysis of mouse genes which affect which hematopoietic cell lineage is transformed by Friend helper virus may provide new insights into post- F-MuLV replication steps in viral leukemogenesis.

Hematopoietic Stem Cells

0531 ESTROGENIC INHIBITION OF CARBOHYDRATE METABOLISM IN MOUSE BONE MARROW,
Michael P. Dieter and John E. French, NIEHS, Research Triangle Park, NC 27709

There is some evidence in animals for an increase in leukemogenesis and lymphoid tumors following estrogenic exposure. These hormones are believed to potentiate cellular response to carcinogenesis, perhaps by modulating biochemical reactions. B6C3F$_1$ female mice were treated with diethylstilbestrol, b-estradiol, a-dinestrol, or zearalenol. Bone marrow enzymes from the pentose shunt were more sensitive to estrogens than glycolytic or Kreb's cycle enzymes. Inhibition of pentose shunt dehydrogenase enzyme activities were proportional to dose and correlated with the estrogenic activity of the chemical administered. There was no effect from b-dinestrol or zearalenone suggesting the 'A' ring in the active steroids was necessary for enzyme inhibition. Ablation experiments showed estrogen inhibition of enzymes was not dependent on thymus or adrenal. The intact bone marrow was then divided into three cell fractions by Percoll density gradient centrifugation. We found the granulocyte-rich portion (density=1.038-1.048 g/ml) accounted for 50% of the pentose shunt enzyme activity with 20% each attributable to the lymphocyte-rich (density= 1.087-1.097 g/ml) and the erythrocyte-rich (density=1.100-1.121 g/ml) portions. Presently, the relative sensitivity of these three cell populations to estrogenic inhibition of pentose shunt enzyme activities is being investigated.

0532 Human long term marrow cultures derived from cells treated with a monoclonal anti-Ia antibody and complement. JW Singer and A Keating, University of Washington and the VA Medical Center, Seattle.

Ia antigen is expressed on more than 80% of human committed hematopoietic progenitors measurable in semisolid culture (CFU-C and BFU-E) and also may be expressed on a CFU-C precursor population. We have established long term marrow cultures from cells treated with a monoclonal antibody to Ia (7.2) and rabbit complement (C') to determine whether the stem cells on this system express the Ia antigen. When marrow cells were treated with 7.2 + C', 84 ± 6 (mean ± SD; n = 3) percent of CFU-C were lost. Long term marrow cultures established from these cells were morphologically normal and produced CFU-C for more than 8 weeks. The 7.2 treated cultures produced significantly fewer non-adherent cells and CFU-C than did the controls for the first 2 weeks. However, after week 3, the 7.2 + C' cultures produced nearly as many cells and CFU-C as did the controls. In 1 of 3 experiments, the treated cultures produced 89% of the number of control CFU-C, despite an initial 90% loss after antibody treatment. In the 3 experiments, the 7.2 treated cultures produced a mean of 57% of control CFU-C. If CFU-C production between weeks 3 and 7 between the treated and control cultures is compared, then the 7.2 treated flasks yielded 89% of control CFU-C. These data suggest the following: 1)CFU-C produced by long term marrow cultures after week 3 are predominantly derived from Ia-negative progenitors, 2)the long term culture system provides a useful, albeit indirect, manner for examining these progenitors, 3)anti-Ia treated marrow cells may be safe to use in autologous marrow transplantation despite committed progenitor toxicity.

0533 MORPHOLOGICAL EXAMINATIONS OF MURINE GRANULOCYTE/MACROPHAGE AND MEGAKARYOCYTE COLONIES GROWN IN CHEMICALLY DEFINED CULTURE MEDIUM, Kazuo Kubota(1), Kazuma Ikeda(2), Sachiko Kajigaya(1), Hiroko Ijima(3), Fumitoshi Taketazu(1), Shigeru Shionoya(1), Masaki Saito(2), Kazuo Motoyoshi(1), and Yasusada Miura(1,2), Division of Hemopoiesis, Institute of Hematology (1), Division of Hematology, Department of Medicine(2), and Department of Pediatrics(3), Jichi Medical School, Minamikawachi-machi, Tochigi 329-04, Japan.

Murine bone marrow granulocyte/macrophage and megakaryocyte progenitor cells proliferated to form colonies consisting of their progeny in chemically defined culture medium in the presence of colony-stimulating activity(CSA). C57BL/6 or BALB/c bone marrow cells were mixed in 0.3% agar in growth medium with CSA. The growth medium used here was Iscove's modified Dulbecco's tissue culture medium supplemented with bovine serum albumin, human transferrin, cholesterol, and L-α-phosphatidylcholine. C57BL/6 spleen conditioned medium stimulated by pokeweed mitogen (SPCM) was used as a source of CSA. No fetal calf serum was used for preparing SPCM. After 7 or 8 days of incubation, colonies were scored under an inverted microscope. Then, whole plate preparations were made with the technique previously described and the morphology of individual colonies were examined after the double esterase stainings. The cloning efficiency for the chemically defined culture system was almost compatible to that for control culture system containing 20% fetal calf serum. However, individual colonies in chemically defined cultures were smaller in size when compared to those in the control cultures. Since the CSA used in this study was crude conditioned medium, further study using purified CSA will be required and such a study is now under way.

0534 CANINE HEMOPOIESIS: THE RESPONSE TO INFECTION WITH E. COLI., T.J. MacVittie, L. Casey, M. Smith, M. Fink, G. Murano, D.F. Gruber, M. Patchen, R. Walker, H. Gelston, and J.J. Conklin, Armed Forces Radiobiology Research Institute and Naval Medical Research Institute, Maryland 20814.

A model of peritoneal sepsis was needed in order to determine the mechanisms of the radiation- and trauma-induced predisposition to sepsis in the canine. Two models were selected, both involving an initial laparotomy followed by the injection of live E. coli (10 ml at 2 x 10^8 organisms/ml) into the wall of the large intestine, or the placement of freshly prepared fibrin clot containing the bacteria into an intraperitoneal space near the liver. Control animals received an injection of saline or the placement of a sterile fibrin clot. The parameters measured were peripheral blood values, white cells (PBL) and platelets (PLT); plasma content of colony stimulating activity (CSA); acute phase reactant, fibrinogen and C-reactive protein (C-RP); bone marrow derived granulocyte-macrophage colony-forming cells (GM-CFC) and erythroid progenitors (CFU-e) and peripheral blood derived GM-CFC. Peripheral blood elements: a rise in PBL was observed to peak at 7 d post infection and then decline to below normal levels by d 14. Platelet levels declined to less than 75% of normal through day 10 irrespective of surgical consequences. Bone marrow: GM-CFC concentration declined during the initial 24 h and rose sharply to values 270% of normal by 48 h then declined to values 200% of normal through d 10 followed by a slow decline to normal by d 21 post-infection. CFU-e concentration declined significantly within 24 h and then increased to values 150% of normal by 7 d. Peripheral blood: GM-CFC increased sharply to peak values nearly 5000% of normal 72 h post-infection. These results, as well as those obtained from clinical measurements will be compared with the fibrin clot approach in order to determine a valid model for canine peritoneal sepsis.

0535 MYELOID ASSOCIATED DIFFERENTIATION ANTIGENS ON STEM CELLS AND THEIR PROGENY IDENTIFIED BY MONOCLONAL ANTIBODIES, Robert G. Andrews, Beverly Torok-Storb and Irwin D. Bernstein, Fred Hutchinson Cancer Research Center, Seattle, WA 98104

We have used monoclonal antibody technology to detect lineage restricted myeloid associated differentiation antigens. We have characterized a series of monoclonal antibodies which react with determinants on committed myeloid stem cells and their progeny. Their reactivity with peripheral blood cells and committed hematopoietic progenitor cells has been determined by both cytotoxic assays and fluorescence activated cell sorting. Antibody 1G10 which reacts with cells of the granulocytic lineage and with a minor subset of mature monocytes was shown to react with CFU-GM. Three antibodies T5A7, L4F3, and L1B2 were shown to react with both granulocytic and monocytic cells and in fluorescence activated cell sorting studies to detectably stain granulocytic cells at different stages of maturation. These three antibodies also react with CFU-GM, two (L4F3 and L1B2) reacting with all CFU-GM, while T5A7 reacts with only a portion of the day 7 CFU-GM. Antibody L4F3 also reacts with a portion of BFU-E. In contrast, antibody 5F1 which reacts with monocytic cells, nucleated erythroid cells, and platelets was shown to react with CFU-E and to block CFU-E growth in vitro in the absence of complement. Strategies using these antibodies to selectively enrich progenitor cells of a single hematopoietic lineage are being developed in order to study regulation of differentiation. In addition, the mechanism by which 5F1 blocks CFU-E growth is also being studied.

0536 CHARACTERIZATION OF FACTOR-PRODUCING AND FACTOR-DEPENDENT CLONES FROM LONG-TERM HAMSTER BONE MARROW SUSPENSION CULTURES, C. Eastment and F. Ruscetti, National Cancer Institute, Bethesda, Maryland 20205

Long term hamster bone marrow (BM) cultures produce stem cells which can be grown in the absence of an adherent layer and without exogenous growth factors or hormones. (Blood 60:130-135, 1982) Cloning of 3-5 month old suspension cultures by limiting dilution method has resulted in the development of factor-producing clones (H_6S_5) and factor-dependent clones (H_7S_{17}). H_6S_5 and its subclones have been in culture 15 months, are macrophage, and grow as either adherent or suspension cells. Analysis of serum-free conditioned medium (CM) from H_6S_5 revealed the production of stimulatory and inhibitory effects on hematopoiesis. The addition of 1-10% CM to either fresh or cultured hamster bone marrow results in the formation of CFU_C and epo-dependent BFU_E in agar and increased numbers of myeloid and erythroid cells in suspension. Addition of 10-15% CM produces substantial inhibition of erythropoiesis. Analysis of several subclones produced the same range of positive and negative stimulation of hematopoiesis. H_7S_{17} is dependent on 5% hamster spleen CM for growth. The clone contains variable numbers of blasts macrophages and myeloid elements. No erythroid or multipotent progenitors could be detected in this clone. Spleen CM stimulates a tenfold increase in growth and differentiation in a dose-dependent manner and stimulates the formation of granulocyte-macrophage clones in agar. CM from H_6S_5 can substitute for the spleen CM in growth of H_7S_{17} but addition of both CM generates 4 times more cells than either CM alone. Existence of these clones allows studies on the interaction of factor-producing and factor-responding cells in the growth and differentiation of hematopoietic progenitor cells.

0537 HUMAN FETAL HEMATOPOIESIS AT AN EARLY STAGE OF DEVELOPMENT, Inna A. Svet-Moldavsky, Svetlana N. Zinzar and Constantin Iliescu, Mt. Sinai Medical Center, New York, N.Y. 10029

Femoral marrow cell suspensions from human fetuses 14-16 weeks old were studied in liquid culture (RPMI 1640 medium supplemented with 20% fetal calf serum) and in a double layer agar system. Normal adult bone marrow cells were used as controls. Fibroblast cloning efficiency was studied using inocula from 10^4 to 10^7 cells per 75 cm^2 flask. After 7 days at 37°C and 5% CO_2 the flasks were stained by May-Grunwald-Giemsa and fibroblast plaques were counted macroscopically. Fetal bone marrow cell suspensions display at least 10 times greater cloning efficiency than adult bone marrow.

In a double layer agar system for each individual fetal or adult bone marrow large variations were found in the number of granulocyte-macrophage colonies formed on feeder layers supplemented with human tumor cell line (#5637) granulocyte-macrophage colony stimulating factor. Mean number of granulocyte-macrophage colonies was 111 for fetal bone marrow (range 39 to 370) and 84 for normal adult bone marrow (range 35 to 146). Human fetal bone marrow fibroblast monolayer or intestinal fibroblast monolayer as feeder cells in a double layer system can support granulocyte-macrophage colony formation from fetal bone marrow CFU-C but not from adult. Adult bone marrow fibroblast monolayer as feeder cells in a double layer agar system supports both fetal and adult bone marrow granulocyte-macrophage colony formation.

0538 IDENTIFICATION AND CHARACTERIZATION OF HEMATOPOIETIC-LYMPHOID DIFFERENTIATION ANTIGENS, Bob G. Sanders, Kimberly Kline, Bradley W. McIntyre, and James P. Allison, University of Texas, Austin, TX 78712 and University of Texas System Cancer Center, Science Park Research Division, Smithville, TX 78757

Monoclonal and polyclonal antibodies recognize hematopoietic-lymphoid membrane glycoproteins that are differentially expressed on cells undergoing normal cellular differentiation and on virally-transformed erythroid and lymphoid cell lines. Antisera to chicken fetal antigens (CFA) and chicken adult antigens (CAA) identify 14 and 7 serological determinants, respectively. Expression of CFA and CAA is developmental and cellular differentiation stage dependent. Avian erythroblastosis virus-transformed erythroid cells express both CFA and CAA but undergo qualitative and quantitative CFA/CAA changes when induced to undergo cellular maturation. Lymphocytes, granulocytes, macrophages, and erythrocytes express distinct subsets of CFA/CAA. An erythroid specific monoclonal antibody reacts with reticuloendotheliosis virus-transformed lymphoid cells, yet no radiolabeled molecule is immunoprecipitated, suggesting that CFA-antigenic sites may exist on different core molecules from cells of different lineages. Two-dimensional IEF-SDS-PAGE analyses with neuraminidase treatment show CFA and CAA to be glycoproteins Limited proteolytic peptide analyses reveal homologies among the different molecular species of CFA and CAA. Contributions of the carbohydrate moiety to CFA and CAA antigenicity and molecular weight are being examined with endo-β-N acetylglucosaminidase F treatments.
NIH Grant CA 12851, BGS; and CA 26321 and CA 26981, JPA.

0539 COMPARISON OF B CELL SURFACE ANTIGENS DETECTED BY MONOCLONAL ANTIBODIES ON NORMAL AND MALIGNANT HEMATOPOIETIC CELLS, Theodore F. Zipf, Gilles Lauzon, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada T2N 4N1, and B. M. Longenecker, Department of Immunology, University of Alberta, Edmonton, Alberta, Canada, T6G 2H7

Monoclonal antibody 41H.16 was produced using cells from a patient with hairy cell leukemia. This antibody is of the mouse IgG2a isotype and reacts strongly with normal B cells while neither resting nor activated T cells show any reactivity. Malignant cells from 40 patients with CLL show strong antibody binding while no reactivity was observed with an equal number of patients with non T/non B ALL. Antibody is bound by cell lines SB, 8392, Raji, K562, ML1, and 6.16. No binding is observed for Daudi, NALM-6, NALM-12, or BALM-1. Determinations by immunoprecipitation and $NaDodSo_4$/polyacrylamide gel electrophoresis yield a molecular weight of 43,500 Daltons. Antibody binding for normal and malignant cells has been measured by quantitative flow cytometry. The density of the antigen detected by antibody 41H.16 has been compared to the densities of antigens detected by previously reported monoclonal antibodies against other B cell surface antigens for both normal and malignant lymphoid cells.

0540 PARENT-PROGENY RELATIONSHIP BETWEEN MURINE CFU-S AND CFU-DG, Eero Niskanen, Division of Hematology-Oncology, University of Virginia, Charlottesville, Virginia 22908

Bone marrow cells were cultured in diffusion chambers implanted into the peritoneal cavity of mice for 5 and 8 days (CFU-DG). After harvest, individual colonies were removed from the clots formed inside the chambers, placed in McCoy's 5A medium, and disrupted by mechanical teasing. The resulting cell suspensions were injected into lethally irradiated mice. Spleens were analyzed on day 8 or day 12 for CFU-S. Of the 5 day diffusion chamber colonies, 74% gave rise to 12 day CFU-S and 13% to 8 day CFU-S. Of the CFU-DG obtained following 8 days of culture, 63% and 23% gave rise to 12 and 8 day CFU-S. In another set of experiments, individual spleen colonies were dispersed in McCoy's 5A medium and placed in diffusion chambers which were implanted in mice. Of the 8 and 12 day spleen colonies, 0% and 30% respectively, gave rise to CFU-DG harvested on day 5. None of the CFU-S gave rise to CFU-DG harvested on day 8. Based on these observations we conclude that the majority of the cells which give rise to colonies in diffusion chambers detected after 5 days of culture may be at least as primitive as CFU-S observed on day 12. Ability of some 12 day CFU-S to give rise to 5 day CFU-DG indicates that these precursors may occasionally be identical.

0541 ROLE OF POLYAMINE BIOSYNTHESIS IN MURINE HEMATOPOIESIS, G. Thomas O'Conor, Jr., and Eero O. Niskanen, University of Virginia, Charlottesville, Virginia 22908

In a previous study murine hematopoiesis was enhanced, as reflected by increased numbers of CFU-S, CFU-DG and CFU-C per tibia, by the administration in drinking water of 2% difluoromethylornithine (DFMO), a specific inhibitor of ornithine decarboxylase (ODC), which results in decreased synthesis of putrescine, a precursor of spermidine and spermine. Methylglyoxal-bis-(guanylhydrazone) (MGBG) is another polyamine synthesis inhibitor which decreases spermidine and spermine synthesis via inhibition of the enzyme S-adenosylmethionine decarboxylase (SAMD). When mice were injected i.p. with MGBG at doses up to 25 mg/kg/day for 11 days, there was no effect on hematopoiesis as determined by peripheral blood counts, bone marrow cellularity and the number of CFU-C, CFU-DG and CFU-S per tibia. No effect was observed on colony formation by bone marrow cells placed in diffusion chambers, implanted in mice and exposed to MGBG by i.p. injection of 25 mg/kg/day for 4 days. The combination of DFMO and MGBG (25/mg/kg/day), while having no effect on bone marrow cells exposed in situ to both drugs for 11 days, did cause a significant decrease in the number of colonies formed (25 ± 8) by cells placed in diffusion chambers in mice and then exposed to both drugs for 4 days, compared to untreated controls (101 ± 15) ($p < 0.01$). Concurrent administration of putrescine (200 mg/kg/day i.p.) abrogated this effect, indicating that it was dependent upon depletion of putrescine. These findings suggest that ODC is a critical enzyme in regulation of hematopoiesis.

0542 SELECTION OF MURINE LYMPHOBLASTS BY CALCIUM AND MAGNESIUM DEPRIVATION. J.K. Brennan. University of Rochester School of Medicine, Rochester, New York 14642.

Neoplastic cells have decreased calcium, magnesium and serum requirements for growth in culture compared to their untransformed counterparts. To gain additional insights on the relationship of these parameters to each other and to neoplasia, I have exposed sequential populations of mouse lymphoma cells (L5178Y) to medium reduced in Ca and Mg and isolated three variant subpopulations, each derived from its predecessor by more stringent deprivation. The maximum doubling times in normal medium (McCoys 5A medium + 10% fetal bovine serum, 1 mM Ca + 1 mM Mg); Ca + Mg and serum requirements for 50% of maximum dt; and tumor deaths following i.p. injection of 1 x 10^4 wild type and variant cells are shown below. These data indicate that

Population	Maximum Doubling Time (Max DT) (hr)	Ca + Mg Req. 50% Max DT (μM)	Serum Req. 50% Max DT (%)	Tumor Deaths per 25 BDF1 Mice at Risk
Wild Type	10	100	4.5	4
Variant 1	12	30	1.5	13
Variant 2	11	25	4.0	7
Variant 3	12	25	1.0	8

L5178Y populations are heterogeneous with regard to calcium, magnesium and serum requirements and, at least in the case of variant 1, reduced requirements are associated with enhanced oncogenicity. L518Y and its variants have stable phenotypes when maintained in normal medium and may be useful in studies of divalent cation metabolism.

0543 HIERARCHY OF GRANULOCYTIC COMMITTED STEM CELLS IN HUMAN LIQUID MARROW CULTURES. F.T. Slovick, C.N. Abboud, J.K. Brennan & M.A. Lichtman, University of Rochester, Rochester, New York 14642.

We have established human long term liquid marrow cultures in order to study the survival of committed granulocytic stem cells and their subtype composition by triple stain histochemical techniques. We quantitated the presence of progenitors (CFU-Cs) at Days 7 & 14 of culture as well as pre-CFU-Cs in a liquid system involving a 7 day incubation period with GCT and Mo cell line derived conditioned media as sources of colony stimulating factors. All analyses were performed on non-adherent cells at the time of biweekly feeding of cultures as well as on the adherent cells treated with collagenase on a weekly basis. In this CFUs were maintained for a mean of twelve weeks in over 10 experiments in the non-adherent cell compartment without recharging. Initially most CFUs on Day 7 of culture were pure neutrophils while at Day 14 they were mixed neutrophil-monocyte. By day 28, few day 14 eosinophil colonies remained and the predominant phenotype was pure macrophagic colonies at day 14. In addition to these changes in colony subtype, pre-CFUs rapidly declined in the non-adherent cell layer over the first 4 weeks. Analysis of the adherent cell compartment showed that although the initial CFU-C content was lower, these progenitors declined more slowly than those in the non-adherent cell compartment, so that by 3-4 weeks there were more CFU-C in the adherent compartment than in the non-adherent one. In addition, pre-CFUs survived longer in the adherent cell compartment. Data reflecting the effect of T-cell addition to this system will be presented.

0544 EXPRESSION OF MURINE LYMPHOCYTE ANTIGENS ON THE SURFACE OF HEMOPOIETIC STEM CELL AND PRECURSOR CELLS, W. Mark and U. Hammerling, Sloan-Kettering Inst., New York, NY 10021

Five murine lymphocyte surface antigens have recently been defined using monoclonal antibodies (Tada et al., 1981, in Monoclonal Antibodies and T-cell Hybridomas; Hammerling, Hammerling & Kearney, editors; Elsevier/North Holland Biomedical Press; Kimura et al., 1981, Immunogenetics 13:547; Kimura, unpublished results). Three of these lymphocyte alloantigens have restricted tissue distribution (Ly-m6*, Ly-m19, Ly-m20) and the other two determinants (Ly-m10, Ly-m18) are expressed on a wide variety of tissues. Since these lymphocyte antigens are found on bone marrow cells, a study was done to determine whether hemopoietic stem cells and precursor cells also express these antigenic determinats. Adult bone marrow cells were treated with the monoclonal antibodies in the presence of complement. The surviving cells were then assayed for their abilities to form colonies in the spleen (CFU-S) and in vitro (CFU-C). Results from these studies indicate that while all 5 antigenic determinants are found on CFU-C, only 4 of the 5 lymphoid alloantigens studied are on CFU-S. The antigen Ly-m10 is present on > 90% of both types of colony-forming cells. On the other hand, antigens Ly-m6*, Ly-m18, Ly-m19 and Ly-m20 are detectable on only a fraction of the CFU-C population (40-60%). Similarly, Ly-m6*, Ly-m18 and Ly-m19 are present on between 40-70% of the CFU-S population. Given these findings we are now investigating the possibility that some of these lymphoid alloantigens mark different subpopulations of hemopoietic stem cell and/or precursor cells.

0545 STUDIES OF NATURAL KILLER (NK) and NATURAL CYTOTOXIC (NC) CELL PRECURSORS IN MOUSE BONE MARROW, J. Hackett, Jr., M. Bennett, and V. Kumar. Univ. of Texas Hlth Sci Center, Dallas, TX 75235. Studies of NK and NC cells have revealed differences with respect to target spectrum, dependence upon bone marrow (BM) microenvironment and surface antigens. To investigate whether this heterogeneity reflects stages of differentiation of a common precursor or derivation from separate committed precursors, we developed an in vivo NK/NC stem cell assay. Transplantation of 1-5 X 10^6 BM cells into lethally irradiated syngeneic recipients results in generation of new splenic NK/NC cells from their precursors. Prior to day 8 no significant lytic activity is detected even if host spleen cells are preincubated with interferon (IFN). Between days 8 and 10 some NK (YAC-1) lytic activity can be detected which is greatly augmented with IFN. There appears to be a transition from an IFN non-responsive state to IFN responsive state of NK cells. Studies to determine whether this reflects a quantitative change or a qualitative change in NK cells are in progress. The NK alloantigen detected by CE X NZB anti-CBA serum is present on mature NK cells. Treatment of BM cells with anti-NK-serum + C prior to transfer into irradiated recipients did not reduce CFU-S or precursors of NK cells. Treatment with the myelotoxic drug dimethyl myeleran (20 mg DMM/kg) reduced marrow cells by 75% , CFU-S 40-fold, spleen cells by 50% and NK activity 75% by day 5. Ten days after transfer of BM cells from mice treated with DMM vs. vehicle revealed an 8-fold reduction in splenic cellularity and NK activity and a complete lack of NC (WEHI-164.1) activity. Analysis of NK/NC cells after a smaller dose of DMM indicated that CFU-S and NC cells may be closely related.

0546 REGULATION OF HUMAN B-CELL COLONY FORMATION AND CELL DIFFERENTIATION Rozenszajn LA, Goldman J, Weiss E. Radnay, J. Clinical Laboratories, Meir Hospital, Kfaf Saba, and Department of Life Sciences, Bar-Ilan University, Ramat-Gan, Israel

To study human B-cell colony growth a two-layer soft agar culture system has been developed which enables human normal and leukemic B-cell enriched population to a colony formation ($SmIg^+$ cells) in the presence of mitogens as PHA or PWM. B-cell proliferation and expansion were dependent on the presence of a limited number of T cells among the seeded cells. This dependency might be substituted, at least partially by a soluble factor produced by T cells. An elaboration of the role of monocytes and the influence of monocyte related substances (PG) of B-cell colony formation was performed. Culturing a monocyte-depleted B-cell enriched population the number of colonies was higher than when a B-cell enriched inoculum was plated. It was shown that T cells and monocytes actively contribute to the physiological mechanism regulating B-lymphocyte proliferation, colony formation and differentiation. The work was supported by a by research grant of the Israel Academy of Sciences and Humanities and Israel Ministry of Health.

0547 RELEASE OF MONOCYTE-DERIVED ACIDIC ISOFERRITIN - INHIBITORY ACTIVITY (AFIA) AGAINST CFU-GM IS CONTROLLED BY HLA-DR RESTRICTED LYMPHOCYTE-MONOCYTE INTERACTIONS, Hal E. Broxmeyer and Bo Dupont, Memorial Sloan-Kettering Cancer Center, New York, NY 10021

AFIA suppresses the formation in vitro of colonies and clusters from an $HLA\text{-}DR^+$ subpopulation of cycling CFU-GM of normal donors, but not from $HLA\text{-}DR^-$ cycling CFU-GM from patients with leukemia. Cell interactions modulating the release of AFIA from monocytes were evaluated. AFIA was assessed in medium conditioned by separated subpopulations of blood mononuclear cells in the presence of 10^{-6} M indomethacin by its capacity to inhibit colony and cluster formation of normal bone marrow CFU-GM stimulated by GM-CSF. AFIA derives from an $HLA\text{-}DR^+$ subpopulation of human monocytes and cell interactions were involved in its release. Constitutive release from 10^5 monocytes/ml was suppressed completely by an $HLA\text{-}DR^+$, $OKT3^+$, $OKT8^+$ subpopulation of T lymphocytes and release of AFIA from 10^3 monocytes/ml, which do not constitutively release AFIA, was induced by an $HLA\text{-}DR^+$, $OKT3^+$, $OKT4^+$ subpopulation of T lymphocytes. These two effects of T-lymphocytes on AFIA release by monocytes were detected in autologous cell mixtures, in mixtures of cells from HLA genotypically identical siblings and in mixtures of HLA-A,B,C,DR phenotypically identical cells from unrelated donors. Matching for only one HLA-DR haplotype provided partial responses. These interactions were not detected with HLA-DR different cell mixtures. Treatment of monocytes with monoclonal anti-DR in the absence of complement did not influence constitutive release of AFIA from 10^5 to 10^6 monocytes/ml, but did block the suppressive and inductive influences of the lymphocyte subpopulations. These results suggest that release of AFIA from monocytes is genetically restricted by HLA-DR at the level of T-lymphocyte-monocyte interactions.

0548 EVIDENCE THAT HUMAN MEGAKARYOCYTOPOIESIS IS CONTROLLED IN VIVO BY A HUMORAL FEEDBACK REGULATORY SYSTEM, Eric M. Mazur, Pedro de Alarcon, Karen South and Laurie Miceli, The Mary Imogene Bassett Hospital, Cooperstown, NY 13326.

We have previously demonstrated that aplastic anemia sera contain an activity (Meg-CSA) that promotes the in vitro proliferation and differentiation of the human megakaryocyte progenitor cell (CFU-M). Using plasma clot cultures of normal human bone marrow (BM) cells, serial serum Meg-CSA levels were assayed in 4 patients during 5 courses of intensive antileukemic chemotherapy (CT). Meg-CSA elevations were first detected 4 to 7 days after initiating CT, during the fall of the peripheral platelet counts (PPC). In 2 instances, Meg-CSA levels of 2½ and 4½ times baseline were seen while the PPC still remained over 100,000/mm^3. Meg-CSA peaked 7-11 days after initiating CT and remained elevated throughout the periods of BM hypoplasia. However, within the 12-hour period following 5 of 6 evaluable platelet transfusions (PTx), Meg-CSA was depressed to 28-64% of pre-PTx levels. This depression was transient, occurring as early as 1½ hours after completion of the PTx and dissipating in all cases within 60 hours. In one instance, Meg-CSA returned to pre-PTx levels within 3¼ hours. Meg-CSA generally returned to pre-PTx levels before the PPC. Complete BM recovery (BMR) was ultimately accompanied by a decrease of circulating Meg-CSA to pretreatment levels. This fall in Meg-CSA commonly lagged behind clinical evidence of BMR and persistently elevated Meg-CSA was seen during two instances of BMR at times when the PPC had risen to over 100,000/mm^3. Meg-CSA is a potent humoral megakaryocytic stem cell regulator which can be induced by intensive CT. The control of circulating Meg-CSA is complex with evidence of influence by both BM megakaryocyte mass and PPC. Its regulatory function may be specific for the platelet-megakaryocyte axis.

0549 REGULATION OF HUMAN MEGAKARYOPOIESIS: LACK OF IN VITRO STIMULATORY ACTIVITY OF HUMAN URINARY ERYTHROPOIETIN. PA de Alarcon, EM Mazur, LA Miceli, K South. The Mary Imogene Bassett Hospital, Cooperstown, N.Y.

The role of erythropoietin (EPO) as a stimulator of in vitro megakaryopoiesis in the plasma clot culture system for colony forming unit-megakaryocyte (CFU-M) is controversial. Some investigators have shown a positive relationship between the dose of EPO and the number of CFU-M. Others have failed to confirm this data. Serum from patients with aplastic anemia has been reported to contain an activity that stimulates in vitro human CFU-M (Meg-CSA). To further clarify the role of EPO in megakaryopoiesis we studied the dose response curve of CFU-M to human urinary EPO in the plasma clot colony assay for CFU-M from human bone marrow mononuclear cells. Human urinary EPO CAT-1 with a specific activity of 1140 u/mg of protein was obtained from the National Heart, Lung and Blood Institute. Bone Marrow mononuclear cells were planted at a concentration of $5x10^5$ cells/ml. In the absence of added EPO, Meg-CSA at concentrations of 0, 20 and 30% induced the formation of a mean of 30, 153 and 250 colonies per culture respectively. When EPO was added to the culture system at final concentrations of 0.5, 1, 2 and 4 u/ml of culture medium a mean of 19, 9, 20 and 18 colonies was produced. When 4 u/ml of EPO were added to a culture containing 30% Meg-CSA a mean of 181 colonies was obtained. Simultaneous colony forming unit-erythroid (CFU-E) and burst forming unit-erythroid (BFU-E) assays planted in plasma clot from the same bone marrow cells produced a mean of 245 CFU-E and 111 BFU-E at EPO concentrations of 1 and 2 u/ml respectively. In our hands, partially purified endotoxin-free human urinary EPO does not stimulate in vitro human megakaryopoiesis. At a concentration of 4 u/ml it has an inhibitory effect.

0550 GENERATION OF LYMPHOCYTES IN A MODIFIED LONG-TERM HUMAN BONE MARROW CULTURE SYSTEM, Carlos A. Izaguirre, Fay Katz, and M.F. Greaves, ICRF Dept Medical Oncology, St. Bartholomew's Hospital and Imperial Cancer Research Fund, London, U.K.

The objective of this study was to establish a method of long-term bone marrow cultures (LTMC) that would permit the study of lymphoid stem cells. Several methods were tested and the following was considered optimum: marrow buffy coat cells was cultured in medium containing 20% human citrated plasma, 10% fetal calf serum (FCS) 5 x 10^{-5}M 2-mercapto ethanol, hydrocortisone, and Iscove's medium. The mixture was placed in a T-25 flask and cultured at 37°C in an atmosphere containing 5% CO_2, 5% O_2, balance N. Cloting ensued providing an extracellular matrix of fibrin where optimum growth of a layer of stromal cells occured. Cultures were maintained weekly using 25% FCS and the other ingredients except plasma. Cells in stromal were Adipocytes, endothelial cells (Factor VIII antigen positive), epitheliod cells and macrophages. Epitheliod cells reacted with anti-human Thy-1 and anti-common ALL(J5) monoclonal antibodies (MoAb). They were HLA-DR negative and negative for other myeloid and lymphoid cell markers. The supernatant was tested at intervals, for the presence of lymphoid stem cells using a lymphoid colony assay (Brit J Cancer 42: 430 1980). Colonial cells were tested for lymphoid markers using immunofluorescence and a panel of MoAb. Lymphoid colonies were consistantly obtained for up to 2 months in culture (maximum time tested so far) 5 experiments, range of colony formation per supernatant: 22 to 53. The cells were positive for HLA-DR, common ALL antigen, B1, Surface Ig, OKT11A and on OKT1 equivalent (UCHT-2) confirming the lymphoid origin of these cells. In summary, we have developed a method of LTMC that permits ... maintenance of lymphoid progenitors for up to 2 months.

0551 USE OF STEM CELL ASSAYS IN MONITORING PLASMAPHERESIS THERAPY OF PURE RED CELL APLASIA. J. Greenberg, H. Schlessinger, F. Gill, and A. Cohen, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, 19104.

Pure red cell aplasia (PRCA) is an uncommon disorder in children. Our patient was 13½ years on presentation with a 3 week history of pallor. No associated disease process could be documented and after failing to respond to prednisone 2-4mg/kg/day, he was maintained on frequent transfusions of PRBC's to maintain a Hb≥8.5gm/dl for an 18 month period. Serum assays of complement dependent serum inhibitory activity (CDSIA) of normal marrow BFU-E and CFU-E growth in vitro documented inhibition, and treatment was begun with an intense plasmapheresis program. Following the 5th treatment, reticulocytes were seen on the peripheral smear which gradually disappeared 14 days following discontinuation of therapy. Reticulocytosis was re-established following every other day plasmapheresis and the patient has been maintained at levels of Hb= 9.5-11.0gm/dl with reticulocytes=0.3-1.4% for the past 18 months, without need for further PRBC transfusions. He currently is maintained on plasmapheresis every 7 weeks. Initial morphology of the bone marrow aspirate (BMA) revealed arrest of erythroid maturation at the early pronormoblast stage with an M:E≥30:1. A BMA done during therapy revealed normal erythroid development with an M:E ratio=2:1. Tests of CDSIA of pre and post treatment samples on the patient's own bone marrow substantiated the loss of inhibitory activity with plasmapheresis therapy. The use of in vitro assays has proved an important tool in selecting and monitoring therapy for patients with PRBC in whom sterilizing doses of cytotoxic therapy might otherwise be indicated. In addition, our patient is the first reported case of PRBC in whom continued maintenance on plasmapheresis therapy has been necessary.

0552 **DIFFUSION CHAMBER CULTURE OF AKR LEUKEMIA CELLS: GROWTH AND DIFFERENTIATION IN RELATIONSHIP TO CELL SIZE, Kathleen Hiller,Barbara Lau,Renate Pelka-Fleischer and Peter Dörmer,Institute of Hematology,Munich,Germany**

The patterns of proliferation and differentiation of spleen cells obtained from mice bearing the long passage AKR leukemia have been studied using the diffusion chamber technique. During the 20-28 days of culture the growth of lymphoid cells and leukemic blasts are most predominant,however,granulopoietic cells and macrophages are also observed. In contrast,normal AKR spleen cells differentiate almost exclusively along the granulocytic pathway. By means of centrifugal elutriation a fraction of GOG1 cells could be isolated from the spleens of leukemic mice whose growth and differentiation potential upon subsequent culture in diffusion chambers far surpasses that of the corresponding unseparated spleen cell population. These observations seem to indicate an inhibitory effect of the malignant cells on the spleen cells responsible for normal hematopoiesis.

0553 CHARACTERIZATION OF mRNA FOR GRANULOCYTE-MACROPHAGE COLONY STIMULATING FACTORS, Aldons J. Lusis, Irvin Chen, David W. Golde, Andrew D. Jacobs, Lawrence Lasky, Kelly O'Donnell and Richard Shadduck, Department of Medicine, UCLA, Los Angeles, CA 90024 and Department of Medicine, Montefiore Hospital, University of Pittsburgh, Pittsburgh, PA

We have translated and partially characterized mRNA for human and mouse granulocyte-macrophage colony stimulating factors (CSFs). The mRNA was isolated from a human T-lymphocyte cell line (Mo), human peripheral blood leukocytes stimulated with phytohemagglutinin (PHA), or mouse L-cells. The Mo line produces CSF-2, which stimulates the formation of both granulocyte and macrophage colonies in cultures of bone marrow cells. When mRNA from the Mo line was injected into frog oocytes, it directed the synthesis of biologically active CSF, which resembled the CSF derived from Mo cells in biolgoical activity and in heat stability. After 2 days of incubation, about 90% of the activity produced by oocytes was present in the incubation medium. As judged by sucrose gradient sedimentation, the size of the CSF-2 mRNA is about 1200 nucleotides, sufficient to encode a protein of molecular weight about 40,000. Mouse L-cells produce CSF-1, which stimulates primarily macrophage colonies in cultures of bone marrow. Injection of L-cell mRNA into oocytes resulted in the production of relatively low levels of activity. We are currently attempting to develop an in vitro translation assay for the CSF mRNA, in which CSF is identified by incorporation of radioactive amino acids into immunoprecipitable CSF. Human peripheral blood leukocytes produce both CSF-1 and CSF-2 when stimulated by PHA. Preliminary results indicate that treatment of leukocytes with PHA results in a rapid (within 48 hours) induction of CSF mRNA. These studies of CSF mRNA are aimed at the molecular cloning of CSF sequences.

0554 LONGEVITY AND SELF-RENEWAL OF MYELOMA COLONIES, Takayuki Takahashi, Nazir Jamal, Bing Lim, Daniel E. Bergsagel and Hans A. Messner, Ontario Cancer Institute and Institute of Medical Science, University of Toronto, Toronto, Ontario, M4X 1K9.

Culture conditions previously described for the growth of multilineage colonies also facilitate the development of plasma cell colonies in some patients with multiple myeloma. Bone marrow and peripheral blood samples of 7 patients with very advanced disease formed myeloma colonies when grown in methylcellulose supplemented with 30% human plasma, 5% PHA-LCM, and 2-mercaptoethanol. Cells within these colonies displayed morphological features of plasma cells and reacted with antibodies directed against the appropriate light chain typical for the patient. The colonies can first be discriminated by day 7. Cells within the colonies continue to proliferate and colonies increase in size to 200-500 cells by day 30. Addition of fresh medium at that time facilitated further cell proliferation and development of colonies that may contain up to 1-2 million cells. Resuspended pooled colonies usually give rise to secondary, tertiary and sometimes further generations of colonies. One sample is currently growing the 4th, and a second sample, the 11th generation of colonies. Recloning experiments of individual colonies yielded considerable heterogeneity ranging from 5-61 secondary colonies. The clonogenic cell population is positive for the typical light chain. Cytoplasmic μ was not seen during any phase of colony development. Colony growth was not observed in 37 patients with multiple myeloma tested during earlier stages of disease and in 5 patients with benign monoclonal gammopathy. In conclusion, long-living and self-renewing myeloma colonies can be grown from bone marrow or peripheral blood samples of patients with very advanced multiple myeloma. Their presence is associated with poor prognosis.

0555 G-6-PD ANALYSIS OF MYELOID AND LYMPHOID CELLS IN MULTILINEAGE COLONIES, Bing Lim, Nazir Jamal and Hans A. Messner, Ontario Cancer Institute and Institute of Medical Science, University of Toronto, Ontario, M4X 1K9.

Some multilineage hemopoietic colonies contain myeloid cells and T-lymphocytes. The latter proliferate extensively in liquid suspension culture under the influence of TCGF (Blood 58: 402, 1981). The clonal origin of myeloid and lymphoid components was determined by the G-6-PD isoenzyme analysis of multilineage colonies grown from peripheral blood of three G-6-PD heterozygous normal volunteers. E-rosette depleted mononuclear cells of density less than 1.077 g/ml were cultured at 2.5 - 5 x 10^4 cells per ml. Multilineage colonies were removed and portions subjected to G-6-PD and T-cell analysis as well as expansion in liquid suspension culture. 191 were of isoenzyme type A, 81 of type B. Both isoenzymes were observed in 9. The isoenzyme distribution was similar to that of single lineage colonies. Eight multilineage colonies contained a sufficiently large number of E-rosette forming cells to generate an isoenzyme band. All 8 colonies produced a single isoenzyme band (3 type A, 5 type B). Seven additional colonies of one single isoenzyme at primary assessment were subsequently expanded in liquid suspension culture. Six contained T-cells of the same single isoenzyme type (3A,3B) as in the original colony. Both isoenzyme types were observed in one expanded colony. The G-6-PD assay is sufficiently sensitive to detect isoenzyme bands contributed by 30 granulocytes and erythroblasts, 4 to 6 megakaryocytes, 2 to 3 macrophages and 50 to 100 T-cells. T-cells can be detected even if myeloid components are present in 10 to 20 fold excess. In conclusion, these findings support that view of a common progenitor for myeloid and lymphoid cells present in the peripheral blood of normal adults.

0556 THE EFFECT OF LITHIUM CHLORIDE (LiCl) ON HIGH PROLIFERATIVE POTENTIAL COLONY FORMING CELLS (HPP-CFC), IN MURINE DEXTER CULTURE, Philip M. Wade and Peter J. Quesenberry, University of Virginia School of Medicine, Charlottesville, Virginia 22908

Studies utilizing murine Dexter cultures have shown that production of multiple stem cells is increased by the presence of LiCl. We have evaluated the effects of LiCl on the "primitive" stem cell HPP-CFC. Cultures were established at 40x10^6/flask from BDF_1 hybrid mice and assayed for supernatant cells, CFU-C, and HPP-CFC weekly for 3 weeks. The stroma was assayed separately at week 3. LiCl (4 mEq/L) stimulated HPP-CFC, CFU-C and cell recovery in both the supernatant and stroma phases (Table 1). Cultures with LiCl (1 mEq/L) were irradiated at 950R at week 3 and then used to condition lithium free media weekly for 9 weeks. Media conditioned on LiCl pre-exposed stroma showed marked increases in CSA activity (compared to media prepared identically from non-LiCl pre-exposed stroma).

TABLE I.

Week	Group	Total Cells (%Control)	CFU-C (%Control)	HPP-CFC (%Control)
1	Supernatant	89	67	230
2	"	222	189	275
3	"	149	152	158
3	Stromal	183	162	247

These findings indicate that LiCl stimulates primitive marrow stem cells in Dexter culture and that stromal elaboration of activity affecting stem cells may be increased by exposure to LiCl.

0557 GENERATION OF MUCOSAL MAST CELLS IN RAT BONE MARROW CULTURE, Ellen E.E. Jarrett, David M. Haig, Thomas A. McKee and Richard Woodbury, University of Glasgow Veterinary School, Bearsden, Glasgow, U.K. and University of Washington, Seattle, Washington, U.S.A.

The connective tissue of mammals contains two distinct types of mast cell that differ in morphology, histochemical staining properties and location. One type - the normal connective tissue mast cell - can be obtained in nearly homogeneous preparation from a mixed cell population in the peritoneal cavity. The other type - the mucosal mast cell - requiring special fixation techniques for its detection in tissues, has been observed only in the mucosae.

Certain conditions are associated with a proliferation of mast cells: the most conspicuous of these is infection with helminth parasites, with parasites of mucous surfaces, in particular, inducing a rapid hyperplasia of mucosal mast cells.

We have found that lymphocytes of helminth infected rats stimulated *in vitro* with specific antigen, or lymphocytes of normal rats stimulated with Con-A release factors causing pronounced mucosal mastocytosis in normal rat bone marrow cultures.

The cultured mast cells show the morphological and histochemical staining characteristics of mucosal mast cells that have previously been defined in *in vivo* systems. Also they contain a very high level of a mucosal mast cell protease which is antigenically distinct from the protease present in normal connective tissue mast cells.

0558 RAPID INDUCTION OF MYELOID LEUKAEMIA BY A NOVEL FeLV. David Onions[1], Oswald Jarrett[1], Nydia Testa[2] and S. Toth[1]. Dept. Vet. Pathology, [1]University of Glasgow Veterinary School, Scotland. [2]Paterson Labs. Christie Hospital, Manchester, England.

The establishment of persistent feline leukaemia virus infections (FeLV) in cats may result in a wide range of haematological malignancies. The latent period between infection and the development of malignancies. The latent period between infection and the development of malignancy is usually over 1 year and may be several years in duration. We report here on a novel FeLV isolated from a field case of erythromyeloid leukaemia which produced myeloid leukaemia in 2 of 6 cats within 12 weeks. A further 2 cats died, one with a preleukaemic syndrome and the other with erythroid hypoplasia.

Virus from one of the experimentally produced myeloid leukaemias was repassaged into 10 newborn cats and from 10 days onwards the formation of bone marrow precursors for erythroid (BFU-E) and granulocyte macrophage cells (GM-CFC) were analysed. The main findings were:

(1) An early expansion of the GM-CFC population occurred in infected cats (infected cats 200-300 GM/CFC 10^5 cells: control 65 GM-CFC/10^5) and the *in vitro* differentiation of these cells was independent of exogenous CSF.

(2) At a later stage GM-CFC declined and showed abnormal, small colony formation.

(3) CSF production from infected marrow was not increased and BFU-E did not display an altered response to erythropoietin.

0559 REGULATION OF GRANULOPOIESIS BY T CELL SUBPOPULATIONS AND T CELL CLONES, Carol L. Reinisch, E. Rena Bacon, Tufts University, Boston, MA 02111

The purpose of this study was to define the role of activated T cells, T cell subpopulations and T cell clones in regulating granulopoiesis. Bone marrow cells were cultured (± T cells), in supplemented McCoy's medium containing CSF. The cells were cultured for 7 days after which time CFU-C growth was evaluated. Cell clusters composed of 20 or more cells were scored as a colony. The first experiment showed that activated T cells stimulated CFU-C differentiation in vitro. For example, mixing 10^7 MLR stimulated T cells with 10^5 bone marrow cells resulted in CFU-C proliferation. (Bone marrow cells: ((47±3)) vs bone marrow + MLR T cells ((85±5))*. T cells were then activated in an MLR, and highly purified Lyl^+ or $Ly2,3^+$ subsets obtained using the Flourescence Activated Cell Sorter. These T cell subsets were cultured at a 1:1 ratio with normal bone marrow cells in a CFU-C assay. The addition of activated Lyl^+ T cells resulted in CFU-C differentiation (bone marrow ((56±5)) vs bone marrow + Lyl^+ T cells ((115±15))). Surprisingly, the addition of activated $Ly2^+$ cells had similar effects: (bone marrow ((45±4)) vs bone marrow plus $Ly2^+$ T cells ((86±5))). Further experiments showed that $Ly2^+$ cells, activated in vitro to suppress the generation of PFC, simultaneously amplified CFU-C differentiation. Lyl^+ T cell clones with inducer function were then analyzed for their capacity to promote granulopoiesis in vitro. The addition of lethally irradiated cloned Lyl^+ T cells also enhanced CFU-C differentiation (bone marrow: ((52±8)) vs bone marrow + clone A ((123±15))). Taken together, these results suggest that the capacity to induce CFU-C differentiation in vitro is not restricted to the "helper" subset of T lymphocytes.

*(Data are presented as CFU-C ± SEM)

0560 STUDIES ON THE ORGANIZATION OF BONE MARROW: STEREOLOGY OF ENDOCLONED HEMATOPOIETIC COLONIES. Richard H. Lambertsen, University of Florida, Gainesville, FL 32610. Hematopoietic colonies were studied in the marrow of alternate fraction irradiated mice by light microscopic stereology to investigate the microenvironmental organization of marrow. Undifferentiated colonies (UC) were detected at 3 days post-irradiation, showed a marked predilection for bone, and disappeared as differentiated colonies developed. Some UC occurred along arteries. Granulocyte colonies (GC) occurred in all areas at 3 days, but grew rapidly only subosteally. Erythrocytic colonies (EC) appeared at 4 days as dispersed populations of cells within localized areas of marrow; these tended to proliferate initially in intermediate and central zones and along arteries. Macrophage colonies (MøC) of two "subtypes" occurred, peaking in frequency at 4 days. Megakaryocyte colonies (MC) originated along bone and differentiated away from bone. From 3-5 days, the frequency of GC > UC > MøC >> MC. All colony types except UC, MøC, and central GC increased in size and became mixed in differentiation by 12-14 days. For several weeks, however, erythropoiesis concentrated towards central areas whereas granulopoiesis and thrombopoiesis concentrated along bone. Some mixed colonies transformed from erythrocytic, centrally, to granulocytic, subosteally. The results are interpreted as evidence that 1) hematopoietic microenvironments for stem cell proliferation and commitment occur in endosteal and periarterial regions, 2) a proliferative and/or chemotactic stimulus to erythroid progenitors exists in intermediate and central marrow 3) some subosteal regions preferentially support nonerythroid differentiation, and 4) study of characteristics shared by endosteal and periarterial marrow may be a valid approach for identifying the determinants of blood cell differentiation in vivo.

0561 SURFACE IMMUNOGLOBULIN (SIg) POSITIVE COLONIES AND SIg POSITIVE CELLS IN MULTILINEAGE HEMOPOIETIC COLONIES (CFU-GEMM) DERIVED FROM BONE MARROW OF PATIENTS WITH LYMPHOCYTIC LYMPHOMAS (WDLL), A.A.Fauser, L.Kanz, and G.W.Löhr, Med. Univ.-Klinik, Albert-Ludwigs-Universität,Freiburg, FRG
Bone marrow cells from patients with lymphocytic lymphoma (WDLL) were grown in the presence of a leukocyte conditioned medium stimulated with phytohemagglutinin (PHA-LCM), a supernatant (B-cell growth factor - BCGF) derived from a human T-hybridoma and erythropoietin (EPO).After 14 days of culture multilineage hemopoietic colonies could be identified by their typical morphological appearance consisting of red cells and translucent cells of various size.Mixed hemopoietic colonies were examined for SIg positive cells (Ig M+D) using the Peroxidase-Antiperoxidase slide technique.It was found that approximately 20-30 % of the analysed mixed colonies contained surface Ig M+D positive cells.The number of positive cells ranged from 0-150 cells per colony.In addition a new type of colony could be identified under the employed culture conditions,consisting of approximately 400-700 cells.Cells derived from these colonies were positive for SIg. Evidence for the single cell origin of SIg positive colonies is provided by linearity studies.The assay was found to be linear with extrapolation through the origin.
The data suggest that B-cells might be part of the differentiation program of multilineage hemopoietic progenitor cells in lymphocytic lymphoma.

0562 ANALYSIS OF IN VITRO CULTURE SYSTEM FOR SEGREGATION OF HEMATOPOIETIC STROMAL AND STEM CELL COMPARTMENTS. SL Mann, DA Crouse, and JG Sharp, Dept. of Anatomy, Univ. Nebraska Medical Center, Omaha, Nebraska 68105.
In order to probe in vitro the interactions between proliferating and differentiating hematopoietic cells and the stromal cells of their microenvironment, a system is required in which the source of stem cells, humoral factors and stromal cells can be defined and manipulated. The culture system introduced by Dexter et al., while useful, is complicated by the fact that both the adherent and nonadherent layers receive stem and stromal cell contributions from the original and subsequent bone marrow inocula. In our hands the modification developed by Cohen and Greenberger which employs a stem cell depleted initial adherent layer, works with an efficiency comparable to the Dexter method yet the stem cells are known to be derived almost exclusively from the second inoculum. However, the second inoculum still contributes both stromal and stem cells to the adherent layer and when derived from the nonadherent population of other similar cultures can form adherent layers which may support CFUs. Other differences in hematopoiesis between the originally described intact monolayers and stem cell depleted initial monolayers include a generally greater production of granulocytes compared to macrophages on the latter. We cannot define differences in fibronection, laminin or Factor VIII positive cells or cellular aggregates in these cultures. Strain differences (F1 combinations are better than parental) and variations between individual donors as well as between individual cultures from pooled donor marrows are often greater than differences observed between these two culture methodologies. Greater enrichment of the stem cell population inoculated onto stem cell depleted adherent layers would now appear to be a required objective. (Supported by NIH grant AM26636.)

0563 HEMATOPOIETIC ABNORMALITIES INDUCED IN RECIPIENTS OF BONE MARROW-DERIVED STROMAL CELLS. JD Jackson, SL Mann, BO Murphy, GA Perry, RW Anderson, DA Crouse, and JG Sharp, Department of Anatomy, University of Nebraska Medical Center, Omaha, Nebraska 68105

In an attempt to confirm and extend the studies of Friedenstein et al. (Exp. Hematol. 1982) that fibroblastoid cells transfer hematopoietic microenvironments, we have transplanted a thymic (T131) and three marrow (MC1, MC3, MC4) stromal cell lines IM, IP and under the kidney capsule of syngeneic (C57Bl/6J) and allogeneic (CBA/CaJ) recipients. The cell lines were isolated by repeated sub-culture of primary cultures and were not deliberately infected with virus. None formed detectable tumors or altered hematopoiesis in allogeneic recipients. In syngeneic recipients they grew to an easily palpable size after one month when transplanted under the kidney capsule, IM, or IP. Although they contained hematopoietic cells, none produced clear evidence of forming specific hematopoietic microenvironments in recipients, however, the recipients of the grafts showed abnormalities of hematopoiesis. IM injection did not lead to an ascites, but subcapsular transplantation did lead to an occasional ascites (20% incidence) and IP recipients had a 100% incidence of a bloody cellular ascites (hematocrit 7-14%). These recipients were anemic, had elevated white cell counts, and splenomegaly with an increased splenic but decreased femoral radioiron uptake. Similarly, but much less pronounced changes were observed in recipients of subcapsular grafts. Although these cell lines do not form specific hematopoietic microenvironments, they do produce CSF-like molecules (Anderson and Sharp, Exp. Hematol. 1981) and it appears that they can modify hematopoiesis in syngeneic but not allogeneic hosts possibly via their factor production. (Supported by NIH grant AM26636.)

0564 THE ROLE OF FIBROBLASTOID CELLS AND DENDRITIC MONOCYTES IN THE INDUCTION OF LONG TERM IN VITRO MYELOPOIESIS. Dov Zipori and Zvi Malik, Cell Biology, Weizmann Institute, Rehovot, Israel 76100 and Life Sciences, Bar Ilan University, Ramat Gan, Israel.

Previous studies have suggested that primary stromal cells from mouse bone marrow produce both an inducer of myelopoiesis (resident CSF?) and a differentiation restraining activity (1). In order to further characterize these factors we attempted to grow permanent producer cell lines. A number of such stromal lines derived from the bone marrow of BALB/c, SJL/J, RF, C3H and Nude ICR mice were obtained and were found to differ both morphologically and functionally (i.e. in their CSF production and ability to restrain differentiation). One of those (MBA-14) maintained a mixed cellular composition following over 30 in vitro passages. The main cell types observed in electron microscope examination were defined as being fibroblastoid cells and dendritic monocytes. Both could be cloned and grown separately. However, the dendritic cells (14M) required L-cell-CM for independent growth. All other cell lines were uniformaly fibroblastoid. Their co-culture with fresh bone marrow cells resulted in elimination of myeloid progenitors within one week incubation. In contrast MBA-14 cells supported the proliferation of myeloid progenitors up to 4-8 weeks culture. It is noteworthy that these cultures were performed in the absence of hydrocortisone or horse serum. One of the dendritic cell lines tested (14M1) was incapable of inducing long term myelopoiesis. Further studies are now in progress with more cloned lines of the MBA-14 parent and with combinations of these clones. This culture system may enable us to define the minimal cellular requirements for continuous myelopoiesis in culture. (1) D. Zipori, J. Supramol. Struct & Cell Biochem. 17:347 (1981).

0565 LODGING OF HEMOPOIETIC CELLS: OBSERVATIONS IN AN EXPERIMENTAL MODEL, Mehdi Tavassoli, Veterans Administration Hospital, Jackson, MS 39216

A major early event in hemopoiesis is selective entrappment and lodging of hemopoietic stem cells (HSC) as a result of their interaction with the endothelium of hemopoietic organs. This is the conceptual basis of marrow transplantation after infusion of marrow cells. Studies were done on cellulose acetate membrane placed in the mouse peritoneal cavity. Sequential SEM and TEM studies indicated coating of the membrane with 10-12 cellular layers consisting predominantly of an equal mixture of macrophages and collagen producing fibroblasts. Within a week the coat underwent organization: Blood vessels penetrated the coat and the proportion of fibroblast to macrophage increased 10:1 and cells in the lower layers flattened. A remarkable finding was the development of an endothelial-like layer on the surface of the coat. This was 1-3 μ in thickness and gradually thinned out forming a veil on the surface. On SEM, short microvilli (>1 μm) appeared on its free surface as early as day 3 and gradually became longer. They were directed randomly. On day 7 animals were given 400 rads (total body) to reduce the size of HSC compartment, followed by ip infusion of 10^5 isogeneic marrow cells. Two days later round lymphoid cells with short surface projections were seen penetrating the endothelial-like layer. At this stage, microvilli were directed toward these cells interacting with their surface projections. In TEM, lymphoid cells penetrated the veil. This was followed by formation of hemopoietic foci underneath the veil. Thus, this model may permit the study of specific interactions between endothelium and HSC with the lodgement of the latter cells.

0566 IN VITRO EFFECTS OF MACROPHAGES ON ERYTHROID PROGENITOR CELLS, Candace S. Johnson and Philip Furmanski, Department of Cell Biology, AMC Cancer Research Center and Hospital, Lakewood, CO 80214

In various studies, macrophages have been shown to either stimulate or inhibit clonal in vitro growth of erythroid progenitor cells (CFU-E's). To clarify the disparate effects of macrophages on in vitro erythropoiesis, plasma clot cultures of CFU-E's were grown over monolayers of macrophages or macrophages suspended in a layer of agar. Normal resident macrophages from N/PLCR mice did not significantly affect CFU-E numbers when attached in monolayers. However, the same macrophages in agar suspension markedly stimulated CFU-E's. Further experiments showed that a component of the Bacto-agar used for the macrophage suspension cultures was responsible for macrophage stimulation of CFU-E's. This activity was inhibited by addition of DEAE-Dextran to the cultures. Less mature macrophages (blood monocytes) or activated macrophages (exudate-induced or peritoneal macrophages from leukemic animals) stimulated CFU-E's in monolayer cultures, and gave greater stimulation than resident cells in agar suspension. Macrophages from various mouse strains were tested for their ability to influence CFU-E's in culture. Most gave a pattern similar to N/PLCR mice. However, resident macrophages from 50% of the CFW outbred strain strongly inhibited CFU-E colony formation, irrespective of whether the macrophages were grown in monolayer or suspension. The results show that macrophages influence in vitro erythropoiesis. However, the effects observed are dependent upon the state of differentiation and functional activities of the cells and the genotype of the animals from which they are derived.

0567 MODULATION OF HUMAN MEGAKARYOCYTIC COLONY FORMATION, L.Kanz, G.Straub, G.W.Löhr, and A.A.Fauser, Medizinische Universitätsklinik, Freiburg,FRG

Pure Megakaryocytic Colonies (CFU-M), Megakaryocytic-Erythroid Colonies (CFU-M/E) and Multilineage Hemopoietic Colonies (CFU-GEMM) can be grown from human bone marrow,using phytohemagglutinin-stimulated leukocyte conditioned medium (PHA-LCM) as a source of thrombopoietin. Megakaryocytes can be identified by their positive reaction with antibodies to human factor VIII-related antigen or anti-human platelet antibodies as well as by serum from a patient with post-transfusion purpura,demonstrating anti-Pl^{A1} antibody activity.

An increased plating efficiancy for Pure Megakaryocytic Colonies (CFU-M) was observed when bone marrow cells of density less than 1.09 g/ml were cultured. In addition the frequency of CFU-M was augmented when nonadherent cells or mononuclear cells depleted of T-cells either by E-rosetting or by "panning" with OKT 3 and OKT 11 were used.

Preincubation of marrow cells (density less than 1.09 g/ml)with antibodies to human μ H chains of immunoglobulins resulted in a significant increase of CFU-M when compared to control experiments.

Whether the modulation of megakaryocytic colony formation by preincubation of mononuclear marrow cells with anti-μ antibodies reflects regulatory control mechanisms or a direct stimulating signal to megakaryocytic progenitors remains to be determined.

0568 IN VIVO REGULATION OF NORMAL ERYTHROPOIESIS BY MACROPHAGES, Philip Furmanski and Candace S. Johnson, Department of Cell Biology, AMC Cancer Research Center and Hospital, Lakewood, CO 80214

Macrophages are known to influence in vitro colony formation by committed erythroid progenitor cells (CFU-E's). To determine whether macrophages are involved in the regulation of in vivo erythropoiesis, N/PLCR mice were treated with macrophages obtained as purified normal syngeneic resident peritoneal cells. Splenic CFU-E numbers, determined by in vitro colony formation, were significantly suppressed by macrophage treatment. The cells responsible for suppression were identified as macrophages by antibody depletion; T cells, B cells and granulocytes did not decrease CFU-E numbers. Macrophages also inhibited the increases in CFU-E's which accompany erythropoietic stimuli (bleeding, phenylhydrazine treatment). Macrophage-induced suppression by CFU-E's was reversed by treatment with human urinary or sheep erythropoietin (epo). To determine the locus of macrophage suppression, numbers of early, epo-independent, erythroid progenitors (BFU-E's) and CFU-E's were compared in macrophage treated and untreated animals. Macrophages suppressed CFU-E's but not BFU-E's. Plasma epo levels were determined in anemic mice and anemic mice treated with macrophages, using the in vitro fetal liver cell assay. Epo levels were significantly reduced in the treated animals. Thus, in addition to their direct effects on in vitro CFU-E colony formation, macrophages regulate erythropoiesis in vivo, likely as a result of an influence on epo levels.

0569 MORPHOLOGICAL AND MOLECULAR CHANGES ASSOCIATED WITH GRANULOCYTE DIFFERENTIATION IN LIQUID CULTURE, Warren H. Evans, Shirley M. Wilson and Vernon Alvarez, National Cancer Institute, National Institutes of Health, Bethesda, MD 20205

The morphological aspects of granulocyte differentiation have been well studied. Relatively little is known, however, about the humoral factors and biochemical mechanisms that control the induction of specific proteins associated with the differentiation of granulocytes in the bone marrow. Further progress in this area depends on the development of direct and rapid assays for detecting and quantifying the many proteins associated with the differentiation process. We are using liquid cultures of immature granulocytes in conjunction with our recently developed high performance liquid chromatography (HPLC) assay for differentiation proteins to study, concomitantly, the morphological and the molecular aspects of cell differentiation. Immature granulocytes, isolated from guinea pig bone marrow by Ficoll density centrifugation, are placed in stationary liquid cultures containing RPMI-1640 medium with various inducing agents and incubated for periods up to one week. In the presence of 10% dialyzed guinea pig serum these cells are almost all converted to mature granulocytes with normal morphology whereas at serum concentrations below 1% mostly macrophages are formed. Using a recently developed HPLC assay for granulocyte differentiation markers, we find that morphological conversion of immature granulocytes to mature cells in vitro is accompanied by the formation of all the major membrane and granule differentiation markers detected by the HPLC assay in extracts of mature granulocytes formed in vivo. This assay is being used to identify the factors in normal serum that control the induction of synthesis of these differentiation markers.

Regulatory Factors

0570 CONTROL OF PROLIFERATION OF CLONED CYTOLYTIC T CELLS BY TCGF, R.P. Sekaly, H.R. MacDonald, M. Nabholz and K. Smith. Ludwig Institute for Cancer Research, Swiss Institute for Experimental Cancer Research, 1066 Epalinges, Switzerland and Dartmouth Med. School, Hanover N.H. 03755.

The specific binding of T cell growth factor (TCGF) to activated T cells induces proliferation of these T cells (1). In order to investigate possible cell cycle regulation of this phenomenon, the interaction between TCGF and a cloned TCGF-dependent cytotoxic T cell line (CTLL) was analyzed in detail. When an exponentially growing population of CTLL was deprived of TCGF for 30 hr, an accumulation of cells in the G1 phase of the cell cycle was observed. Using radiolabeled purified TCGF, the number of TCGF receptors on arrested CTLL was shown to be similar to that of an exponentially growing population ($\simeq$ 10.000 receptors/cell) G1 arrested cells also maintained their cytotoxic activity. Following addition of TCGF to CTLL arrested in G1, a majority of cells entered S phase in a semi-synchronous fashion after a lag phase of 10-12 hours. The number of cells entering S phase and the rate at which they were passing through mitosis were dependent on the concentration of TCGF ; however, the duration of the lag phase was independent of TCGF concentration. The presence of serum was not required for cells to progress through S phase. Experiments are currently underway to correlate TCGF receptor occupancy and proliferation rate using flow microfluorometry.

1) R.J. Robb et al. J. Exp. Med. 154, 1455-1574 (1981).
2) R.P. Sekaly et al. J. Immunol. 129, 1407-1416 (1981).

0571 CHARACTERIZATION OF MURINE AND HUMAN CSF ACTIVITIES, Diane Mochizuki, Steve Gillis and Paul J. Conlon, Immunex Corpoartion, Seattle, WA 98101

The characterization of CSF activites has been hampered by the length of time (5-7 days) required for in vitro biological assays. Recently we have developed two rapid (24 hours), quantitative and sensitive assays for CSF activities utilizing CSF dependent cell lines. One assay detects murine mast cell CSF. The second assay measures human GM-CSF activities. The two assays have facilitated our efforts to biologically and biochemically characterize both murine and human CSF activities. We will describe the rapid CSF assays and biochemical properties of both murine mast cell CSF derived from an IL-2 producing T cell line as well as human GM-CSF derived from stimulated peripheral blood leukocytes and appropriately stimulated tumor cell lines.

0572 MONOCLONAL ANTIBODY REACTIVE WITH ACUTE MYELOGENOUS LEUKEMIA CELLS BUT NOT WITH NORMAL BLOOD OR BONE MARROW, David S. Askew, Hayley M. Broker, and Fumio Takei, Department of Pathology, University of British Columbia, and the Terry Fox Laboratory, B.C. Cancer Research Centre, Vancouver, B.C., Canada

A monoclonal antibody has been raised to the human promyelocytic leukemia cell line HL-60, and characterized using radioimmunoassay and the fluorescence activated cell sorter. To date, the antibody has been found to react with blood and/or bone marrow cells from 5/6 patients with acute myelogenous leukemia (AML) and one patient with chronic myelomonocytic leukemia. It has shown no detectable binding to cells from AML in remission (2/2), chronic myelogenous leukemia (6/6), normal peripheral blood (4/4), normal bone marrow (4/4), or the erythroleukemia cell line K562. When HL-60 is induced to differentiate along the granulocyte pathway by incubating with dimethylsulfoxide, or along the monocyte/macrophage pathway by incubating with 12-o-tetradecanoylphorbol-13-acetate, the cells lose reactivity with the antibody. It is not yet known whether the antibody reacts with a small population of normal hemopoietic cells.

0573 RESTORATION OF THE DEFECTIVE T CELL MITOGEN RESPONSE IN PATIENTS AFTER BONE MARROW TRANSPLANTATION BY HIGHLY PURIFIED INTERLEUKIN 2(IL 2) IN VITRO.

Karl Welte, Niculae Ciobanu, Roland Mertelsmann, Malcolm A.S.Moore, Richard J.O'Reilly Memorial Sloan-Kettering Cancer Center, New York,N.Y.10021

Using mitogenic monoclonal antibodies to T Cell surface antigens (OKT 3, Pan T2)and PHA as mitogens, we studied the proliferative response of peripheral blood mononuclear cells from patients one or more months after bone marrow transplantation in vitro in the presence or absence of IL 2 (10 U/ml). All patients had a defective T-cell response to the mitogens tested This response could be restored by adding highly purified (Welte,et al. J.Exp.Med.156:454)IL 2 to the culture system.

Mitogen:	PHA(0.5 %)		OKT 3(125 ng/ml)		Pan T2(500 ng/ml)	
	- IL 2	+IL 2	-IL 2	+IL 2	-IL 2	+IL 2
	^{3}H-thymidine incorporation at day 3 [(cpmx10^{-3})(median)]					
Patients(5)	3.2	17	4.5	42	1.3	26
Controls(20)	80	90	61	82	59	79

Evidence shows that the defective mitogenic response is secondary to the abnormally low endogenous IL 2 production.

0574 THE ROLE OF IA-LIKE ANTIGEN IN RESPONSE OF ACUTE NON-LYMPHOCYTIC LEUKEMIA(ANLL)BLASTS TO COLONY STIMULATING FACTOR(CSF)AND PHA-CONDITIONED MEDIUM(PHA-LCM), Raymond Taetle and J.Michael Honeysett,University of California Medical Center,San Diego,CA 92103.

Previous studies have demonstrated Ia-like antigens on normal granulocyte/macrophage colonies (CFU-GM)grown with CSF.ANLL leukemia blasts also proliferate in liquid & colony culture in response to CSF & PHA-LCM.The presence of Ia-like antigens may distinguish PHA responsive blast colony cells & those responding to CSF(Hiroaka,Blood 56:859).We examined effects of CSF(placenta conditioned medium,PlCM),& PHA-LCM on proliferation of T cell depleted,blasts before & after treatment with a monoclonal anti-Ia antibody & complement:& compared responses to normal cells.As expected,treatment of 4 normal marrows reduced 7 day colonies & clusters to 7±4%($\bar{x}$± SE)& 21±10%,& 14 day colonies to 8±10% & 4±5% of control. ^{3}H-TdR incorporation by normal marrow was reduced(50%,d3;25%,d6),suggesting Ia+ cells were important in sustaining growth in liquid culture.In contrast,ANLL cells treated with anti-Ia & complement showed variable reduction in colony growth.Percent Ia+ cells was determined by immunofluorescent staining in 4 cases & averaged 29.5±7%;cytotoxicity with Ia+/complement averaged 24±5% by dye exclusion.Leukemia colonies from 5 pts were reduced to 64±20% with PlCM(range 9-110%)& 50±25%(range 6-100%) with PHA-LCM. ^{3}H-TdR incorporation was reduced:d3,32±12%(range 27-114%)with PlCM;88±36% with PHA-LCM(range 27-194%),with similar values on d6.No separation of CSF & PHA-LCM responsive cells was achieved in colony or liquid culture.Proliferating ANLL cells are heterogeneous with respect to Ia-like antigen & organization of leukemia hemopoiesis with respect to Ia thus differs from normal. Treatment of leukemia marrow with anti-Ia antibody would not consistently eradicate proliferating leukemia cells.

0575 DEVELOPMENT OF AN IN VITRO ASSAY FOR THROMBOPOIETIN USING ^{3}H-THYMIDINE UPTAKE OF MEGAKARYOCYTIC PRECURSORS, Patricia Williams-Lumsden, James R. Jeter, William H. Baricos*, and I-Li Chen, Department of Anatomy and Department of Biochemistry*, Tulane Medical Center, New Orleans, Louisiana, 70112

Megakaryocyte differentiation is believed to be controlled in part by a humoral thrombopoietic stimulating factor (thrombopoietin). Characterization of thrombopoietin has not yet been accomplished. Determination of the site of production and the mechanism of action of thrombopoietin is not possible at this time due to the lack of purified thrombopoietin. Purification attempts have been limited by the present in vivo assays for thrombopoietin. An in vitro assay for thrombopoietin-induced megakaryocytopoiesis utilizing ^{3}H-thymidine uptake has been developed. Thrombopoietin-rich serum from thrombocytopenic guinea pigs is used to initiate megakaryocytopoiesis in vitro. Autoradiography is utilized to demonstrate radioisotope incorporation by megakaryocytes in culture. This in vitro assay offers rapid and direct measurements of cellular events during megakaryocytopoiesis. In addition, the use of cellular kinetics in vitro provides an assay with the needed sensitivity for use in further investigations of thrombopoietin purification, its mode of action and receptor studies. (Research supported by Grants from the Louisiana Chapter of the American Heart Association.)

0576 TWO BIOLOGICALLY DISTINCT COLONY-STIMULATING FACTORS ARE SECRETED BY A T LYMPHOCYTE CLONE. M.B. Prystowsky, J.N. Ihle, I. Rich, J. Keller, G. Otten, M. Naujokas, M. Loken, E. Goldwasser, and F.W. Fitch. Depts. of Pathology and Biochemistry, and the Comm. on Immunology, Univ. of Chicago, Chicago, IL; Frederick Cancer Research Facility, Frederick, MD. The mouse T lymphocyte clone, designated L2, secretes at least 10 lymphokine activities affecting hemopoietic stem cells, T and B lymphocytes, macrophages, and fibroblasts. The present work compares the biologic properties of one of the colony-stimulating factors (CSF) secreted by L2 cells to the biologic properties of purified interleukin 3 (IL3). Conditioned medium from L2 cells subjected to ammonium sulfate precipitation, Sephadex G-100 filtration (Mr 10,000 - 55,000), and DEAE chromatography, yields two peaks of CSF. CSF activity in the effluent, representing 1% of the total CSF, coelutes with IL3; 99% of the CSF elutes with 0.08 - 0.14 M NaCl and can be separated into two forms using concanavalin A-Sepharose. This major CSF has been enriched further to a specific activity of 4×10^{8} colonies/mg protein and induces mainly granulocyte/macrophage colonies. Homogeneous IL3, purified from WEHI-3-conditioned medium, has CSF activity (3×10^{10} colonies/mg protein), induces 20 alpha hydroxysteroid dehydrogenase in lymphocytes, induces Thy-1 expression by bone marrow cells, and has mast cell growth factor activity and P cell-stimulating factor activity. IL3 also potentiates erythroid burst formation, while the major T cell CSF suppresses erythroid burst formation. Anti-IL3 antiserum inhibits IL3-induced thymidine incorporation by bone marrow cells but has no effect on thymidine incorporation induced by the major L2 cell CSF. Thus the T lymphocyte clone L2 secretes at least two biochemically and biologically distinct proteins affecting hemopoietic cells. Supported by USPHS Grants.

0577 MONOCLONAL ANTIBODY WITH SPECIFICITY FOR INTERLEUKIN-2, Hans-Joachim Feickert, Karl Welte and Roland Mertelsmann, Memorial Sloan-Kettering Cancer Center, New York, NY 10021

Using highly purified human Interleukin 2 (IL2) for immunizations, a mouse-mouse hybrid secreting an antibody with specificity for IL2 was obtained and subsequently subcloned. This monoclonal antibody (mAb-Fw-4) is of an unusual immunoglobulin class (IgA). It shows high neutralizing activity in IL2 dependent cultures of murine and human origin. Using a murine IL2 dependent cell line, neutralizing activity could be demonstrated for rat, mouse IL2 and human IL2 purified from normal and lymphoblastic blood cells as well as from an IL2 producer line (JM). The antibody immuno-precipitates from conditioned medium all human IL2 molecular subtypes with molecular weights of 14,000, 16,000 and 17,000, as well as what appears to be a biologically inactive aggregate or precursor molecule of higher molecular weight.

0578 IN VIVO EFFECTS OF HUMAN INTERLEUKIN 2, Roland Mertelsmann, Karl Welte, Niculae Ciobanu, Vincent J. Merluzzi, Neal Flomenberg, Nancy A. Kernan, Stuart P. Feldman, Gerard Kruger, Herbert F. Oettgen, Malcolm A.S. Moore and Bayard Clarkson, Memorial Sloan-Kettering Cancer Center, New York, NY 10021

The in vivo effects of highly purified human Interleukin 2 (IL2) from mitogen-stimulated normal lymphocytes is being investigated in a mouse model system as well as in a Phase I/II clinical trial in patients with acquired immunodeficiency syndromes (AIDS). In the mouse model, IL2 generates non-specific cytotoxic cells in vitro from spleen cells of normal and cyclophosphamide (CY) treated mice. IL2 delivered in vivo restores cytotoxic activity in Cy-immunosuppressed mice. IL2 was found to be free of side-effects in mice up to the highest dose levels tested (4000U/day equivalent to 300,000U/m^{2}/day). We have previously shown, that patients with AIDS exhibit defective in vitro proliferative responses to PHA and mitogenic monoclonal anti-T cell antibodies (OKT3, Pan T2). This defect is secondary to defective IL2 production, and can be restored in vitro by addition of IL2. Based on these observations we have initiated a phase I/II trial of IL2 for patients with AIDS. The preliminary results demonstrate that in vivo administration of highly purified human IL2 is without side-effects up to 2500U/m^{2}/day in man (up to 300,000U/m^{2}/day in mice), and that IL2 at 800-2500U/m^{2}/day appears to have immunemodulating effects in man in vivo.

0579 INTERLEUKIN-2 DEPENDENCE OF COMMON ALL COLONY FORMATION IN METHYLCELLULOSE, Stuart P. Feldman, Karl Welte, Salvatore Venuta, Carlos A. Izaguirre, Malcolm A.S. Moore and Roland H. Mertelsmann, Sloan-Kettering Institute, New York, NY 10021

The concept of autostimulation in human leukemogenesis has been suggested by Brennan et al in the HL-60 cell line model and more recently by Gallo and Wong-Staal in the HTLV model. We have previously reported that peripheral blood mononuclear cells from patients with non-B non-T ALL produce IL-2 upon stimulation with PHA but in contrast to normal PBL, not in response to the mitogenic anti-T-cell monoclonal antibody PanT2 (Venuta et al, Blood, in press). We report here the utilization of the blast cell progenitor colony assay for common ALL to assess the response of E-rosette depleted leukemic cells to partially-purified (ppIL-2) and highly purified IL-2 (hpIL-2). The assay, as described by Izaguirre et al (Blood 57:823), requires E^- ALL in the presence of irradiated normal E^+ feeder T-cells, as well as PHA-T-cell conditioned medium (PHA-TCM) mixed together in 0.8% methylcellulose. We tested cells from five patients with common ALL in this assay and substituted ppIL-2 or hpIL-2 for PHA-TCM, both in the presence and absence of feeder T-cells. Colony growth ($\geq$ 40 cells/colony) was observed in all leukemic samples when grown in the presence of hpIL-2 plus feeder cells. In two samples (ALL-223,-12) we observed moderate colony growth with ppIL-2 alone. ALL cells grown with hpIL-2 (from ALL-12) also required feeder T-cells for optimal colony growth. Monoclonal antibody cell surface marker characteristics of pooled colonies were similar to those obtained originally from the patients prior to therapy; i.e. 25-50% J5-positive, 40-60% B1-positive and 0-15% OKT3-positive. These studies suggest that autostimulation is not only operative in cell lines but also may be important in vivo and could be the basis for new therapeutic strategies.

0580 PRESENCE OF T CELL GROWTH FACTOR (TCGF) RECEPTORS ON HUMAN T CELL LEUKEMIA VIRUS (HTLV) POSITIVE LEUKEMIC CELLS; RECEPTOR CHARACTERIZATION. Warren J. Leonard, Joel M. Depper, Jeri S. Roth, Thomas A. Waldmann, and Warner C. Greene, NIH, Bethesda, MD

We have identified a monoclonal antibody (anti-Tac) that is able to distinguish HTLV positive leukemias (Adult T Cell Leukemia) from other cutaneous lymphomas such as Mycosis Fungoides and the Sezary syndrome. In HUT102-B2 cells, a cell line derived from a patient with an HTLV positive leukemia, an aberrant but functional TCGF has been identified by others. We have shown that anti-Tac blocks the action and membrane binding of human TCGF, and therefore appears to recognize the human TCGF receptor (Nature, in press). The production of TCGF by and presence of TCGF receptors on these cells may be critical in permitting their uncontrolled growth. In HUT102-B2 cells, the receptor identified by anti-Tac is a glycoprotein of approximately 50,000 daltons and has a pI of 5.6-6.1. By crude estimates, it represents 0.05% of total cellular protein synthesis. In contrast to HUT102-B2 cells where the receptor is constantly present, resting cells do not bear receptors or produce TCGF. Using ^{125}I-anti-Tac, we have shown that within 4-8 hours after PHA activation, these putative TCGF receptors appear on normal peripheral blood T lymphocytes, indicating the rapid induction of TCGF receptor gene expression. The receptor in these normal cells is 5,000 daltons larger; thus it appears that not only do HUT102-B2 cells produce an aberrant TCGF, but may also express an aberrant TCGF receptor. We are currently using anti-Tac diagnostically and are evaluating therapeutic applications of the antibody.

0581 MIXED COLONY STIMULATING FACTOR. I. A 12,000 MOLECULAR WEIGHT PROTEIN STIMULATES HEMATOPOIETIC COLONY FORMATION IN VIVO AND IN VITRO, John C. Cambier and Tani Fernández-Albornoz, Duke University Medical Center, Durham, NC 27710

We report the purification and partial biochemical and functional characterization of a mixed colony stimulating factor (CSF-mixed) secreted by cells present in high frequency in murine hematopoietic tissues. The factor was purified from bone marrow and spleen cell culture supernatants using a combination of ammonium sulfate precipitation, gel filtration and ion-exchange chromatography, or affinity chromatography using an antiserum specific for CSF-mixed. The purified radioiodinated factor migrates as a single 12,000 dalton species in SDS-polyacrylamide gel electrophoresis. The functional activity of the purified factor was examined using the in vivo endogenous colony assay for transient erythroid colonies (TE-CFU) and the in vitro mixed colony assay (CFU-mixed). Results indicate that the factor promotes colonies in vitro, suggesting it may be a mixed colony stimulating factor (CSF-mixed) important in the positive regulation of pluripotent stem cells or of early erythroid progenitors.

0582 Monoclonal antibodies to mammary carcinoma membrane proteins generated by in vitro immunization, John Morrow, Robert Bjercke, George Sledge, Dean Edwards, and William McGuire, University of Texas Health Science Center, San Antonio, Texas 78284.

Monoclonal antibodies to a crude membrane fraction from the human breast cancer cell line MCF-7 have been developed. BALB/c mouse spleen cells were cultured for 5 days in a thymocyte conditioned medium in the presence of a 3M KCl membrane extract as the immunogen. The cells were fused with NS-1 plasmacytoma cells and hybridomas were selected with HAT medium. Wells in microtiter plates containing hybridomas were screened by reacting supernatants with a rabbit antibody against mouse IgG, IgM and IgA, and then probing with (125)/I protein A. Positive wells were cloned and those clones which retained the ability to produce monoclonal antibodies against the 3M KCl membrane extract were characterized using immunohistochemistry (Avidin-Biotin-Complex). Although none of the antibodies tested are tumor specific, several appear to be tissue specific, reacting against some tissues and not others. Thus the in vitro immunization procedure can be used to study the properties of the cancer cell membrane.

0583 GROWTH FACTOR STUDIES ON HUMAN NON-HODGKIN'S LYMPHOMAS, Richard J. Ford, Frances Davis, James Reuben, Nicola Kouttab, M.D. Anderson Hospital, Houston, Texas, 77030.

Non-Hodgkin's lymphomas (NHL) are a very heterogeneous group of T and B cell neoplasms that are thought to represent frozen stages in human lymphocyte differentiation. These tumor cells have shown recently to , in some cases, retain functional activities characteristic of their normal lymphocyte counterparts. We have studied a large group of monoclonal lymphoma cells derived from fresh biopsy material in untreated patients. These cell populations were depleted of normal inflammatory or other reactive cells and then characterized by a battery of monoclonal antibodies. The human malignancy-associated nucleolar antigen (HMNA), which we have recently shown to discriminate between neoplastic and normal lymphocytes was used to determine that the cell population being studied was actually comprised of neoplastic lymphoid cells. The lymphoma cell populations were then studied for in vitro proliferative capacity utilizing either mitogens or purified human growth factors, including IL-1, IL-2 (TCGF, or B cell growth factor (BCGF). The same cell populations were also studied for differentiative capabilities using the same factors in a microelisa assay for Ig production. Our studies showed that the small cell type of NHL (PDL, WDL, etc.) would often respond to growth factors alone or after anti-immunoglobulin-priming in the B cell types. The responses seen usually involved proliferation without differentiation. Differentiation usually required the presence of either autologous or allogeneic T cells plus factor(s). The large cell lymphomas ("Histiocytic", Immunoblastic, etc.) were found to be generally refractory to growth factor stimulation for either proliferation or differentiation. The results of these experiments will be discussed in regard to immunoregulation in the lymphomas. Supported in part by Grant CA31479.

0584 **AUTOSTIMULATORY GROWTH FACTORS PRODUCED BY HUMAN B CELL LINES**

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Epstein Barr virus (EBV) transformed human B cells grow poorly at low cell density and clone with an inefficiency less than 0.5% in the absence of feeder layers. When these cells are grown at higher densities or in the presence of B lymphoblastoid conditioned media their proliferation is markedly enhanced. By using a micro ^{3}H-thymidine incorporation assay we have investigated growth factors produced by human B lymphoblastoid cell lines. Clonal growth of B lymphoblasts is supported by the addition of conditioned media from human myeloma cell lines and from mitogen stimulated normal PBLs, in addition to CM from B lymphoblastoid cell lines. The relationship of these factors to B cell growth factor(s) is under investigation. The growth stimulatory factors can be absorbed by washed, rapidly growing B lymphoblastoid cells. The factors are protease sensitive and are 25-50K M_r by gel filtration. Further cellular and biochemical studies of these autostimulatory B lymphoblastoid growth factors is in progress.

0585 A T CELL DERIVED GLUCOCORTICOSTEROID RESPONSE MODIFYING FACTOR ($GRMF_T$). Sally S. Fairchild, Karen Shannon, Elaine Kwan, & Robert I. Mishell. U. Calif., Berkeley, CA.
Interleukin 1 (IL-1) blocks glucocorticosteroid suppression of helper T cell function and granulocyte/macrophage progenitor cell proliferation in vitro. Supernatants from murine spleen cells or a T cell hybridoma, FS6 14.13.1, stimulated with Con A also have glucocorticosteroid response modifying factors (GRMF) that act on steroid-sensitive helper cells and granulocyte/macrophage precursors. T cell derived GRMF ($GRMF_T$) is distinct from other well defined leukocyte hormones. Gel exclusion chromatography and isoelectric focusing data indicate that $GRMF_T$ has a molecular weight of approximately 25,000 daltons and is heterogeneous in charge (pI 5.5-8.5). It is completely separated from IL-2 by isoelectric focusing, is larger than B cell growth factor, and is more basic than MIF. Since FS6 14.13.1 does not make IL-1 and CSF, $GRMF_T$ is different from these molecules. Furthermore, purified IL-3 does not have GRMF activity indicating that $GRMF_T$ is distinct from IL-3. We have identified several helper T cell lines that do not proliferate in the presence of dexamethasone; supernatants from FS6 14.13.1 block the dexamethasone suppression. We are characterizing these cell lines, and the molecule(s) that protect them from steroid suppression. Results of these experiments and of those on the physiochemical properties of $GRMF_T$ will be presented. We will also report data on $GRMF_T$ production by MRL/lpr mice and their normal congenic partner. MRL/lpr mice develop autoimmune and lymphoproliferative disease and are severely deficient in their capacity to produce IL-2. They may thus allow us to dissociate $GRMF_T$ production from the synthesis of other leukocyte hormones.

0586 REGULATION OF THE BIOLOGICAL ACTIVITY OF INTERLEUKIN 1, A HORMONE THAT MAY INTERFERE WITH CHEMOTHERAPY OF LEUKEMIA. Stephen R. Martin and Robert I. Mishell, Department of Microbiology and Immunology, University of California, Berkeley, CA.
Interleukin 1 (IL-1) is a polypeptide hormone normally synthesized and secreted by macrophages and keratinocytes in response to products of microbial origin. It is also secreted by cells of most patients with myeloid and myelomonocytic leukemia and by several human and murine monocytic cell lines. IL-1 is a central mediator of inflammation and specific immune responses. One of the biological properties of IL-1 discovered in our laboratory is that it prevents the immunosuppressive and myelosuppressive effects of glucocorticosteroids. The available data indicate that leukemic cells from most patients with myeloid leukemia are resistant to known effects of glucocorticosteroids yet possess apparently normal glucocorticosteroid cytosol receptors. These observations led us to postulate that the apparent resistance of myeloid leukemia cells to glucocorticosteroids is due to the IL-1 they secrete. The literature suggests the existence of substances that inhibit the synthesis of IL-1 and others that antagonize its biological effects. Studies are being conducted to verify these reports and to examine the effects of these substances in reversing IL-1 antagonism of glucocorticosteroid suppression of the formation of granulocyte/macrophage colonies in vitro. Results of these studies and details of the proposed pathways will be presented.

0587 LEUKOCYTE HORMONES THAT MODIFY CELLULAR RESPONSES TO GLUCOCORTICOSTEROIDS. Robert I. Mishell, Sally S. Fairchild, Elaine Kwan, Stephen R. Martin, Barbara B. Mishell, and Karen Shannon. Dept. of Microbiology & Immunology, U. California, Berkeley, CA. 94720.
Glucocorticosteroids kill or functionally inhibit many lymphoid and myeloid cells. We found that supernatants of adjuvant-activated macrophages contain a polypeptide hormone, subsequently identified as Interleukin 1 (IL-1), that blocks the suppressive effects of glucocorticosteroids on T helper cells and on the progenitor cells of granulocyte/macrophage colonies. IL-1 potentiates both inflammation and specific immunity by virtue of its stimulatory effects on several different target tissues and cells. In addition to its protective effects against glucocorticosteroid suppression it elevates body temperature by direct stimulation of the hypothalamus, stimulates the synthesis and secretion of acute phase reactants by hepatocytes, collagenase and prostaglandins by synovial cells and IL-2 by a subpopulation of T lymphocytes. It is also a growth factor for fibroblasts. Recently we found that T cells synthesize another glucocorticosteroid response modifying factor ($GRMF_T$) that also blocks the immunosuppressive and myelosuppressive effects of steroids. $GRMF_T$ appears to be distinct from other well characterized leukocyte hormones. Current studies are directed to understanding the functional relationship of IL-1 and $GRMF_T$, their mechanisms of action, and the means by which their production and elimination is regulated. The results of these studies will be presented and the possible role of the two hormones in leukemia and in diseases characterized by abnormal host immune and inflammatory responses will be discussed.

0588 EXPRESSION OF MONOCYTE-SPECIFIC ANTIGENS AND CYTOTOXIC FUNCTIONS IN HUMAN PROMYELOCYTIC CELL LINES TREATED WITH LEUKOCYTE-CONDITIONED MEDIUM, Elahe Talieh Dayton, Bice Perussia and Giorgio Trinchieri, The Wistar Institute, Philadelphia, PA 19104

Human promyelocytic cell lines treated with conditioned medium from PHA-stimulated leukocytes acquire several phenotypic and functional markers of differentiated monocytes. Promyelocytic cells treated with conditioned medium express, among other markers, monocyte-specific and HLA-DR antigens absent from the parental cells and become potent effectors of antibody-dependent cell-mediated cytotoxicity against erythrocytes and tumor cells. In cultures of promyelocytic cell lines maintained in the presence of conditioned medium, an equilibrium between proliferation and differentiation is established and two cell populations can be separated on the basis of expression of differentiation surface markers. One population has a differentiated morphology, expresses non-specific esterase activity, Fc receptors, C receptors, monocyte-specific and HLA-DR antigens, is able to mediate antibody-dependent cytotoxicity and has a very limited ability to proliferate. A second population retains the phenotype of undifferentiated promyelocytes and continues to proliferate. The differentiated monocyte-like cells originate from a proportion of the proliferating promyelocytes which respond to the differentiation inducers contained in the conditioned medium.

0589 BINDING OF ERYTHROPOIETIN TO ERYTHROPOIETIC CELLS. Tania L. Weiss, Charles K-H Kung and Eugene Goldwasser, The University of Chicago, Chicago, Illinois 60637.

We have found by fluorescence microscopy that 1.3% of the live nucleated cells of normal rat and mouse is capable of binding, erythropoietin (epo). Further study of epo binding was done with ^{3}H-labeled epo prepared by the method of Van Lenten and Ashwell.[1] The preparation had a specific activity of 2.6×10^{5} Ci/mole and retained 95% of its biological activity when tested in vitro. The labeled epo was not distinguishable from native, unlabeled epo by HPLC or SDS gel electrophoresis. Normal rat marrow incubated with ^{3}H-epo (17 f moles/ml) reached a plateau of binding by 4 hours at 37°. Dilution of ^{3}H-epo with a 500 fold excess of unlabeled epo reduced binding to the background level. No specific binding was found in 3 hours at 0°. Binding of ^{3}H-epo to mouse spleen cells that had been enriched 7 fold for CFU-E also plateaued at 4 hours. Binding to this preparation of CFU-E was proportional to epo concentration between 17 and 85 f moles/ml (3×10^{5} cells/ml).

Supported in part Grants CA 18375 from the National Cancer Institute and HL 16005 from the National Heart Lung and Blood Institute; NIH DHHS

1) Van Lenten, L. and Ashwell, G., J. Biol. Chem. 246, 1889, (1971).

0590 CLONING OF HUMAN INTERLEUKIN-2 GENE, Ernest Kawasaki,Michael McGrogan,Michael Doyle, David Mark, Cetus Corporation,Berkeley,CA 94710

Human PBLs, isolated by ficoll-hypaque centrifugation, were stimulated to produce IL-2 by incubation with ConA and PMA. Total mRNA isolated from the induced PBLs was translated in Xenopus oocytes and the product assayed on murine HT-2 cells. Induced mRNA, containing high levels of IL-2 activity, was subjected to preparative gel electrophoresis under denaturing conditions and the size fractionated RNA was analyzed for IL-2 activity in the oocyte bioassay (IL-2 mRNA was identified in the region corresponding to 1.1 to 1.2 kb). The IL-2 mRNA fraction was used to construct a cDNA bank and induction specific clones were identified by +/- hybridization screening with cDNA probes made from induced and uninduced PBL mRNA. The induction specific clones were then analyzed by hybridization-translation methods and 5 clones were found which gave positive signals in the IL-2 assay. The DNA sequences of these clones appear to be derived from two different genes, unrelated at the nucleotide level. Although the regulation and expression of these two genes appear very similar, their structure and genomic representation are quite different. The significance of these findings will be discussed.

0591 THE EFFECTS OF FISCHER RAT LEUKEMIC SERUM ON BONE MARROW FIBROBLAST COLONY FORMATION, Sue A. Bauldry, Floyd D. Wilson, Paul C. Stromberg, G. Adolph Ackerman, Battelle Memorial Institute, Columbus, OH 43201

Myelofibrosis, a common complication of human leukemias, is characterized by an overgrowth of bone marrow fibroblasts (adventitial or reticular cells). Marrow fibroblasts have also been implicated in the regulation of hematopoiesis, therefore, the growth potential of this cell was investigated. Liquid culture of isolated bone marrow cells leads to the formation of discrete fibroblastic colonies. In the culture of rat bone marrow cells, we have found that the addition of 2-mercaptoethanol (2-ME) to culture media causes a 2-fold increase in fibroblast colonies and an increase in the number of cells per colony. We also found that 2-ME may be acting through the release of a serum component. In further studies we have been investigating the role of serum factors on marrow fibroblast growth in the interrelationship between myelofibrosis and leukemia in Fischer rats. Fischer Rat Leukemia (FRL) is a spontaneous neoplasm which occurs in 20 to 25% of aged Fischer rats. Although the primary organ involved in FRL is the spleen, there is a high incidence of myelofibrosis developing late in the disease. We have found that the serum of leukemic rats contains a substance that enhances normal rat marrow fibroblasts as shown by a 175% increase in the number of fibroblast colonies and a 325% increase in the number of colonies containing more than 80 cells. After implantation of leukemic cells, the serum of transplanted rats also contains factors which augment fibroblast proliferation. Our studies may be useful in eliciting the connection between leukemia and myelofibrosis.

0592 INCREASED FREQUENCY OF LYMPHOKINE RESPONSIVE CELLS IN PRELEUKEMIC SPLEENS: POTENTIAL USE AS A SENSITIVE SCREEN FOR POTENTIAL LYMPHOKINES, John C. Lee, Smith Kline & French Laboratories, Philadelphia, PA 19101

Balb/c mice inoculated with Moloney Leukemia virus at birth generally developed acute viremia and subsequent lymphoma. We have previously demonstrated the correlation of viremia and leukemia with chronic blastogenic responses to viral antigens in preleukemic mice. An immediate effect of chronic immune response was the increase in the frequency of lymphocytes capable of responding to lymphokines in vitro (PNAS 78, 7712). We now report that the enhanced response of preleukemic splenic lymphocytes can be used to detect potential lymphokines with growth-promoting activity. Con A conditioned supernatant from normal splenic cultures or antigen pulsed immune spleen cells were partially purified through sizing and ion-exchange chromatography. Individual fractions were assayed in the lymphocyte proliferation assay using spleen cells from normal or preleukemic mice. Distinct multiple peaks of activity were detected when preleukemic spleen cells, but not normal spleen cells, were used. The proliferative activities correlated with fractions that were previously shown to contain known lymphokines such as IL2, IL3 or CSF. Furthermore, using highly purified lymphokines, titration of functional activities correlated with preleukemic splenocyte proliferation activity. Culture supernatants from a variety of hematopoietic lines were examined and several, notably that of RAW 264, were active, although their exact function was unknown. However, the initial identification of such cytokine was greatly facilitated by the use of the preleukemic splenocyte proliferation assay as a prescreen.

0593 RESPONSE OF MALIGNANT HEMATOPOIETIC CELLS TO NORMAL GROWTH FACTORS, J.M. Heard, N. Casadevall, J. Choppin, S. Fischelson, C. Lacombe, B. Varet, INSERM U 152, CHU Cochin, 75674 Paris Cedex 14 France

We have previously shown that "endogenous" erythroid colonies observed in polycythemia vera were in fact derived from erythroid progenitors dependent on exogenous erythropoietin and exquisitely sensitive to the hormone. Murine myeloblastic leukemic cells, apparently able to grow in suspension culture and in agar without added growth factors also exhibited a response to purified CSF with a higher cloning efficiency without significant cellular maturation. Moreover some data suggested that murine myeloblastic leukemic cells synthetise a CSF like factor. The sensitivity of myeloblastic cells to exogenous and autologous CSF is under study. These data suggest that two kind of abnormal behavior in regard to growth factor might be distinguisted in malignant hemotopoietic cells : 1) hypersensitivity to an exogenous growth factor, 2) synthesis of an autostimulation (normal ?) growth factor. Whether such abnormalities are a constant finding in malignant hematopoietic cells and what is their significance in the process of malignant transformation remain open questions.

0594 HLA-DR ANTIGEN POSITIVE MONOCYTE/MACROPHAGES (MØ) INTERACT WITH T LYMPHOCYTES (TL) AND ITS SUBPOPULATIONS FOR BURST PROMOTING ACTIVITY (BPA) ELABORATION AND THE ACTIVITY STIMULATING MIXED COLONIES (GMEEM-CSA), Dharmvir S. Verma, Gary Spitzer and Karel A. Dicke, The University of Texas System Cancer Center, Houston, TX 77030

We have demonstrated that MØ interact with TL for the control of GM-CSA elaboration. Therefore, we studied this interaction for the elaboration of BPA and the factor stimulating mixed colonies. Conditioned media (CM) were prepared in the presence of MER (methanol extraction residue of BCG) from either MØ ($0.5x10^6$/ml) or TL (0.75, 1.5, 3.0, or $4.5x10^6$/ml) alone, or both coincubated at various ratios (1:1.5, 1:3, 1:6, or 1:9) and assayed in methyl cellulose cultures. A significant synergistic elaboration of BPA was observed at MØ:TL ratios of 1:1.5 and 1:3 ($p<0.01$). Further increases in TL decreased BPA. BPA in the CM from MØ plus 3000 R-irradiated TL was even higher at 1:1.5 and 1:3 ratios; also it increased progressively at 1:6 and 1:9 ratios. The proportion of mixed colonies in cultures containing MØ plus irradiated TL CM was also higher than in the cultures containing CM from MØ or TL alone (37%vs14%). We furthermore demonstrated that the TL subpopulation that synergized with MØ was part of the OKT8$^-$, Tnon-γ fraction and it was radioresistant. The subpopulation that did not synergize was in the OKT8$^+$, and Tγ fractions. It was found that only HLA-DR antigen positive MØ collaborated with Tnon-γ. Further experiments revealed that addition of the Tγ subpopulation to the coincubation mixture of MØ plus Tnon-γ at the beginning of CM preparation completely abrogated the synergism for BPA and GMEEM-CSA elaboration, indicating an active suppressor role for Tγ fraction. These data indicate that MØ, and its helper and suppressor subpopulations actively regulate BPA and GMEEM-CSA elaboration.

0595 THE DEVELOPMENT OF A MORE RAPID AND SENSITIVE ASSAY FOR THE DETECTION OF INTERLEUKIN-1, Paul J. Conlon, Staff Scientist, Immunex Corporation, Seattle, WA

The rapid detection and quantification of interleukins has greatly enabled advances in the purification and elucidation of the mechanism(s) of action of these potent immunoregulatory molecules. One of these factors, macrophage derived IL-1, has traditionally been measured by the ability of this substance to augment the proliferative response of murine thymocytes to suboptimal concentrations of T cell mitogens. Although a reproducible test for IL-1 activity, this assay requires a longer time period (72 hours) and deals with a heterogenous responder cell population (mature versus immature thymocytes) than is desired. Another biological assay useful in the measurement of IL-1 activity involves the ability of this factor to convert an IL-2 nonproducer murine tumor cell line, LBRM-33-1A5, to an IL-2 producer. This assay was shown to be more rapid and more sensitive (1000 fold) than the traditional thymocyte proliferation assay. The phenotypic conversion of the IL-2 nonproducer to a producer cell, in the presence of human IL-1, has been modified by direct addition of the IL-2 dependent continuous T-cell line (CTLL-2) to metabolically inactivated LBRM-33-1A5 cells. This assay enables the rapid (24h) analysis of macrophage derived IL-1 from culture supernatants. The current conversion assay is approximately 100,000 times as sensitive as standard thymocyte mitogenesis protocols.With this assay we hope to be capable of screening monoclonal antibodies specific for this monokine as well as measuring the presence of IL-1 in serum of auto-immune or immunocompromised patients.

0596 NEUTRALIZATION OF BURST PROMOTING ACTIVITY (BPA) WITH ANTIBODIES TO PLASMA MEMBRANES, Nicholas Dainiak and Carl M. Cohen, St. Elizabeth's Hosp., Tufts Univ., Boston, MA.

Leukocyte conditioned medium (LCM) contains both soluble and vesicular growth factors that stimulate erythroid burst formation (Blood 60:583, 1982). The vesicles are spontaneously shed from leukocyte plasma membranes that are also rich in BPA. To test the hypothesis that soluble and membrane-associated BPA may cross react, we tested the effects of antimembrane antibodies on soluble BPA in culture. Plasma membranes were purified 10-12 fold from human leukocytes by centrifugation on a sucrose step gradient. Membrane purity was monitored by membrane bound ^{125}I-wheat germ agglutinin. 100-350 μg of membrane protein emulsified with Freund's complete adjuvant was injected into rabbits intradermally and antisera were collected. Rabbit IgG was precipitated with ammonium sulfate, purified on DEAE cellulose and tested for BPA neutralizing effects in serum-restricted fibrin clots. Human marrow cells were cultured with 0.5-4.0 IU/ml erythropoietin (Ep) and 9.0% (v/v) unseparated LCM, high-speed LCM supernatant, LCM vesicles or α-medium. While post-immune anti-membrane IgG reduced BPA in a dose-related fashion, pre-immune IgG had no effect. As little as 0.001 mg/ml abolished all BPA expressed by whole LCM and its fractions. When added to cultures containing α-medium plus 4.0 IU/ml Ep, post-immune IgG at ≤0.1 mg/ml did not alter colony growth. Inhibition of burst but not of colony forming unit-erythroid (CFU-E) growth was found at lower Ep doses. Moreover, post-immune IgG did not alter in vivo activity of Ep in the hypertransfused, polycythemic mouse assay. We conclude that antibodies raised to leukocyte membranes neutralize BPA present not only in LCM-derived vesicles but also in high-speed LCM supernatants. Thus, soluble and membrane-derived growth factors in LCM may share antigenic determinants.

0597 ERYTHROPOIETIN-DEPENDENT DNA SYNTHESIS BY ERYTHROID PRECURSORS IN A COMPLETELY DEFINED CULTURE MEDIUM, Raymond Kaempfer, Arnon Rosenthal, Susan Marsh and Daphna Manor, The Hebrew University-Hadassah Medical School, Jerusalem, IL 91 010.

A cohort of highly erythropoietin-responsive red cell precursors appears in bone marrow of phenylhydrazine-treated, anemic rabbits after treatment with a single dose of actinomycin D. When placed in a completely defined culture medium that totally dispenses not only with serum but also with serum-replacing factors, these cells incorporate [^{3}H]thymidine in total dependence on erythropoietin. A stimulation index of up to 40-fold is obtained at 50 mU/ml of the hormone. The ability to follow erythropoietin-dependent DNA synthesis and multiple cell divisions by a cohort of erythroid precursors in completely defined culture conditions may find application in controlled studies of red cell development.

0598 INDUCTION OF GM-CSF BY RETRCVIRUSES IN FIBROBLASTS, Ian B. Pragnell, Allan Balmain and Mark J. Koury, Beatson Institute for Cancer Research, Glasgow, Scotland.

Murine retroviruses which cause sarcomas also induce abnormal proliferation of haematopoietic cells leading to death in newborn and in some cases adult mice. Cloned murine leukaemia viruses (MuLV) also cause abnormal haematopoietic cell proliferation. Such proliferative response may be the direct effects of retroviruses on haematopoietic cells or they could be an indirect effect on other cells which in turn provide the stimuli for the proliferation of haematopoietic cells. Results are presented which show that specific fibroblast cell lines produce GM-CSF, required for growth in vitro of granulocyte-macrophage colony forming cells GM-CFC[1]. Infection of fibroblast cell lines with MuLV or sarcoma virus isolates results in large increases in GM-CSF in most cases. Other cell lines which did not produce detectable GM-CSF when infected do not produce it after sarcoma virus or MuLV infection. However, treatment of these cell lines with phorbol ester (TPA) did result in a large induction of activity (M-CSF) supporting growth of macrophage colony forming cells. We have also found that in vivo treatment of mouse skin with TPA results in a dramatic increase in M-CSF. The mechanism of this induction is being studied using frog oocytes as an mRNA assay for GM-CSF[2]. The results suggest and confirm that the haematopoietic proliferations associated with retrovirus infection can occur secondary to markedly increased production of GM-CSF by virus infected fibroblasts and long term marrow cultures[3].

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0599 HUMAN T CELL HYBRIDOMAS PRODUCING T CELL AND B CELL GROWTH FACTORS, Elaine C. DeFreitas, Hilary Koprowski, and Carlo M. Croce, The Wistar Institute, Phila., Pa., 19104

We have produced tetanus toxoid [Tet]-specific human T cell hybridomas which "help" autologous B cells produce anti-Tet Ig in vitro [PNAS 79:6646, 1982]. Clones of these T hybridomas were examined for their ability to produce interleukin 2 [Il-2] and B cell growth factor [BCGF] after mitogenic or antigenic stimulation. Il-2 was assayed on mouse CTLL cells, an Il-2 addicted T cell line. BCGF was assayed on normal peripheral blood B lymphocytes, extensively depleted of T cells by SE_{AET} rosetting and treatment with Leu 1 and complement. Several clones were found to produce Il-2 only. Two clones produced BCGF in the absence of any detectable Il-2. In addition, one clone [6K3] made Il-2 when stimulated with mitogen, and BCGF [but not Il-2] when stimulated with antigen [Tet]. Induction of both lymphokines was optimal in the presence of irradiated DR-positive accessory cells.

Cell Lines, Lymphopoiesis, Leukemia

0600 INDUCTION, GROWTH AND DIFFERENTIATION SIGNALS OF CYTOTOXIC T LYMPHOCYTES, Peter Scheurich, Conny Hardt, Martin Rollinghoff and Hermann Wagner, Institute of Medical Microbiology, University of Mainz, 6500 Mainz, West Germany

The in vitro induction of a primary cytotoxic T cell (CTL) response is strictly controlled by the functional activity of T helper cells or their soluble products. Based on the observation that purified interleukin 2 (Il 2), which is considered to be the major helper product, appeared to be insufficient to generate a cytotoxic response, the participation of other lymphokines was postulated. We have obtained evidence, that the induction of cytolytic activity from CTL precursors requires three distinct soluble mediators, one of which acts together with antigen as an early signal to induce sensitivity to IL 2. Il 2 in turn drives clonal expansion of such preactivated T cells. Differentiation into cytotoxic effector cells is independently controlled by a mediator (cytotoxic T cell differentiation factor, CTDF) distinct of the former two lymphokines. This conclusion is derived from experiments, in which proliferative and cytotoxic responses of antigen or mitogen stimulated Lyt 2^+ T cells are initiated by supplementing the cultures with selected preparations of mediators.

0601 Two new Ph^1+ myeloid cells lines. A Keating, P Martin, I Bernstein, T Papayannopoulou, W Raskind, JW Singer. Univ. of Washington and VA Medical Center, Seattle.

Two new cell lines, EM-2 and EM-3, were established from a patient who relapsed with CML in blast crisis after an allogeneic marrow transplant. EM-2 derived from a long-term marrow culture established 28 days after transplantation from a "remission" marrow. EM-3 was established after a relapse at day 47. Cytogenetic analysis of EM-2 and EM-3 shows that both lines are hyperdiploid with multiple copies of Ph^1. The lines have been in continuous culture for > 7 months (> 25 passages) with current doubling times of under 3 days. The frequency of NBT dye reducing cells increased after a 5 day incubation with retinoic acid (RA) (5×10^{-7} M) from 2 to 8% for EM-2 and from 1 to 4% for EM-3 ($p < 0.01$). The lines were examined for expression of determinants recognized by a panel of monoclonal antibodies for myeloid antigens and the BB-2, an antibody recognizing only activated B-cells, before and after incubation with RA or TPA. (See table.) We conclude that both EM-2 and EM-3 have myeloid features based on both NBT reduction and reactivity with T5A7, T3A3, L1B2, 1G10, and Myl. Both lines can be induced to undergo further myeloid differentiation with TPA and/or RA. EM-3 expresses antigenic determinants associated with activated B-cells when preincubated with TPA.

Line	Additive	T5A7	T3A3	L1B2	1G10	Myl	BB2
EM-2	-	+++	+	+	+	+++	nt
	TPA	+	+	+	++	nt	nt
	RA	+++	+	+	+	nt	nt
EM-3	-	+	+	+	+	+++	±
	TPA	+	+	-	+++	nt	+++
	RA	+++	-	-	+++	nt	nt

0602 COMPUTER ANALYSIS OF A CYTOGENETICS REVIEW OF THE LITERATURE ON LEUKEMIA. **Marc De Braekeleer, Marc Cugnon, University of Calgary, Calgary, Alberta T2N 4N1 Canada.**

The purpose of the study is to find some particular association between chromosome or part of chromosome and leukemia of different types. To do that, an IBM34 computer was used to classify leukemias and karyotypes according to sex, age, race and techniques of preparation.

The poster will show diagrams concerning chromosome banding in acute non lymphocytic leukemia(according to FAB classification), acute lymphoblastic leukemia, chronic lymphocytic leukemia, chronic myelocytic leukemia, blast crisis of CML, and other leukemia less common such as eosinophilic leukemia, basophilic leukemia, megakaryocytic leukemia ...

The results show that in each type of leukemia non random chromosomes are involved and very often are different from one type to another type of leukemia.

0603 DIRECT HPLC ANALYSIS OF DIFFERENTIATION PROTEINS OF NORMAL AND LEUKEMIC GRANULOCYTES AND T-LYMPHOCYTES, Vernon L. Alvarez, Warren Evans, and Michael Mage, NCI, NIH, Bethesda, Md. 20205

Reverse phase high performance liquid chromatography (RP-HPLC), a powerful tool for protein analysis, has been applied to the study of normal and leukemic granulocytes and T-lymphocytes. Each cell type is lysed with detergent (or other lysis buffers or solvents), and the centrifuged lysate applied directly to an RP-18 column. Separations of proteins in regions of interest on the chromatogram could be optimized by varying extraction procedures and gradients. In the case of guinea pig granulocytes, a typical gradient can detect seven maturation-linked proteins in less than 20 minutes. Subcellular fractionation coupled with RP-HPLC further identified these peaks as membrane or granule in nature. Leukemic granulocytes, like normal blast cells, are characterized by greatly diminished amounts of these proteins. Different lysis conditions can be used to extract different populations of proteins; this is especially advantageous to study proteins from leukemic cells. Maturation-linked proteins could similarly be quantified in normal and leukemic human granulocytes.

Murine T-lymphocytes, purified using a plating technique, have also been studied, as have cloned T-cell lines. Preliminary data indicate that RP-HPLC can readily detect changes due to stimulation with lectins such as con-A and growth factors such as IL-2. Functional studies are being carried out in an attempt to identify a receptor for IL-2.

0604 P388AD TUMOR CELLS PULSED WITH HAPTENATED-TOLEROGEN PRIME NORMAL B CELLS: CONVERSION OF A TOLEROGENIC TO AN IMMUNOGENIC SIGNAL, Richard P. Phipps and David W. Scott, Duke University Medical Center, Durham, NC 27710

The P388AD lymphoid dendritic cell-like tumor line can present the tolerogen fluorescein-ated sheep gamma globulin (FL-SGG) in immunogenic fashion to B cells. Tolerogen bound to P388AD cells could specifically augment the plaque forming cell response of spleen cells as assessed by challenge with FL-thymus independent (TI) antigens. This augmentation occurred when P388AD cells were pulsed with FL-SGG or FL-F(ab')$_2$ fragments thereof, but not consistently with other FL-antigens. The ability of P388AD cells to augment a TI response required histocompatible T cells during the initial 24 hours of presentation. Pretreating P388AD cells with LPS prior to antigen presentation enhanced the priming effect, possibly due to IL-1 production by P388AD cells. Several tumor cell lines, some with known antigen presenting ability, were tested for their ability to prime B cells to FL-TI antigens. However, only an IA$^+$ J774 tumor clone was capable of priming to FL-TI antigen. Studies are currently in progress to assess the role of both Ia antigens and IL-1 in the stimulation of the response of B cells to TI antigens.

0605 DIFFERENTIATION AND METASTATIC PROPERTIES OF T LYMPHOMA CELL LINES IN VIVO, E. Hays, C. Streifinger and C. MacLeod, UCLA, Los Angeles, CA and Salk Institute, San Diego, CA

Two T-lymphoma cell lines, SL12.1 and SL12.4 have been cloned from AKR SL12, a cell line established from the bone marrow of an AKR mouse with spontaneous T cell lymphoma. SL12.4 is sensitive to dexamethasone induced lysis and displays high thy 1.1 and ThB antigens on its surface. SL12.1 is resistant to dexamethasone displays low thy 1.1, and H2K on its surface. The *in vitro* doubling times of these cell lines are similar (12 hrs). However, the *in vivo* growth characteristics are remarkably different. 10^6 cells from each cell line were injected intravenously into young AKR mice. Groups of 5 mice were killed at weekly intervals for 5 weeks after inoculation and bone marrow spleen and thymus were cultured to recover tumor cells. In SL12.4 injected mice, lymphoma cells were not recovered at weeks 1-5. All of the organs appeared normal. Between weeks 6 and 9, all of the remaining 5 inoculated animals developed lymphoma with gross tumor involvement of kidneys and liver (diffuse). In addition tumor cells were recovered from the three test tissues. Some of the recovered tumor cells had become resistant to dexamethasone. In SL12.1 injected mice lymphoma cells were found in the bone marrow at one week, in marrow and spleen at week 2, and in marrow, spleen and thymus at week 3, when the first observation of splenomegaly was made. At week 4, all of the animals were moribund or dead. Gross tumor was found primarily in liver (nodular), spleen and retroperitoneal nodes. Thus, the differentiated cell line (SL12.1) had more aggressive growth properties and different patterns of metastasis than the less differentiated SL12.4 line. (Supported by DOE and NIH grants to EH, and National Leukemia Association grant, Leukemia Society of America and NIH fellowships to CM).

0606 PHENOTYPIC STATUS AND FUNCTIONAL CAPABILITIES OF CELLS RECENTLY MIGRATED FROM THE THYMUS, Roland Scollay, Walter and Eliza Hall Institute, Melbourne, Australia, 3050

One important step in the production of T cells is the release of newly formed lymphocytes from the thymus. Despite a wide variety of approaches to this question, many issues remain controversial. The number of cells released is clearly very low compared to the intrathymic production rate, but arguments concerning the phenotypic and functional status of the cells that do leave are probably a result of the indirect methods used to examine them. We have resolved these questions by directly analysing cells leaving the thymus. These are identified as the fluorescein positive cells which accumulate (in a linear manner) in blood, spleen and lymph nodes, 1-6 hours after intrathymic injection of fluorescein isothiocyanate, a procedure which labels 30-50% of thymocytes, but no cells outside the thymus (1,2). We have used a wide range of markers (Thy 1, Ly 2, H-2, TL, PNA, size, density) to show that these migrant cells are phenotypically distinct from cortical thymocytes (the predominant thymocyte population), but the same as medullary thymocytes or peripheral T cells. For markers which distinguish medullary thymocytes from T cells, (size, density and a new monoclonal antibody, B2A2) the migrants are like medullary cells. In limit dilution, functional assays which measure the ability of single cells to respond to Con A by proliferation and development of cytotoxicity, purified thymus migrants perform as well as peripheral T cells. These data show that the major maturation events occur before cells leave the thymus.

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2. R. Scollay (1982) J. Immunol. 128, 1566.

0607 MONONUCLEAR CELL LEUKEMIA OF FISCHER 344 RATS: AN ANIMAL MODEL FOR HUMAN MYELOMONOCYTIC LEUKEMIA, R.R. Maronpot, John E. French and Michael P. Dieter, NIEHS, Research Triangle Park, North Carolina 27709

A spontaneous leukemia of male and female Fischer 344 rats is the principal cause of death for up to 50% of rats between 18 and 26 months of age. The disease is characterized clinically by marked weight loss, severe anemia, and icterus. Salient pathologic features include hepatosplenomegaly, occasional lymphadenopathy, and infiltration of malignant cells into many tissues often resulting in microinfarcts. Malignant cells initially proliferate in splenic red pulp. The cell of origin of this mononuclear cell leukemia is unknown but cytomorphologic and histochemical features are compatible with those of the monocytic series. It is proposed that this spontaneous leukemia represents an animal model of human myelomonocytic leukemia. Results of current efforts to characterize the biological behavior, light and ultrastructural morphologic features, and biochemistry of the leukemic cells will be presented.

0608 SPONTANEOUS, IN VITRO, MALIGNANT TRANSFORMATION OF A BASOPHIL/MAST CELL LINE, Jean-François Conscience and Philip E. Ball, Friedrich Miescher-Institut, P.O. Box 2543, CH-4002 Basel, Switzerland.

In several laboratories, including our own, permanent cell lines displaying a number of basophil/mast cell traits have been isolated in vitro from spleen, bone marrow, fetal liver, thymus and lymph node of a variety of mouse strains. These lines are strictly dependent for their continued proliferation on growth factor(s) present in medium conditioned either by the WEHI-3 myelomonocytic leukemia cell line, or by lectin- or antigen-stimulated spleen cells. They fail to give rise to tumors in vivo. We have now observed and documented a case of spontaneous, in vitro transformation of such cells. The transformed cells, in contrast to their untransformed counterparts, no longer require the addition of factor(s) for continuous growth but still respond to it by an increase in growth rate and cloning efficiency. When injected in vivo, they have become highly tumorigenic. Interestingly, they differ little from the factor-dependent cells in the expression of several basophil/mast cell differentiated traits (presence of histamine and IgE receptors, positivity for specific stains) and they have remained diploid. No retrovirus appears to be involved. Together with their untransformed counterparts, these cells should represent a useful tool to study cell differentiation and transformation in the basophil/mast cell lineage.

0609 A DEFECT IN L-SYSTEM AMINO ACID TRANSPORT IN B-CELL CHRONIC LEUKEMIC LYMPHOCYTES: MULTICOMPONENT ANALYSIS. G.B. Segel, W. Simon & M.A. Lichtman, University of Rochester Rochester, New York. The L-system of amino acid transport in chronic lymphocytic leukemic lymphocytes (CLL) is markedly diminished when compared to blood or tonsillar lymphocytes (J Biol Chem 257:9255, 1982). Since CLL cells are of B-lymphocyte origin, we now have examined amino acid transport in isolated B-lymphocytes from human blood and tonsils. L-system transport was measured with BCH, which is a synthetic amino acid whose transport is limited to the L-system. Amino acid uptake was subjected to a multicomponent computerized analysis which partitioned the total uptake into the saturable carrier-mediated transport system and the uptake by diffusion. The maximal velocity of L-system transport in CLL cells, 81 μmol/L-cell water per min, was less than 10% that of blood B-lymphocytes, 1422 μmol/L-cell water per min. Normal blood B-lymphocytes may be the best homologue of the CLL B-cell. BCH uptake by tonsillar B-cells, a B-lymphocyte cell line (RPMI 1788), and blood T-lymphocytes also was ten times that observed in CLL cells. Similarly, the L-system uptake of leucine, a naturally occurring amino acid that is usually transported primarily by the L-system, was reduced in CLL B-cells to 10% of normal B-cells. Total leucine uptake by CLL cells, however, was 40% of that expected because of transport via an alternative transport system. We did not observe a defect in CLL cells in the A-system, another major amino acid uptake pathway. Our data indicate that there is a specific, profound decrease in L-system carrier-mediated amino acid transport in CLL B-cells, and this abnormality in the membrane of CLL lymphocytes is related to their neoplastic nature rather than to their immunologic subtype. These findings imply an absent or functionally defective membrane protein in the CLL cells of each of 8 patients studied thus far.

0610 EARLY T CELL DIFFERENTIATION ANTIGEN EXPRESSED ON MOUSE T LEUKEMIA CELL LINES, Fumio Takei, Department of Pathology, University of British Columbia, and the Terry Fox Laboratory, B.C. Cancer Research Centre, Vancouver, B.C., Canada

Three surface antigens expressed on a mouse T leukemia line, EL-4, have been identified by rat monoclonal antibodies. The antigens are not expressed at a significant level on normal adult thymocytes, spleen cells, lymph node cells, bone marrow cells, or mitogen activated T and B cells as tested by radioimmunoassay or by FACS analysis. The first antigen (YE1/7.1) is strongly expressed on at least 3 T cell lines, but none of the Thy-1 negative lines express this antigen. The antigen is also expressed on a subpopulation of fetal and neonatal thymocytes, suggesting that the antigen may be a differentiation antigen uniquely expressed on cells in early stages of the T cell lineage. The antigen molecule has an apparent molecular weight of 60 Kd. The other two antigens (YE1/19.1 and YE1/48.10) are not detectable on fetal or neonatal thymocytes, but a small number of cells in fetal liver seem to express the antigens. Whether those positive cells in fetal liver represent a distinct cell population is unknown. YE1/19.1 antigen molecule has an approximate molecular weight of 230 Kd under non-reducing conditions and 115 Kd under reducing conditions. YE1/48.10, too, seems to be a dimer with a molecular weight of 95 Kd under non-reducing conditions, decreasing to 50 Kd under reducing conditions.

0611 *IN VIVO* EFFECTS OF A CLONED CELL LINE WITH NK ACTIVITY ON MARROW GRAFTS AND TUMOR DEVELOPMENT, John F. Warner and Gunther Dennert, The Salk Institute for Biological Studies, La Jolla, CA 92037

Natural killer (NK) cells represent a class of cells which are capable of lysing a variety of tumor cells, virus infected cells and hemopoietic cells *in vitro* without intentional prior exposure to antigen. We demonstrate that a cloned murine cell line with NK activity exhibits *in vivo* effects in three experimental animal models. First, we show that NK-cell deficient C57BL/6-beige mice, injected with cloned histocompatible NK cells, develop the ability to reject allogeneic bone marrow grafts. The marrow graft rejection is specific for target structures primarily encoded in the H-2D region of the major histocompatibility complex. Second, using the lung colony assay, we show that lung implantation of i.v. injected B16 melanoma tumor cells in NK-deficient C57BL/6 mice is markedly inhibited in those mice previously given syngeneic cloned NK cells. Third, we demonstrate that the incidence of radiation-induced thymic leukemia in C57BL/6 mice is markedly reduced by i.v. administration of syngeneic cloned NK cells three months prior to the onset of thymic leukemia. These results support a role for NK cells in bone marrow graft rejection and in the surveillance of neoplasia.

0612 ABNORMAL INSULIN BINDING AND MEMBRANE PHYSICAL PROPERTIES OF A FRIEND ERYTHROLEUKEMIA CLONE RESISTANT TO DMSO-INDUCED DIFFERENTIATION, Ido Simon, Thomas Brown Barry Ginsberg, Dept. of Int. Med. and Biochem., DERC VAMC, Univ. of Iowa, Iowa City, IA.

We have compared insulin binding, plasma membrane fluidity, and membrane composition of three different Friend Erythroleukemia clones. The non-differentiating DMSO-resistant clone (R3) had dramatically altered insulin binding properties when grown under normal serum concentrations (15%). The receptor of R3 bound insulin as if it possessed a single class of low affinity receptors that lack the property of negative cooperativity. The Scatchard plot was linear and there was no ligand-induced acceleration of dissociation. The Hill coefficient for R3 was 1, implying "no cooperativity," whereas the Hill coefficient for two DMSO-inducible clones (FLC and F+) was 0.8, implying "negative cooperativity." In addition, the insulin receptor of R3 had a decreased affinity for insulin manifested as a 40-fold increase in the amount of insulin required to compete for half of the tracer binding (41 nM for R3 vs 1 nM) for FLC and F+. Growing R3 in 2.5% serum partially reversed the abnormal properties of the insulin receptor. Electron spin resonance measurements with the 5-nitroxy stearate spin probe demonstrated that after growth in 15% sera, R3 had a more fluid plasma membrane than the FLC and F+ clones and that the membranes of all clones were less fluid when grown in 2.5% serum. The weight ratio for polyunsaturated fatty acids to monounsaturated fatty acids for R3 was 0.47 and 0.20 when grown in 15% and 2.5% serum, respectively. The FLC clone had higher ratios of 0.61 and 0.42 when grown simularly. These results suggest that the abnormal binding properties for the insulin receptor of the DMSO-resistant clone, R3, may be related to very high membrane fluidity.

0613 A SPONTANEOUS AKR B-LYMPHOMA EXPRESSING LY1 AND LY2 ANTIGENS, E. Richie, J. Allison, L. Lanier, R. Nairn, A. Howell, J. Brown, University of Texas System Cancer Science Park, Smithville, Tx 78957 and Becton Dickinson, Mountain View, CA 94043

A spontaneous lymphoma, designated 225, arising in an aged AKR mouse was found to be of B cell origin by phenotypic analysis using monoclonal antibodies and flow cytometry. Greater than 80% of spleen cells from the original host expressed u heavy and k light chains as well as I-A I-E/C, E2, ThB, DNL, DNL 1.9, and B220 antigens. The 225 cells did not express Thy or T30 antigens. An unusual feature of this B-cell lymphoma was expression of Ly1 and Ly2 antigens. After enzymatic stripping to remove cell surface Ig, Ly1 and Ly2 molecules, the 225 cells regenerated these markers proving that their expression was not due to passive absorption. The surface marker profile of the 225 lymphoma was maintained during serial passage into syngeneic recipients. Upon adaption to in vitro culture, the 225 cell line maintained a surface phenotype (u+ Ly1+ Ly2+ Thy-) consistent with that of the in vivo passaged lymphoma cells. Preliminary studies indicate that the 225 cultured cell line is secreting IgM.

Immunochemical analysis of MuLV env proteins isolated by specific immunoprecipitation revealed that the 225 cell line expresses multiple gp70 molecules related to xenotropic and ecotropic genomes. Electrophoretic analysis suggested that one of these represents the product of a recombination between ecotropic and xenotropic MuLV env genes. Restriction endonuclease analysis of cellular DNA revealed the presence of a specific recombination in MuLV env sequences resulting in generation of a novel XbaI/BclI linkage similar to that seen in MCF induced AKR T-lymphomas. This data suggests that this unusual Ly2+ B-cell lymphoma has a similar recombinant etiology as that associated with AKR T-lymphomas.

0614 RB VIRUS: A VARIANT FRIEND VIRUS THAT CROSSES THE *Fv-2^r* BARRIER, Roy W. Geib, Rita Anand, Kenneth Blumberg, Kathryn Jones, and Frank Lilly, Albert Einstein College of Medicine, Bronx, N.Y. 10461, and Morehouse School of Medicine, Atlanta, GA 30314.

Friend erythroleukemia virus (FV) is a complex of at least two distinct viruses, a replication-competent helper virus (FMuLV) and a replication-defective virus (SFFV). Strong resistance to SFFV is a trait governed by a single recessive gene, *Fv-2^r*. We have isolated and partially characterized a new FV variant, RB, which is capable of crossing the *Fv-2^r* barrier. The variant virus differs from the standard FV strains in several aspects. When RB is injected into *Fv-2^s* mice, it produces a rapid ery throleukemia (10-30 days) similar to that produced by FV. In contrast, injection of RB into *Fv-2$^{r/r}$* mice produces an erythroleukemia with a significantly longer latent period (2-4 months in about 80% of the injected mice). Another difference between wild-type FV and RB is that the RB virus produces at least one variant protein encoded for by the SFFV *env* gene. Wild-type SFFV produces an *env* gene product with a molecular weight of 52K. The variant RB virus produces two proteins, 52K and 45K. Pulse-chase experiments indicate that the 45K protein is not a breakdown product of the 52K protein.

0615 FOLATE TRANSPORT BY ERYTHROLEUKEMIA CELLS, Robert E. Corin, Howard C. Haspel, Richard A. Rifkind, and Martin Sonenberg, Memorial Sloan-Kettering Cancer Center, New York, NY 10021

Induced differentiation of murine erythroleukemia cells(MELC)is characterized by expression of the erythroid phenotype including commitment to terminal cell division. The relationship between nutrient transport and proliferative capacity of MELC during differentiation was studied by examining the ability of MELC to transport the nonmetabolized folate analog methotrexate(MTX). MTX transport by MELC has the following characteristics:a)influx and efflux are temperature-dependent,b)influx is trans-stimulated, stimulated by azide, effectively competed for by reduced folates but not folic acid, and saturable with a Km of ~ 5 uM. When cells were cultured with hexamethylene bisacetamide(HMBA,5mM) or dimethyl sulfoxide (DMSO, 210mM) for 4-6 days there was a >90% decrease in their ability to transport MTX. The loss of MTX transport was dose-dependent with respect to HMBA after 5 days of induction. MELC strain DR-10(HMBA-sensitive;DMSO-resistant)lost 94.5 and 29.3% of MTX transport activity after 5 days of exposure to HMBA and DMSO, respectively. Loss of MTX transport during induction was dependent upon the kinetics and extent of induction. Under optimal conditions of induction significant decreases of transport activity were noted by 40 hours of exposure to HMBA. Decreased folate transport appears to be an induction-related event, and the extent of decline in folate transport appears greater than the decline of other transport functions.

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0616 HUMAN CUTANEOUS T-CELL LYMPHOMA: CONSTITUTIVE PRODUCTION OF INTERFERON IN CULTURED T-LYMPHOCYTES, J.L.Moore, B.J.Poiesz, K.W.Zamkoff, S.Hanna, A.Gazdar, R.L.Comis, BKRC, Auburn, NY 13021, UMC, VAH, Syracuse, NY 13210, NCI-Navy, Bethesda, Md. 20205.
Human T-cell lymphoma leukemia virus (HTLV) is a novel exogenous retrovirus associated with T-lymphocyte neoplasia in man. We examined the relationship between constitutive production of interferon, lymphocyte phenotypes, and HTLV expression in cell cultures from patients with cutaneous T-cell lymphoma (CTCL). Serial passages of TCGF independent cell line HUT 102 showed that HTLV expression was inversely correlated with constitutive interferon production with early passages producing more interferon and little HTLV and later cloned cell passages producing high titer HTLV and no interferon. Early passages of HUT 102 were strongly positive for OKT3 and OKT4 but negative for OKT8. With prolonged passage the expression of OKT3 and OKT4 is greatly decreased. Cell cultures of in vivo activated lymphocytes were established with TCGF from patients with CTCL and were tested for HTLV expression, surface lymphocyte phenotypes, and constitutive interferon production. When the culture is derived from neoplastic cells, cells grown are OKT4 and HTLV positive, OKT8 negative, and do not elaborate interferon. When the culture is derived from non-malignant lymphocytes, the cells grown are OKT4 and/or OKT8 positive, HTLV negative, and do elaborate interferon. From patient K.W., samples obtained from his neoplastic lymph node were highly positive for HTLV expression, whereas his non-neoplastic peripheral blood T-lymphocytes were rarely positive. The data indicates that in the presence of in vitro endogenous interferon in cell cultures from CTCL patients, expression of HTLV is low or negative. Interferon producing cultures contained OKT4 and/or OKT8 positive lymphocytes, whereas neoplastic, HTLV positive cultures were negative for the OKT8 phenotype.

0617 EXPERIMENTAL PROGRESSION OF T LYMPHOBLASTOMA CELLS TO AUTONOMOUS T LYMPHOMA CELLS. M.Haas[1,3], A.Altman[4], E.Rothenberg[5], and O.W.Jones[2,3]. Dept.of Biology[1], Genetics[4], and Cancer Center[2], Univ.of California, San Diego; Scripps Clinic Research Foundation[4], La Jolla; and Division of Biology, California Institute of Technology[5], Pasadena, California.
X-irradiated and Radiation Leukemia virus-inoculated C57Bl/6 mice harbor different T cell lymphomas, a factor-dependent T cell lymphoblastoma (TCLB) and an autonomous, malignant T cell lymphoma (TCL). Often both types can be grown from the same diseased mouse. We have grown both types of lymphoma cells in culture.

Proliferation of TCLB cells is dependent on the presence of T cell growth factor (TCGF,IL-2). The cells possess receptors for IL-2, produce the hormone and synthesize large amounts of it upon stimulation with lectins. In mass culture TCLB cells propagate in an autostimulatory fashion if plated at $>5x10^5$ cells/ml. Single cell cloning of TCLB cells requires the addition of IL-2 to the medium. Cells of the TCL variety neither require nor produce IL-2, and can be quantitatively cloned. TCLB and TCL cells can differ in a number of cell surface markers. In vivo TCLB cells home for the spleen, proliferate and kill the animal. In contrast, TCL cells can grow into a local tumor at the site of injection.

TCLB cells are normal diploid and have remained so through four years of in vitro passage. The autonomous TCL cells possess an aneuploid karyotype of 41 chromosomes, some of which are trisomic for chromosome # 15. We have experimentally transformed the ("non-malignant") TCLB cells to the autonomous (malignant) state. Transformation was accompanied by changes in most of the parameters mentioned above, including a shift from hormone-dependence to autonomy as well as a change from the normal diploid karyotype to aneuploidy

0618 SPECTRIN-DEFICIENT SPHEROCYTOSIS, IS SPECTRIN SYNTHESIS REDUCED OR IS THE SPECTRIN UNSTABLE?, Peter Agre and Vann Bennett, Johns Hopkins University, Baltimore, MD 21205
An unusually severe form of spherocytosis has recently been described (Agre etal, N Engl J Med 306:1155,1982). The inheritance appears to be mendelian recessive, and the RBC membranes are relatively deficient in spectrin: spectrin/band 3 (sp/b3) = 0.5 when measured by SDS-PAGE. It is unresolved whether the deficiency is due to reduced spectrin synthesis or due to progressive in vivo loss of an unstable spectrin variant. Bone marrow aspirates showed extreme erythroid hyperplasia although the RBC progenitors appeared normal while RBCs were markedly spherocytic. Patient spectrin was examined for qualitative abnormalities. No evidence of fragmentation was seen, and spectrin binding and binding sites were normal. Anti-spectrin antibodies reacted with patient and control spectrin with identical affinities. Rotary shadowing e.m. analysis and 2 dimensional maps of patient spectrin and digests are in progress. Patient and control blood were fractionated over percoll gradients (1.090-1.120 g/ml). Patient blood contained relatively more RBCs in the top fractions (MCHC 34.6) and bottom fractions (MCHC 38) while control blood had much less variation. Patient RBCs at the top demonstrated a median osmotic fragility (MOF) at 0.52% NaCl while those at the bottom were more fragile, MOF at 0.57%. Control RBCs from top and bottom had MOFs at 0.43%. Patient membranes from top and bottom showed sp/b3 = 0.6, and control membranes from top and bottom showed sp/b3 = 1.0. Thus younger RBCs from patients are already deficient in spectrin and more fragile than the control RBCs. Older RBCs from patients are even more fragile although the spectrin deficiency does not progress. Thus neither reduced synthesis nor early degradation of a defective subpopulation of spectrin can be ruled out.

0619 MODULATION OF LYMPHOCYTE RESPONSES BY A PHORBOL ESTER TUMOR PROMOTER, Andrea M. Mastro, The Pennsylvania State University, University Park, PA 16802

The phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA) is a strong tumor promoter in vivo and a potent regulator of cell differentiation and division in vitro. The particular effect of TPA depends on the kind of cell and its stage in development. Of interest to us, TPA has pleiotypic effects on various lymphoid cells. We originally found TPA to be a weak mitogen but a strong co-mitogen with the lectins PHA or ConA for lymph node lymphocytes (Exp. Cell Res. 88:40, 1974). The synergistic effect of TPA (10^{-8}M) on DNA synthesis was most pronounced with a suboptimal concentration of lectin or in macrophage depleted cultures. In cultures in which macrophages were eliminated by physical means or with anti-macrophage serum and complement, we found that TPA replaced the requirement for macrophages in lectin stimulated cultures. However, this was not true in the mixed lymphocyte reaction. In this system TPA is a strong inhibitor of cell division and blast formation. The effect is reversible. TPA does not destroy the responding cell population. Treatment of lymphocytes for 24 hrs before they are combined in the MLC or before they are stimulated with lectins also suppresses DNA synthesis. Again the effect is reversible. In these cases where TPA is inhibitory it appears to act directly on the responding cells. We are presently testing for changes in cell surface antigens brought about by TPA.

0620 CORRELATIONS OF CELL SURFACE MARKERS AND IMMUNOGLOBULIN GENE REARRANGEMENTS IN ACUTE LYMPHOCYTIC LEUKEMIAS OF T AND B CELL PRECURSOR ORIGINS. A. Bakhshi, A. Arnold, S.O. Sharrow, T.W. LeBien, J.H. Kersey, D.G. Poplack, P. Leder, T.A. Waldmann, and S.J. Korsmeyer. NIH, Bethesda, Md. 20205, Univ. of Minn., Minneapolis, Minn. 55455, and Harvard Medical School, Boston, Mass. 02115

We have examined the relationship between immunoglobulin (Ig) gene rearrangement and cell surface antigen expression within leukemic lymphocytes or cell lines from 37 patients with acute lymphocytic leukemia (ALL). Twelve cases were classifiable as T-cell in type as they rosetted with sheep RBCs and/or displayed T-cell associated antigens detected by the 3A-1, OKT3, OKT4, OKT6, or OKT8 monoclonal antibodies. All 12 T-cell type ALLs had germline κ and λ genes and 11 of 12 had germline heavy chain genes. In contrast, all 25 of "non-T, non-B" type which lacked both definitive T cell markers and surface Ig demonstrated rearranged heavy chain genes with the C_μ or J_H gene region probes. Eleven cases also demonstrated light chain gene recombinations when analyzed with the C_κ and C_λ probes. All of the "non-T, non-B" cases demonstrated HLA-DR and many displayed B-cell associated antigens detected by the BA-1 (20 of 24) and BA-2 (22 of 24) monoclonal antibodies. Comparisons here indicate that "non-T, non-B" ALLs represent early cells in B-cell development and suggest a coordinate sequence of events in which heavy chain gene rearrangement and HLA-DR expression precedes the p30/BA-1 antigen, CALLA, and any subsequent light chain gene rearrangements which themselves proceed in an order of κ before λ. The cases of most controversial classification which bear HLA-DR but lack CALLA appear to represent the earliest recognizable B-cell precursors having rearranged heavy but germline light chain genes.

0621 HAIRY CELL LEUKEMIA: MALIGNANT B CELLS WHICH APPEAR TO EXPRESS MEMBRANE RECEPTORS FOR T CELL GROWTH FACTOR (TCGF). Warner C. Greene, Jeffrey Cossman, Warren J. Leonard, Thomas A. Waldmann, and Stanley J. Korsmeyer, NIH, Bethesda, Md. 20205

Despite extensive study, the cellular origin of hairy cell leukemia (HCL) has remained controversial. In prior studies, we found that all 10 cases of HCL studied had rearranged heavy and light chain immunoglobulin (Ig) genes when hybridized with J_H, $C\kappa$ or C_λ DNA probes. Where examined, these Ig genes were transcriptionally active; and in many cases, cells displayed the appropriate surface immunoglobulin. These findings indicated that HCL cells are committed to the B cell lineage. Unexpectedly, all HCL cases examined also reacted with the anti-Tac monoclonal antibody. We have previously shown that this antibody appears to bind to the membrane receptor for TCGF (interleukin-2). We have not observed such anti-Tac reactivity with normal human B-cells or most other B-cell leukemias. In ^{3}H-anti-Tac binding assays, HCL cells contain 3-10 fold fewer receptors than are present on most activated T cells. Further, anti-Tac binding to HCL cells is not inhibited by monoclonal antibodies of the same subclass as anti-Tac indicating specific rather than F_C receptor mediated binding. Utilizing cell surface iodination of HCL cells, immunoprecipitation with anti-Tac, and SDS-PAGE; the HCL receptor has an M_r of 53-57,000. This receptor size is identical to that observed for the receptors present on activated normal human T-cells. We are presently evaluating potential function of these TCGF receptors in short term proliferative assays using partially and completely purified human TCGF. If functional, the presence of TCGF receptors on these cells may allow the preparation of HCL lines which can be used in further studies of this unique leukemia.

0622 HEMOGLOBIN PRODUCTION ON STIMULATION OF K562 HUMAN PLURIPOTENT LEUKEMIA CELLS, A.E. Felice, S.M. Mayson, A.L. Reese and T.H.J. Huisman, Hemoglobin Research Laboratory, V.A. Medical Center, Augusta, GA and Department of Cell and Molecular Biology, Medical College of Georgia, Augusta, GA, U.S.A.

Programs pertaining to normal erythropoiesis, such as Hb synthesis, could be organized abnormally in the K562 blasts and perhaps also in other leukemic blast cells. Thus, an evaluation of the abnormal regulatory properties of these cell types may contribute to understand various aspects of the normal regulation of erythrocytic differentiation and Hb synthesis.

K562 cells produce low levels of hemoglobin spontaneously. After the addition of hemin, Na butyrate or dimethyl sulfoxide either the MCH, or the proportion of B^+ cells, or both increase. Pre-incubation with L-ethionine, or co-culture with hydroxyurea lead to increased, hemin stimulated, hemoglobinization. Such interactions between stimulating agents could influence the types of Hb chains synthesized. With hemin alone, these are the embryonic ε and ζ chains, the α chains, and a large though variable excess of G_γ, $A_\gamma I$ and $A_\gamma T$ chains. A β or δ chain production has not been documented thus far. Although the production of γ chains varies between experiments, the proportion of $G_\gamma/[A_\gamma I + A_\gamma T]$ chains remains relatively constant at levels often found in the peripheral blood erythrocytes of patients with leukemia. The possible production of β chains is evaluated among K562 cells frozen at much earlier passages. (Samples through the courtesy of Dr. Lozzio.)

Further studies utilize cloned K562 cells which have been adapted for growth in a serum-free medium. At low initial cell densities, hemin appears to have mitogenic properties in this system. Neither erythropoietin nor Prostaglandin E_2 can substitute for hemin.

0623 REGULATION OF CYTOTOXIC ACTIVITIES MEDIATED BY A HUMAN MONOCYTIC CELL LINE (U937), Hillel S. Koren and Gina Burkhardt, Duke University Medical Center, Durham, NC 27710

We have previously shown that various characteristics of the U937 line can be modulated by lymphokines. In the present study we have investigated the effects of supernatants from a human cell line (GCT) containing colony stimulating factor (CSF), partially purified human urinary CSF-1 (from E.R. Stanley) LPS, and α interferon (IFN) on antibody dependent cellular dytotoxicity (ADCC) and non-specific tumor killing of the U937 cell line. The results demonstrate that CSF augments ADCC activity in a dose-dependent fashion. Whereas control unstimulated U937 cells had almost no detectable ADCC activity in most experiments, cells incubated for 48 to 72 hours in the presence of 7% CSF (or 1000u/ml of CSF-1) reached 30% specific lysis at 20:1 E:T ratios in a 2 hr ^{51}CR release assay. Cells activated with 25-100 μg/ml of LPS exhibited similar high levels of ADCC activity. Intermediate levels of ADCC activity were achieved with IFN (500 units/ml). Treatment with any of these reagents did not interfere with cell viability or with their division which would suggest that the cells were not terminally differentiated. The U937 line exhibits appreciable levels of non-specific cytotoxic activity against P815 tumor targets in a 20 hr ^{51}Cr release assay. This activity could only marginally be modulated by any of the tested reagents.

Our data suggest that CSF, CSF-1, IFN, and LPS can modulate the ADCC activity of the U-937 cell line, but have little effect on its direct cytolytic activity.

0624 HUMAN PRE-B LEUKEMIA CLONES WITH VARIANT PHENOTYPES, R. Graham Smith and Peter E. Lipsky, University of Texas Health Science Center, Dallas, Texas 75235.

We established an unusual pre-B leukemia cell line (SMS-SB) from a 15-year-old girl with lymphoblastic lymphoma-leukemia (J. Immunol. 126:586, 1981). These cells are of the pre-B phenotype but do not express the common ALL antigen (CALLA), and only 1-2% express terminal deoxynucleotidyl transferase (TdT). Thus, SMS-SB cells are phenotypically similar to the majority subpopulation of normal bone marrow pre-B cells. Treatment of SMS-SB cells with subtoxic doses of 5-azacytidine (5-A) induces low amounts of membrane Ig. The 5-A treated cells can then be induced to express larger amounts of membrane Ig by co-cultivation with normal allogeneic T cells. The co-cultivated tumor cells express membrane light chain determinants detectable by immunofluorescence. We have now cloned the 5-A treated cells. Ten clones have been analyzed for expression of membrane Ig and TdT. One clone expresses TdT in 41% of the cells and bears very sparse membrane Ig. Other clones express 0.3-1% TdT positive cells and a larger amount of Ig. A third kind of clone bears virtually no Ig and contains TdT in only 1% of the cells, thus resembling the majority of the parent cells. The differential induction of membrane Ig on these cells after co-cultivation with normal T cells will be presented. In summary, we have generated stable variant pre-B and nascent B cells that may be useful in analyzing early steps in B lymphocyte development.

0625 CYTOGENETIC ANALYSIS OF MYELOID PROGENITORS IN Ph^1-POSITIVE ALL, D.K. Kalousek, I.D. Dubé, C.J. Eaves and A.C. Eaves, B.C. Cancer Research Centre, Vancouver, Canada

The Ph^1chromosome has been detected in 5% of childhood ALL and in 25% of adult ALL. In both age groups the presence of the Ph^1 chromosome is usually associated with a poor prognosis. Some cases presenting as Ph^1-positive ALL may represent patients with CML in lymphoid blast crisis whose chronic phase was not clinically recognized. Other cases may represent a variant form of ALL without involvement of the myeloid lineages. Some support for this latter possibility comes from the finding that the standard Ph^1 translocation is found less frequently in Ph^1-positive ALL than in Ph^1-positive CML.

We have recently developed a method that allows cytogenetic analysis to be performed on individual colonies derived from BFU-E, CFU-C and CFU-GEMM. Application of this technique to 2 CML patients in lymphoid blast crisis revealed that all in vitro assayable myelopoietic progenitor cells were members of the 46,XX,Ph^1 chronic phase clone whether the patients were in relapse or remission. In 1 patient, the appearance of lymphoid blasts correlated with the presence of a chromosomally altered subclone. More recently we have investigated a patient who was diagnosed as a Ph^1-positive ALL 2 years ago and is now in CNS relapse. Cytogenetic analysis of 27 recently cultured marrow BFU-E and CFU-GEMM colonies showed these to be all 46,XX,Ph^1. It is therefore likely that in this patient, the chromosomal lesion occurred in a stem cell with both lymphopoietic and myelopoietic potential, as in CML. These results suggest that cytogenetic analysis of myelopoietic progenitors may help to establish the extent and frequency of myeloid stem cell involvement in Ph^1-positive ALL.

0626 THE CALMODULIN ANTAGONIST, W-13, SYNERGISTICALLY ENHANCES COMMITMENT TO DIFFERENTIATION IN HL-60 CELLS, M.L. Veigl, W.D. Sedwick, P.M. Conn and J.E. Niedel, Duke University Medical Center, Durham, NC 27710

In an attempt to demonstrate a role for Ca++ in commitment to differentiation of human promyelocytic leukemia cells, we studied the effect of the calmodulin antagonist W-13 [N-(4-aminobutyl)-5-chloro-2-napthalenesulfonamide] on this process. HL-60 cells were induced with either 1.25% dimethyl sulfoxide (DMSO), 500uM dibutyryl cyclic AMP(dbcAMP) or 100nM retinoic acid (RA). Commitment to terminal differentiation occurs after 18 to 30 hrs of exposure to these inducers. W-13, or the less potent analog W-12, were incubated with the cells at 50uM for the 6 hr window from 24 to 30 hrs following addition of inducers. The cells were then cultured free of inducers or antagonists from 30 to 96 hrs. Differentiation was assessed by expression of chemotactic formyl peptide receptors (CFPR), reduction of nitroblue tetrazolium (NBT) and morphology. Striking synergy was evident between RA and W-13. CFPR was increased 100 fold, NBT positive cells were increased 10-fold and metamyelocytes were increased 3-fold over cells treated with RA alone. Synergy between DMSO and W-13 was also evident with CFPR increased 3-fold and NBT increased 2-fold. W-13 did not alter dbcAMP induced differentiation. W-13 alone did not induce differentiation, nor did W-12 demonstrate any synergistic effect with inducers. Preliminary experiments also suggest that induction of differentiation with DMSO results in an initial decrease in calmodulin (CaM) content/cell. Differential extraction procedures utilizing EDTA or EGTA as chelators, further suggested that changes in the Ca++ dependent bound fraction of CaM occur between 24 and 30 hrs after induction.

0627 CYCLIC NUCLEOTIDE-INDUCED MATURATION OF HUMAN PROMYELOCYTIC LEUKEMIA CELLS: COMPARISON WITH OTHER INDUCERS, James Niedel and Thomas Chaplinski, Duke University Medical Center, Durham, NC 27710

We have recently described granulocytic maturation of the HL-60 cell line in response to dibutyryl cAMP (dbcAMP) or agents that raise intracellular cAMP levels by activation of adenylate cyclase (JCI 70, 953). The properties of cells induced with 500 μM dbcAMP or 10 nM PGE_2 plus 500 μM theophyllin have been compared to those of cells induced with 1.25% DMSO or 100nM retinoic acid (RA). Formyl peptide stimulated chemotaxis and β-glucuronidase release peaked on day 3 for the dbcAMP and PGE_2 treated cells and day 5 for DMSO and RA. The maximum responses for dbcAMP, PGE_2 and DMSO cells were similar, but 3-to 5-fold greater than those of RA cells. Superoxide production peaked on day 3 to 5, with RA cells showing the least superoxide production, PGE_2 and DMSO approximately 5-fold greater production than RA and dbcAMP 15-fold greater production than RA. Expression of formyl peptide receptor at day 5 was 200 fmol/10^6 cells for DMSO and RA, 300 fmol for PGE_2 and 500 fmol for dbcAMP. At day 5, 95% of dbcAMP cells were positive for PMA-induced NBT reduction, 70% of PGE_2 and DMSO cells were positive and only 12% of the RA cells were positive. DMSO and dbcAMP induced cells were 70% adherent, PGE_2 cells were 50% adherent and RA cells were 20% adherent. These data demonstrate that HL-60 cells treated with either dbcAMP or PGE_2/theophyllin, differentiate into cells with functional properties typical of mature granulocytes. These inducers demonstrate efficacy similar to DMSO and RA.

0628 SOLUBLE PHORBOL DIESTER RECEPTOR CO-PURIFIES WITH PROTEIN KINASE C, G.R. Vandenbark and James Niedel, Duke University Medical Center, Durham, NC 27710

A chemically diverse group of stimuli will induce in vitro maturation of myeloid cell-lines, but the phorbol diesters are the most potent and efficacious. The phorbol diester receptor present in the particulate fraction of rat brain and human promyelocytic leukemia cells (HL-60) was solubilized by divalent ion chelation in the absence of detergents. The soluble receptor from both sources was partially purified by $(NH_4)_2SO_4$ precipitation and DEAE chromatography. The rat brain receptor was further purified by S-200 chromatography and sizing and ion exchange HPLC. Receptors from both sources required exogenous phospholipid for activity and displayed a K_D of 7-10 nM for [^{3}H]PDBu. Active phorbol analogs inhibited [^{3}H]PDBu binding with an apparent K_i of 3 nM for PMA, 60 nM for PDBu and 70 nM for PDD. The inactive analog 4α PDD did not inhibit at 500 nM. The Ca^{2+}-dependent, phospholipid-sensitive protein kinase C co-purified precisely with the soluble phorbol diester receptor. Partially purified protein kinase C was activated directly by phorbol analogs with an apparent K_m of 2 nM for PMA, 50 nM for PDBu and 50 nM for PDD. 4α PDD did not activate. These data suggest that protein kinase C is the phorbol diester receptor and that macrophage maturation of human myeloid leukemia cells is due to activation of this kinase.

0629 MARKER CHROMOSOME DETECTION BY BIVARIATE FLOW KARYOTYPING, Loh-Chung Yu, Richard G. Langlois, and Joe W. Gray, Biomedical Sciences Division, Lawrence Livermore National Laboratory, Livermore,, CA 94550

Dual beam flow cytometry has been shown to be useful for classification of human chromosomes. In this process, called bivariate flow karyotyping, chromosomes are isolated from mitotic cells, stained with two dyes binding preferentially to AT and GC rich DNA and processed one by one through a flow cytometer where the chromosomes are classified according to their dye contents. All normal human chromosomes except chromosomes 9 throuth 12 and sometimes chromosomes 14 and 15 can be unambiguously classified using this procedure. In addition, chromosome changes on the order of one band can be detected reliably.

We now show that this procedure is useful in the detection of marker chromosomes in human cancer cells. Specifically, we have measured bivariate flow karyotypes for human colon carcinoma cells (LoVo cell line) and have detected an abnormal marker chromosome present in a large fraction of the LoVo cells. This study of an aneuploid, karyotypically unstable cell population (49 ± 3 chromosomes per cell) demonstrates the power of bivariate flow karyotyping in the detection of marker chromosomes in human malignancies. This method makes it possible to analyze marker chromosomes at the molecular level, since any chromosome that can be resolved cytometrically can be purified by flow sorting.

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0630 EXPRESSION OF HEMATOPOIETIC CELL ANTIGENS BY K-562 CELLS. Albert T. Ichiki, Bismarck B. Lozzio, Carmen B. Lozzio, Carl J. Wust, and Elena G. Bamberger, University of Tennessee, College of Medicine/Knoxville and College of Liberal Arts, Knoxville, TN 37920.

The expression of hematopoietic cell differentiation-linked and/or associated antigens by K-562 cells has been determined with a variety of monoclonal antibodies. These antibodies were obtained commercially or as gifts from a number of investigators and detect cell surface antigens characteristic of hematopoietic precursor cells as well as the erythrocytic, monocytic, granulocytic, megakaryocytic and lymphocytic lineages. The determinations were made by the indirect immunofluorescence assay in which fluorescein conjugated $F(ab')_2$ fragment of sheep anti-mouse IgG was used as second antibody and the cells analyzed in a Cytofluorograf. Values for all monoclonals studied appeared to be significantly higher than background, i.e., the values for second antibody added to cells which were not first treated with monoclonal antibody. However, the range of percentages varied from 5 to 82%. We tentatively concluded that, for the most part, K-562 cells express differentiation-linked antigens of the different lineages although the total of all the specificities is greater than 100% suggesting that some cells express antigens of two lineages. We are evaluating this by the use of combinations of monoclonal antibodies. The results support the contention that the K-562 cells in this laboratory are totipotential stem cells that spontaneously tend to partially differentiate into progenitors of the five bone marrow cell series. However, the differentiation is incomplete but totally reversible when the cells are cultured in fresh medium. The finding by others that describe K-562 cells as erythroleukemic leads us to believe that these investigators have selected for progenitor cells of this lineage, which do not revert to the totipotential state.

0631 LEUKEMIC CELL COLONY FORMATION IN VITRO, K. Dicke, S. Tindle and S. Jagannath, The Bone Marrow Transplantation Center, M.D. Anderson Hospital, Houston, TX 77030

We have reported preliminary results of an in vitro colony assay for leukemic cells obtained from marrow and peripheral blood of untreated acute leukemia patients (acute myelogenous leukemia and acute lymphocytic leukemia) (1). The leukemic origin of the colonies was proven on the basis of cytogenetic analysis. The cells in those colonies had nucleoli containing the human malignancy-associated nucleolar antigen(s) (HMNA). The presence of this antigen in nucleoli in many different types of human tumors, including leukemia, was observed by Davis et al (2). We have developed a technique with which colonies can be incubated in situ with antibodies against HMNA. HMNA is not found in cells of CFC-GM colonies obtained from marrow of hematologically normal individuals. In contrast, colonies from marrow of untreated leukemic patients contained many HMNA-positive cells. Furthermore, cells in many of these colonies were at least partially differentiated. The HMNA assay was used to analyze cells from colonies of 4 leukemic patients in remission. The HMNA-positive as well as HMNA-negative colonies were found. The range of HMNA-positive colonies was 16-69%, and exceeded the percentage of HMNA-positive cells in the original suspension plated. These colonies also contained differentiated cells. These data indicate that in remission a certain number of colony forming cells contain this malignant marker. This test will be used to monitor the efficacy of separation of normal stem cells and leukemic colony-forming cells in remission marrow. In addition, we will use this assay to monitor the activity of monoclonal antibodies against leukemic colony forming cells in the individual untreated and relapsed acute leukemia patient. 1) Dicke KA, et al., Exp Hemat 9(9) 1981; 2) Davis FM, et al., Proc Natl Acad Sci 76(2) 1979.

0632 CONTROL OF TRANSFERRIN RECEPTOR FUNCTION AND EXPRESSION IN HL60 CELLS, Caroline A. Enns, Jeffery E. Shindelman and Howard H. Sussman, Stanford University Medical Center, Stanford, CA 94305

Quantitative studies of the change in the number and the binding properties of the transferrin receptor during induced differention and cessation of growth were conducted with HL60 cells, a continuous proliferating cell line.

There cells were induced to differentiate and to stop proliferating by incubating them in the presence of 1.25% dimethyl sulfoxide. The number of receptors was measured by analysis of specific transferrin binding and by direct measurement of the receptor using a radioimmune assay. The results from the binding measurements amd from the radioimmune assay showed a five fold decrease in the receptor which reached a basal steady state level in three days, although the cells did not stop dividing until days 6-7. The decrease in the transferrin binding values occured sooner after the addition of dimethyl sulfoxide than did the decrease in receptor antigenicity as measured by radioimmune assay.

0633 THE ACTIVATION OF B CELLS REQUIRES BOTH ANTIGEN-SPECIFIC AND NON ANTIGEN-SPECIFIC T CELL DERIVED PRODUCTS, M.A. Jaworski, C. Shiozawa, and E. Diener, University of Alberta, Edmonton, Canada.

We examined the nature of the signals given to B cells by antigen-specific, T cell-derived helper factor (Hf), the polyclonal activator lipopolysaccharide (LPS) and by non-antigen specific factors of macrophage or T cell origin. We used a low density system, highly enriched for B cells, resulting from the positive selection of unprimed murine spleen lymphocytes which formed rosettes in the presence of chicken erythrocyte antigen. In each well, 2×10^3 rosette forming cells (RFC) were cultured, in the presence of one or more of the above factors and their IgM production measured in a direct plaque-forming cell (PFC) assay. In the presence of either Hf or LPS, RFC undergo proliferation but not differentiation into PFC. We could not reconstitute the PFC response of Hf- or LPS-stimulated B cells by adding either purified cultured macrophages, macrophage culture supernatant, or the monokine, interleukin 1. However, we could reconstitute the PFC response of Hf-stimulated RFC by adding TRF containing supernatant derived from the cloned T cell line C.C3.11.75. We also found that the interaction between the Hf and the B cells in the IgM response was I-A subregion restricted, and that in the absence of Hf, B cells, even those which had bound antigen, were not responsive to the TRF containing supernatant. This supernatant only partially reconstitued the PFC response of LPS-stimulated RFC; the response could be completely reconstituted using an IL2-containing supernatant derived from a cloned EL4 thymoma cell line.

0634 EFFECTS OF ETOPOSIDE (VP16-213) ON CELL-CYCLE TRAVERSE, DNA SYNTHESIS AND DNA STRAND BREAKAGE IN HUMAN MALIGNANT LYMPHOCYTES. A. Fridland, D. Kalwinsky, T. Look and J. Ducore*, St. Jude Children's Research Hospital, Memphis, TN 38101, and University of Texas Health Sciences Center*, Dallas, TX 75230

The kinetic and biochemical effects of 4-methyl-epipodophyllotoxin ethylidene-β-D-glucoside (VP16-213), a semisynthetic analog of podophyllotoxin with clinically important antineoplastic activity, was analyzed in a human T-lymphoblastoid cell line (CCRF-CEM) in vitro. Serial studies with 0.25 to 2 μM of VP16-213 for 0-6 hr indicated that the cytotoxic mechanism of this compound involves a primary effect on DNA. The most striking early change shown by flow cytometry in VP16-213 treated cells was a delay of the S-phase transit preceding a later G_2 phase arrest. Coincident with the S-phase delay was a selective inhibition of thymidine incorporation into nascent DNA molecules as well as concentration-dependent scission of DNA. Using the alkaline elution method, we were able to detect DNA breakage at concentrations of the drug below the level required to demonstrate kinetic effects. These data suggest that DNA strand scission is the initial event in the sequence of kinetic and biosynthetic changes leading to growth inhibition and death of VP16-213 treated cells. These data also suggest that the disruption of the DNA by VP16-213 is affecting some putative event(s) regulating the initiation of new replicons of DNA synthesis and propose that this effect is primarily responsible for the lethal properties of the drug.

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0635 MYELOID MATURATION STUDIED IN HUMAN AND RAT LEUKEMIC CELL LINES, A.K. Sullivan, L. Fitz-Gibbon, and A. Brox, McGill University, Montreal, CANADA H3G 1Y6

The study of human myeloid cell lines derived from leukemic patients has shown that some characteristics of differentiation may be maintained in culture and that in one system (HL60) terminal maturation can be induced to occur. We have attempted to extend the capabilities of this model by deriving sublines of HL60 and BN rat leukemia that exhibit different capacities for maturation.

The following have been developed:

1) Sublines of HL60. a)HL60-D. "Undifferentiated" line that lacks granules, peroxidase, promyelocyte surface antigens and is unable to mature with DMSO stimulation. b) HR9. Polyploid line which lacks peroxidase, does not mature in culture but bears promyelocyte antigens. c) 20/20A7t. Has promyelocyte antigens, shows a lower level of spontaneous maturation and matures in presence of DMSO at a slower rate than the parent HL60.

2) Established lines of BN rat myeloblastic leukemia. The leukemia of the BN rat is very similar to the human acute promyelocytic leukemia but had required in vivo passage for propagation. We have established a long term line in culture that exhibits myeloblastic characteristics.

3) Maturation-related surface markers. These lines, both rat and human, have been used to develop hybridoma antibodies that are related to stage of myeloid maturation. Some show cross species reactivity.

These complementing human and rat systems now extends the possibilities for study of how leukemic cells interact with their host.

0636 MYELOBLASTIC SUBLINES OF BN RAT LEUKEMIA SHOW AN ALTERED PATTERN OF DISEASE. A. Brox, and A.K. Sullivan. McGill University, Montreal, Que., CANADA. H3G 1Y6.

The chemically induced promyelocytic leukemia of the Brown Norway rat, propagated by in vivo passage,bears many similarities to the human disease. Such features include growth in the marrow, liver and spleen and, sparing of the central nervous system. We have derived two sublines which are now myeloblastic and show an altered pattern of disease.

The first was obtained by adaptation of the in vivo passaged parent cells to long term culture; the second after six sequential passages through rats treated with daunorubicin. Both of these lines appeared to be lessmature, at the myeloblastic stage, than the parent. On reinjection of either of these cell lines a leukemic syndrome evolves with the new complication of hind limb paralysis. If the disease is allowed to progress until the death of the animal the following incidence of paralysis wae noted:

Cell injected	Paralytic/leukemic
Early passage parent	0/16
In vitro adapted	13/14
In vivo daunorubicin	16/16

On spinal cord dissection tumour nodules were noted which on microscopic examination revealed epidural invasion.

In addition to demonstrating an unusual neurologic complication these studies indicate that less matured forms of a leukemia can be selected through pressures exerted through chemotherapy or growth factors that may give rise to an altered pattern of clinical disease.

0637 FRIEND CELLS ON THE ROAD TO TERMINAL ERYTHROID DIFFERENTIATION. David E. Axelrod and Fatema R. Haider, Rutgers University, New Brunswick, NJ 08903.

We have determined the cell division times of proliferating and differentiating Friend erythroleukemia cells by TV time lapse photography. The observed distribution of cell division times in proliferating populations is not adequately described by the transition probability model of Smith and Martin (PNAS 70:2363 (1973) which assumes stochastic completion of one rate limiting step after a minimum time period. However, the experimental data are well described by the Erying-Stover formalism (Murphy PNAS 75:4404 (1978)) which assumes that cell division times are determined by two opposing steps. Exposure to dimethylsulfoxide (DMSO), which induces terminal erythroid differentiation, results in a time dependent increase in median cell division times, and an increased heterogeneity of cell division times. However, even after exposure to DMSO for 72 hours, all cells are capable of re-entering the cell cycle with nearly normal kinetics if plated at high cell densities. These results suggest that the road to terminal erythroid differentiation, induced by DMSO, is not a dead end, but is a cell cycle detour of increasing length.

0638 CHARACTERIZATION OF ALTERED SURFACE ANTIGEN EXPRESSION ON RF THYMOCYTES INDUCED BY CHEMICAL CARCINOGENS, Marie Metlay, Kelly Switzer-Timmons, Maureen Goodenow, and Frank Lilly, Albert Einstein College of Medicine, Bronx, New York 10461

Skin painting with 3-methylcholanthrene(MCA) induces T cell lymphomas in mice of the RF/J strain. These tumors are morphologically and clinically similar to spontaneous lymphomas in mice of the AKR strain. Specific changes in the expression of cell surface determinants, including virus-related molecules, are observed in spontaneous lymphomas. Previous studies in this laboratory have characterized the surface antigens on MCA-induced primary lymphomas. We have extended these findings to an examination of the alterations in cell surface antigen expression which occur prior to lymphomagenesis, as the target cell progressed towards the transformed state. Parallel analyses of the effects of benzene, the traditional solvent for MCA, have been performed. While benzene treated mice do not develop lymphomas within the time span of MCA treated mice, they do exhibit changes in the expression of cell surface determinants. Within two weeks of either type of treatment, there was a marked reduction in the frequency of cortical thymocytes and a decrease in the total amount of cell surface antigens. The distribution of Lyt-1, Lyt-2, and Thy-1 was followed using FACS analysis. Viral antigen expression was studied with the use of partial peptide mapping and two-dimensional gel analysis of ^{125}I labelled surface molecules which were immune precipitated with both heteroantisera and monoclonal antibodies. These combined analyses of the effects of two known carcinogens, MCA and benzene, during tumor progression, allowed us to follow the process at the molecular level.

0639 CELL SURFACE DIFFERENTIATION MARKERS OF HUMAN ERYTHROID AND MYELOID CELL LINES DETECTED BY MONOCLONAL ANTIBODIES – RELATION TO PROLIFERATION AND DIFFERENTIATION, Patrice Mannoni, Judith Sutherland, Anna Janowska and Robert A. Turner, University of Alberta, Edmonton, Canada

Mouse monoclonal antibodies (MoAb) against human cell surface myeloid and erythroid antigens have been produced by the hybridoma technic. Myeloid MoAb detect different stage of myeloid differentiation. Some recognize progenitor cells of the myeloid and erythrocid pathway, some others detect antigens expressed only on mature myeloid lineage. The expression of these antigens on myeloid leukemic cells depends to a certain extent on their maturation and differentiation.

In order to correlate marker expressions with the events of the proliferation and/ or differentiation- maturation process, erythroid cell lines (K562, HEL) and myeloid cell lines (HL 60, KG 1, ML 1) were used as models. Cell line differentiation or selection were induced by phorbol esters (TPA), Retinoic Acid, DMSO, Na Butyrate and by MoAb. Qualitative and quantitative MoAb binding was maesured by microfluorometry analysis using an EPICS V cell sorter. The expression of these markers was either increased or decreased depending on the inducer type, the cell line and the antibody specificity. In some cases it has been possible to sort negative and positive populations and to study the DNA content, the self renewal capacity and the colony forming efficiency of each subset. Some markers (80H5, 82H6, glycophorin) appeared to be more related to the maturation-differentiation process whereas some others or the weak expression of the first ones were related to the proliferative capacity of the cell line.

0640 HISTOCHEMICAL AND ULTRASTRUCTURAL CHARACTERIZATION OF HL-60 PROMYELOCYTIC LEUKEMIA CELLS INDUCED TO BECOME EOSINOPHILS, Steven A. Fischkoff, Avrom Pollak, and Timothy Reber, University of Maryland Cancer Center, Baltimore, MD 21201

HL-60 progranulocytic leukemia cells can be induced to differentiate to cells with many of the properties of mature neutrophils and macrophages. Recently, several methods of inducing eosinophilic differentiation have been reported. Using histochemical staining techniques, several enzyme activities can be localized within the cell by light and electron microscopy. In the earliest stages of eosinophilic differentiation, peroxidase activity could be demonstrated within the endoplasmic reticulum and perinuclear space. In more mature cells, large, peroxidase-positive granules appeared, either within the cytoplasm or in vacuoles formed from dilated segments of endoplasmic reticulum. Secondary granules, which may contain multilamellar structures, also stained uniformly for peroxidase activity. This enzyme activity was not inhibited by 10^{-3} M KCN. The Golgi apparatus did not stain. Acid phosphatase activity was only minimally detected in the Golgi apparatus and endoplasmic reticulum. The primary granules stained heavily, but in contrast to peroxidase, acid phosphatase activity was usually confined to the periphery of the granule. In secondary granules with multilamellar structures, activity was again confined to the periphery of the granule and internal areas where the multilamellar structure was not well formed. Arylsulfatase B activity could also be demonstrated preferentially at the exterior of the granules. The HL-60 eosinophil may be a useful model in the study of normal and abnormal eosinophilopoiesis.

0641 DIFFERING PHENOTYPIC RESPONSES OF FRESHLY ISOLATED HUMAN LEUKEMIA CELLS TO PHORBOL DIESTERS: A POST-RECEPTOR PHENOMENON, B.J. Goodwin and J.B. Weinberg, VA and Duke Medical Centers, Durham, NC 27705

The phorbol diesters (PDEs) exert a myriad of in vitro biological effects on cells including normal and leukemic human blood cells. Recent studies support the hypothesis that the pleiotypic effects elicited by the PDEs are mediated by specific cellular receptors. Studies using freshly explanted human leukemia cells have demonstrated the induction of macrophage-like characteristics in myeloid leukemia cells in response to phorbol 12-myristate 13-acetate (PMA), but not in lymphoid leukemia cells. We have characterized the binding of the PDEs to freshly isolated human leukemia cell using (20-^{3}H) phorbol 12,13-dibutyrate (^{3}H)PDBu). Despite the marked differences in the phenotypic responses of myeloid and lymphoid leukemia cells to PMA, all leukemic cells studied had specific high affinity receptors for (^{3}H)PDBu, with comparable binding affinities, numbers of binding sites, and binding kinetics at 4°C and 37°C; no catabolism of (^{3}H)PDBu or (^{3}H)PMA by either lymphoid or myeloid leukemia cells was demonstrated using thin layer chromatography. This data suggests that the control of the type of phenotypic leukemic cellular response exists at steps subsequent to initial ligand-receptor binding.

Leukemia Type	Kd	Sites/Cell	PDE Catabolism	Response to PDEs
ANLL(9)	77nM	470,000	None	Macrophage-like
ALL(4)	96nM	617,000	None	Aggregation
CLL(6)	126nM	495,000	None	Aggregation
HCL(1)	54nM	370,000	----	Macrophage-like
Blastic CGL				
myeloid (1)	87nM	218,000	None	Macrophage-like
lymphoid (1)	90nM	600,000	----	Aggregation

0642 PHORBOL DIESTER (PDE)-INDUCED DIFFERENTIATION OF HUMAN LEUKEMIA CELLS: COMPARABLE PDE RECEPTORS IN HL-60 CELLS AND MUTANT, UNRESPONSIVE HL-60M CELLS, J. Brice Weinberg and Bonnie J. Goodwin, VA and Duke Med. Ctrs., Durham, NC 27705

The human promyelocytic cell line HL-60 differentiates to macrophage-like cells in response to PDEs, but a mutant blast subclone (Leuk Res 5:429, 1981) resists the effects of PDEs. PDEs mediate their differentiating effects via membrane receptors. The purpose of this study was to determine if the differing responses were due to differences in the membrane receptors for PDEs as determined by studies using tritium labeled phorbol dibutyrate (^{3}H)PDBu) for binding studies. Phorbol myristate acetate (PMA), PDBu, and phorbol diacetate (PDA) (but not phorbol--PHR), in decreasing order of potency, induced adhesion and spreading of HL-60 cells and inhibited ^{3}H thymidine incorporation with ED_{50} of 5nM (PMA), 15 nM (PDBu), and 1 uM (PDA). However, these effects were not seen in the HL-60M cells. Receptor binding parameters were essentially identical in both HL-60 and HL-60M cells: maximum binding at 37°C (10 min) and 4°C (30 min); fall in bound (^{3}H)PDBu after incubation at 37°C after 60 min; Kd of binding and number of binding sites/cell (50nM and 1.3×10^5 for HL-60, and 80nM and 1.4×10^5 for HL-60M, respectively); reversibility of binding, specificity of binding (PMA)PDBu)PDA, PHR inactive); and lack of degradation of (^{3}H)PDBu or (^{3}H)PMA. Pretreatment of either HL-60 or HL-60M with unlabeled PDBu at 4°C or 37°C for 2 hours did not result in loss of subsequent ability of the cells to bind (^{3}H)PDBu (down regulation). Thus, the differing phenotypic responses of HL-60 and HL-60M cells to PDEs are apparently due to post-PDE receptor differences in the cells.

0643 PRODUCTION OF FRIEND-VIRUS INDUCED CELL LINES IN C57BL-6 MICE, Rita Anand, Roy W. Geib and Frank Lilly. Albert Einstein College of Medicine, Bronx, NY and Morehouse School of Medicine, Atlanta Ga.

The FV-2 locus represents an almost absolute barrier to Friend virus (FV) induced erythroleukemia. The resistance cannot be overcome by increasing the multiplicity of infection; however, variant viruses can be isolated which can overcome the barrier. BSB virus, isolated by Steeves in 1970, is one such variant. In order to better understand the mechanism of FV-2 gene and variant virus, we established FV-induced erythroleukemia cell lines in C57BL-6 (B6) mice (the prototype $FV\text{-}2^r$ strain). The cell lines were produced by injecting BSB into B6 neonates. Three to four months later the enlarged spleens were homogenized and cells placed into tissue culture. The resultant cell lines were characterized. Most of the cell lines produce infectious virus as detectable in both the XC plaque assay and the spleen focus assay. The cell lines are all of the erythrocytic lineage as determined by Wright's stain and hemoglobin production. The production of viral gene products was examined using immunoprecipitation and PAGE analysis. Each cell line produces env gene products of FMuLV and SFFV origin.

0644 **T CELL-LIKE INDUCTIVE SIGNALS FROM C8-DERIVATIZED GUANINE RIBONUCLEOSIDES.** Michael G. Goodman and William O. Weigle, Scripps Clinic and Research Foundation, La Jolla, CA. We have recently described an entirely new class of activator, the C8 substituted guanine ribonucleosides, that traverse the cell membrane, bypassing classical triggering mechanisms to activate the lymphocyte at an intracellular site. In the current studies we have evaluated the capacity of the C8 substituted guanine ribonucleosides to provide T cell-like signals to cultures of splenic B cells. The results clearly demonstrate that 8 mercaptoguanosine (8MGuo), when added to cultures of B cells and macrophages in the presence of antigen, is capable of supplying a "second signal" for B cells, enabling them to generate high numbers of specific plaque-forming cells against the immunizing antigen. This effect is duplicated in cultures of spleen cells from congenitally athymic mice. Inhibition of the effect of interleukin 2 (IL-2) by cyclosporin A, such that the antibody response of normal spleen cells is entirely abrogated, has minimal effects on the T cell-replacing activity of 8MGuo. Additivity studies with MLC supernates as well as kinetic analyses with IL-2-associated lymphokines substantiate that these factors act by a mechanism distinct from that of 8MGuo and 8BrGuo. These observations establish these nucleoside activators as exciting new probes for T helper cell activity and an effective non-T cell source of T cell-like signals for B cells.

0645 DIFFERENTIATION INDUCTION OF HUMAN LEUKEMIC CELL LINE BY LITHIUM CARBONATE. H.C. Kim, I. Heller, M.A. Brostrom, and P. Saidi. UMDNJ-Rutgers Medical School, New Brunswick, New Jersey 08903.

We studied the differentiation-induction ability of lithium using human myeloid leukemic cell line (HL-60) and explored its relationship to the intracellular cyclic nucleotide (cAMP) as a possible modulator for cellular differentiation. HL-60 cells were cultured with various concentrations of lithium carbonate (1;2.5;5.0; 10mM) or without lithium in liquid suspension for up to 7 days. To assess the induction of differentiation by lithium, we measured the inhibition of cell growth by viable cell counts and ^{3}H TdR uptake, and the induction of cellular function by spectrophotometric assay of non-specific esterase, Fc receptors and latex phagocytosis. Compared to the control, lithium caused inhibition of cell growth by 70% and inhibition of ^{3}H TdR uptake by 95% in a concentration-dependent manner. Lithium-treated cells became macrophage-like in morphology with increased non-specific esterase activity up to 3 times of control. Phagocytosis of latex particles and Fc receptors in lithium-treated cells increased during the culture. However, chloroacetate esterase-positive cells remained unchanged during the culture with or without lithium. These findings indicate that lithium induces Hl-60 cells to differentiate to morphologically and functionally macrophage-like cells. Intracellular cAMP levels in lithium-treated cells increased 4-told during the culture of the logarithmic growth phase, while cAMP levels in untreated cells decreased during the same period. The result suggests that lithium induces differentiation in some human leukemia cells, possibly by modulating cellular cyclic AMP.

0646 DIFFERENTIATION OF A HUMAN MONOCYTE-LIKE CELL LINE BY (2'-5') OLIGO-ISOADENYLATE, Azi Schmidt, Toshio Hattori, Thomas Hoffman, National Cancer Institute, Frederick, MD 21701

Treatment of a human monocyte-like cell line (U-937) by (2'-5')ApApA, the 5' dephosphorylated product of an interferon induced enzyme; (2'-5')oligo-isoadenylate [oligo(A)] synthetase, was able to induce differentiation, mimicking the effect of interferon treatment. Treatment of U-937 cells with (2'-5')ApApA resulted in morphologic changes, new (monocyte-associated) membrane antigen expression, and acquisition of the capacity to mediate antibody-dependent cellular cytotoxicity (ADCC). (2'-5')ApA and (3'-5')ApApA were without effect. A myeloid cell line (HL-60) which differentiates in response to other agents, but not to interferon, was not able to differentiate in response to (2'-5')ApApA, despite the ability of interferon to induce (2'-5')oligo (A) synthetase.

0647 T CELL HYBRIDS WHICH PROLIFERATE IN THE ABSENCE OF TCGF REQUIRE BOTH A "CYTOTOXICITY" INDUCING FACTOR" AND TCGF TO EXPRESS CYTOLYTIC FUNCTION, F. Erard, P. Corthésy, K. Smith, A. Conzelmann and M. Nabholz, Swiss Inst. for Exp. Cancer Research, 1066 Epalinges, Switzerland, and Dartmouth Med. School, Hanover NH 03755.

A hybrid (PC60) between a murine cytolytic T lymphocyte (CTL) line and a rat T cell lymphoma, proliferates in the absence of T cell growth factor (TCGF) and is not cytolytic. However, it can be induced, by culture for 48 hrs in mixed leukocyte culture supernatants or ConcanavalinA activated rat spleen cell supernatants (CS), to lyse ^{51}Cr labeled tumor target cells (1). In order to characterize the factor(s) responsible for this reversible induction, serum free CS was fractionated by reverse phase HPLC. None of the eluated fractions induced by themselves the cytolytic activity of PC60. However when they were combined with TCGF, which itself has no inducing activity, an additional cytotoxicity inducing factor (CIF) was identified, which could be separated from γ-interferon and macrophage activating factor. Using purified human ^{3}H-TCGF in a radioreceptor assay (2), we found that non-induced PC60 cells expressed no detectable TCGF receptors (< 100) but that they acquired such receptors during induction (1800 ± 200 receptors per cell, compared to 9500 for the parental CTL line). These data suggest that, in addition to growth promotion, TCGF expresses additional biological activities, and that induction of cytolytic activity of CTL precursors depends of both TCGF and CIF.

1. A. Conzelmann et al. Nature 298:170-172 (1982).
2. R.J. Robb et al. J. Exp. Med. 154/1455-1474 (1981).

0648 HYBRID CELLS PROTECT SYNGENEIC MICE FROM LEUKEMIA, Edward P. Cohen, Sandra L. Garber, and Isabel Chang, University of Illinois at Chicago, Chicago, Il. 60612

Hybrids of ASL-1 murine leukemia cells ($H\text{-}2^{a}$) and LM(TK)$^{-}$ cells, a cultured line of C3H/He mouse fibroblast origin ($H\text{-}2^{k}$), stimulate partial immunity toward ASL-1 cells in (A/J x C3H/He)F_1 mice ($H\text{-}2^{a/k}$). The hybrid cells, the mice used in the experimental studies and the leukemia cells used for challenge all share the same histocompatability antigens. Ordinarily, F_1 mice exhibit no resistance to the proliferation of ASL-1 cells; as few as 200 ASL-1 cells invariably kills them in about 14 days. F_1 mice injected with hybrid cells and then challenged with up to 10^{7} ASL-1 cells survive longer ($p < .001$) than mice who had not received hybrid cells previously. Some mice challenged with lesser number of ASL-1 cells survive indefinitely (>85 days). In no instance have the mice developed a tumor of hybrid cells; they appear to have lost the malignant characteristics of the parental cells and are rejected by immunocompetent syngeneic recipients. Mitomycin-C treated ASL-1 cells suppress ^{3}H-thymidine incorporation by spleen cells from F_1 mice. The suppressive effects are partially reversed if the spleen cells are obtained from mice injected previously with hybrid cells. The hybrid cells used in these experiments, after three years in continuous culture, possess 70 (modal, range 65-77) chromosomes including "marker" chromosomes of each parental source. They form a tumor associated antigen which is cross reactive with an analogous antigen of ASL-1 cells. (Supported by USPHS Grant number CA 27579-02).

0649 MITOGEN INDUCED CLONAL EXPANSION OF T CELLS UNDER LIMITING DILUTION CONDITONS: STUDIES ON THE ONTOGENY OF T CELL FUNCTION AND SPECIFICITY, Klaus Pfizenmaier, Walter Däubener, Hermann Wagner and Martin Röllinghoff, Institute of Medical Microbiology, University of Mainz, 6500 Mainz, West-Germany

We have established a culture system which non selectively allows the activation, differentiation and clonal expansion of virtually all mouse T cells. This is achieved by polyclonal stimulation of limited numbers of T cells with concanavalin A in the presence of nonlimiting concentrations of syngeneic irradiated feeder cells, T cell differentiation and growth factors. The system allows not only to quantify functional T cell subsets within different lymphoid populations of unprimed mice, but is also suitable to estimate their specificity repertoire. Using this approach, we are currently investigating the cytotoxic T cell reactivity of newborn and adult mice. In particular, we compare both quantitatively and qualitatively neonatally expressed T cell specificities with that found in adult mice. Thus, we hope to obtain further insights into mechanisms which influence the expression of the peripheral T cell repertoire.

0650 THE ROLE OF THE LYMPHORETICULAR SYSTEM IN THE PATHOGENESIS OF SCRAPIE DISEASE, Richard I. Carp, George S. Merz and Sharon M. Callahan, Institute for Basic Research in Developmental Disabilities, Staten Island, NY 10314.

The importance of the lymphoreticular system (LRS) in scrapie pathogenesis has been suggested by the finding that following peripheral (non-CNS) injection of scrapie large quantities of infectious agent are found in spleen and lymph nodes long before agent reaches the brain. In the present studies three types of in vivo experiments support the importance of the LRS in scrapie: (1) Splenectomy prior to intraperitoneal (IP) injection leads to a lengthening of incubation period. (2) For IP injected neonatal mice, incubation periods were longer and the proportion of scrapie positive mice was lower than for adult mice injected with the same dose. A postulated mechanism involves the maturation early in life of an LRS cell type that is sensitive to scrapie agent replication. (3) Prior treatment of mice with substances which increase the number of peritoneal exudate cells leads to a lengthening of the incubation time following IP injection with scrapie. In vitro studies show that scrapie infectivity readily attaches to peritoneal exudate cells and that the association occurs to a greater extent at 37°C than at 4°C. Continued incubation at 37°C leads to inactivation of scrapie infectivity. Results with bone marrow cells, splenocytes, and purified T and B cells have failed to consistently show either replication or inactivation of infectivity following extended incubation at 37°C. Extensive cell loss was seen in both cultures exposed to scrapie and in those used as controls. For this reason, current experiments are aimed at improving cell viability so that there is greater possibility for agent replication. Results with scrapie infected mouse myeloma cells will also be reported.

0651 LYMPHOMA INDUCTION IN MICE BY BNU AND MNU (BUTYL- AND METHYLNITROSOUREA), Hans J. Seidel, Ludwika Kreja, Felix Carbonell, Department of Clinical Physiology and Occupational Medicine, University of Ulm, 7900 Ulm, W. Germany

In protocols with BNU and MNU T cell lymphomas can be induced with very high incidence and reproducibility in BDF_1 mice and other strains. The purpose of the experiments was to study the relevance of those changes in the hematopoietic-lymphopoietic cell system which precede the development of the leukemias. The parameters include CFU-S numbers, bone marrow to thymus relationship, T-cell functions, NK cell activity and cytogenetic analysis. Leukemogenesis could be modulated in vivo by endotoxin, cortisone and bone marrow transplantation, but not by enhancing the NK cell activity, as achieved by corynebacterium parvum or Tilorone. The various parameters were influenced during and after the exposure to the leukemogens but studies with groups as well as individual mice showed e.g. a recovery of T cell functions and of CFU-S numbers during the latency period. The NK cell activity was reduced only to a small extent. Findings in mice with leukemia were controlled in studies with a transplanted T cell leukemia. These results demonstrate that the presence of leukemic cells in the cell suspension under study, as also obtained by the in vitro admixture of leukemic cells, influences the experimental results, e.g. the leukemic cells act as cold targets in the NK cell assay.

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0652 T4-DIFFERENTIATION ANTIGEN-EXPRESSION ON HUMAN T CELLS IS NOT REQUIRED FOR HELPER CELL FUNCTION, Werner Solbach, Martin Röllinghoff and Hermann Wagner, Institute for Medical Microbiology, Joh.-Gutenberg-University, D-6500 Mainz, West-Germany

Human T lymphocytes expressing the T4-differentiation antigen consist of at least two different functional subsets, first Interleukin-2 (Il-2) producing T helper cells and second, cytotoxic T cells (CTL) recognizing class II HLA-antigens (1,2). Blocking studies using anti T4-antibody suggest that the T4 molecule is associated with T cell recognition of class II antigens by cytotoxic T lymphocytes.

We investigated whether or not the T4-molecule is also associated with the functional activity of T helper cells, i.e. whether the capacity to produce Il-2 is correlated with the expression of the T4 antigen.

We have shown recently that the expression of the T4 marker can be selectively abrogated by phorbol myristate acetate (PMA). Lectin-stimulation of $T4^+$-lymphocytes in the presence of PMA in nanomolar concentrations caused total abrogation of the T4-marker-expression within 8 hours. Despite the failure to express the T4 antigen, these cells were potent Il-2 producers. High Il-2 levels were obtained in the cell culture supernatants after 24 hours. Thus, it is concluded that the expression of the T4 differentiation antigen is not required for helper cell function.

1) Meuer et al., PNAS 79, 4395 (1982)2) Biddison et al., JEM 156, 1065 (1982)

0653 A TRINITROPHENYL (TNP) SPECIFIC T-CELL CLONE SECRETES ANTIGEN SPECIFIC AND NONSPECIFIC B CELL HELPER FACTORS. Rosemarie DeKruyff and Harvey Cantor, Sidney Farber Cancer Institute, Harvard University, Boston, MA 02115

0^+, Lyl^+ T cell clones specific for the hapten TNP were derived from lymph nodes of immunized Balb/c mice. Clone El responds to TNP on a variety of carrier proteins in the absence of Interleukin 2. Cells of this clone induce pure B cells from fluorescein isothiocyanate (FITC) primed mice to produce an *in vitro* anti-FITC PFC response in the presence of the double conjugate FITC-KLH-TNP but not FITC-KLH plus TNP-KLH. This indicates that clone El induces antigen specific help and this help requires an antigen bridge for maximal antibody production. Cells of Clone El produce both antigen specific and nonspecific helper factors which promote antibody production by purified B cells. After stimulation of cells of Clone El with antigen pulsed adherent cells or Concanavalin A, unfractionated supernatants: 1) contain antigen specific, H-2 restricted helper factor(s) which promote an *in vitro* anti-TNP PFC response by purified TNP primed B cells and 2) contain H-2 unrestricted, nonspecific helper factors which facilitate an *in vitro* antibody response by unprimed B cells to sheep erythrocytes (SRBC) or to Type 2 T-independent antigens. The antigen specific and nonspecific helper activities may be separated on antigen coated columns. The TNP specific help may be absorbed to and eluted from a TNP-Sepahrose column, and is not present in the flowthrough. In contrast, the antigen nonspecific helper activity is contained in the flowthrough and not in the eluate from this column. Biochemical analysis of the factors which mediate the TNP specific helper activity is in progress.

0654 DIFFERENTIATION OF T-CELLS IN *IN VIVO* AND *IN VITRO* MODEL SYSTEMS, G.A. Perry, G.C. Udeaja, and D.A. Crouse. Dept. of Anatomy, Univ. Nebr. Med. Center, Omaha, NE 68105

Pure thymic epithelium results from the treatment of 14 d fetal thymus by low temperature (7 d at 24^oC + 7 d at 37^oC) organ culture (LTOC). These epithelial LTOCs are free of morphologically or surface marker identifiable lymphoid cells and have been used in syngeneic and allogeneic transplantation studies as well as in *in vitro* transmembrane migration studies. In syngeneic C57Bl and allogeneic (C57Bl—CBA) transplants, the LTOC was found to repopulate with lymphoid cells of host origin as supported by Ia phenotype and acquired a normal surface marker and thymocyte functional profile by 30 d post-transplantation. However, no significant reconstitution of mature T-cells was evident in the periphery at 100 d post-transplantation. By 150 d minimal mature T-cell reconstitution was present. This suggested a failure of either peripheralization or peripheral maturation of thymocytes possibly due to the loss of an essential thymic component in the LTOC protocol. Repopulation of such LTOC epithelial "targets" has also been evaluated by transmembrane migration. Marker development paralleled that seen *in situ* with bright Thy 1^+ cells appearing early, followed by Lyt 1^+ then Lyt 2^+ cells. Repopulating abilities of fetal spleen, liver, thymus and adult bone marrow clearly differed, with adult bone marrow and 16 d fetal thymus as the best sources of pre-thymic progenitors. In contrast, fetal liver which is a good source of B cell precursors appeared to be a poor source of pre-thymic progenitors suggesting the possibility of a dichotomy in the optimal sources of pre-B (fetal liver) and pre-T (yolk sac?) cells. Clearly there are still some poorly characterized stages in early T lymphocyte differentiation which these models highlight for further investigation. (Supported by NIH AI15819 and UNMC Seed Grant funds).

0655 LINEAGE SPECIFIC ORGANIZATION OF MEMBRANE PROTEINS IN HEMOPOIETIC CELLS. Robert W. Allen and Beverly A. Hoover, American Red Cross Research Laboratory, St. Louis, MO 63108

The biochemical properties and membrane organization of several polypeptides common to all hemopoietic lineages was examined using in vitro translation and immunoprecipitation. Messenger RNA was extracted from the K562 cell line; a human leukemic cell line displaying characteristics of an erythroid progenitor cell type. The RNA was translated in vitro and the translation products reacted with an antiserum raised against erythrocyte ghosts. Polypeptides of 37,000 (p37), 20,000 (p20), 19,000 (p19), 18,000 (p18), 14,000 (p14), 13,000 (p13), and 11,000 (p11) daltons were immunoprecipitated from the translations. The localization of these antigens to the inner or outer membrane surface of various hemopoietic cell types was determined by absorption of the antiserum with intact cells or red cell ghosts followed by immunoprecipitation of translation products. Results showed that p37, p14, and p13 are expressed on the outer membrane surface of cells from the lymphoid, myeloid, and megakaryocyte series. In the erythroid series, K562 cells express these antigens on the outer membrane surface but in mature erythrocytes these antigens are located on the inner membrane surface. These results suggest that membrane proteins common to all lineages can be organized in the cell membrane in a lineage specific manner.

0656 DIFFERENTIATION OF A T LYMPHOMA CELL LINE IN VITRO. C. L. MacLeod, R. Hyman, S. Bourgeois and E. Hays. Salk Institute, San Diego, CA and UCLA, Los Angeles, CA.

We have characterized an AKR derived T lymphoma cell line SL12·4 which spontaneously undergoes several coordinate changes in vitro. SL12·4 is sensitive to glucocorticoid induced lysis and displays ThB and Thy1.1 surface antigens. Selection in 1 μM dexamethasone (dex) reveals three hormone resistant phenotypes: (1) dex "r" cells which grow and form colonies in 1 μM dex; (2) dex "ga" cells which are growth arrested, but not lysed, and do not form colonies in the presence of hormone; and (3) dex "receptorless" cells which lack glucocorticoid binding to receptor. Fifteen subclones of SL12·4, when separately subjected to dex selection, produced dex "r" and dex "ga" cells at frequencies of between 10^{-2} and 10^{-4}, with normal receptor binding function, but all express reduced levels of cell surface Thy1 and ThB. Further, these cells can be converted to dex sensitivity at high frequency (~10%) by treatment with the DNA demethylating agent 5-azacytidine (5-AzaC). In contrast, the dex "receptorless" phenotype was isolated only once in over 10^9 cells examined, and appears to be the result of (a) spontaneous mutation(s) in the hormone receptor gene(s). The "receptorless" cells retain the parental SL12·4 surface antigen pattern and no dex sensitive cells were recovered following 5-AzaC treatment. We conclude that the dex "r" and dex "ga" phenotypes result from epigenetic events. The changes are consistent with a model of thymocyte differentiation in which a hormone sensitive, high Thy1 cell is the progenitor of a dex "r" thymocyte expressing lower amounts of Thy1. (Supported by NIH grants to S.B., R.H., E.H., a Whitehall Foundation grant to S.B., and National Leukemia Association grant, Leukemia Society of America and NIH fellowships to C.M.)

0657 PROLIFERATION AND DIFFERENTIATION IN VITRO OF MYELOID CELLS IN ACUTE MYELOID LEUKEMIA (AML). Hans W. Grünwald, Div. of Hematology, Queens Hospital Center Affiliation of the Long Island Jewish-Hillside Medical Center, Jamaica, N.Y. 11432 and the Dept. of Medicine, SUNY at Stony Brook, N.Y.

Bone marrow blasts from many AML patients proliferate in vitro when placed in soft agar cultures (CFUc assay) with varying abnormal growth patterns (microclusters, macroclusters, high cluster:colony ratio). In addition to the immediate soft agar plating, we have placed bone marrow blast cells from AML patients into McCoy's 5A medium with 10% FCS at $5x10^5$ cells/ml, and have subcultured these cells in the soft agar assay after 7 days of incubation @ 37° C in a humidified 5% CO_2 atmosphere. Of 48 consecutive AML marrow samples with greater than 10^5 viable cells/ml on day 7 (approximately 75% of the total AML samples) and abnormal growth pattern or no growth in agar initially, 12 yielded morphologically normal granulocyte and macrophage colonies and clusters in the agar subcultures, with normal or near-normal cluster: colony ratios, albeit low plating efficiency. The day 7 liquid cultured cells revealed granulocytic differentiation and reduction in the proportion of blast cells in 29 of the 48 samples when compared to the initial marrow cells. We have so far not been able to ascertain whether the precursors of these differentiated cells and normal colonies are leukemic or residual normal cells. Correlation of remission induction results in these patients with the in vitro studies is presently underway to evaluate whether the findings have prognostic implications.

0658 EVIDENCE FOR LYMPHOCYTIC INVOLVEMENT IN ACUTE MYELOGENOUS LEUKEMIA, Patricia M. Logan, Andrew J. Malcolm and Julia G. Levy, Dept. of Microbiology, University of British Columbia, Vancouver, B.C., Canada, V6T 1W5.

Positive results have been obtained from FACS IV analyses of mononuclear-enriched peripheral blood cell populations from 10 AML remission patients which were tested with myelogenous leukemia-specific antisera (rabbit heteroantisera and one monoclonal antibody). These results led to speculation that lymphocytes may be involved in an underlying pathological event in AML, in terms of carrying a tumor-associated antigen (TAA) on their surface. This putative TAA, a cell surface protein of molecular weight 68,000 daltons and pI 7.2, has been isolated from peripheral blood leucocyte membrane extracts from patients with myelogenous leukemia and has been used to raise our antisera. This antigen is not found in equivalent peripheral blood leucocyte membrane extracts from normal people or from patients with lymphoid leukemias, as previously reported (Malcolm, Shipman and Levy, J. Immun. 128:2599, 1982).

FACS IV sorting studies have verified that lymphocytes, as well as granulocytes and monocytes, from patients with AML in remission react positively with the myelogenous leukemia-specific antisera, indicating that these lymphocytes carry the TAA on their surface. The precise correlation between myeloid neoplasia and the presence of this TAA is unclear at the present time. The nature of the possible significance of lymphoid involvement in AML is of great interest to both experimental and clinical hematopathologists. Investigations are underway to determine whether or not mitogen stimulated remission lymphocytes produce this membrane protein constitutively.

0659 SPECIFIC ALTERATIONS OF CATION TRANSPORT IN HL-60 CELLS UNDERGOING DMSO-INDUCED GRANULOCYTIC DIFFERENTIATION, J.J. Gargus, C.W. Slayman and E.A Adelberg, Yale University, New Haven, CT 06510

The human promyelocytic cell line HL-60 can be induced to undergo differentiation towards mature granulocytic forms by the addition of 1.5% DMSO to the growth medium. Morphological differentiation is discernible by 3 days and is complete by 6 days. Throughout this process, in the face of a decreasing cell volume, the cells maintain a constant high intracellular K^+ concentration of 154 +/- 29 mM and a constant low intracellular Na^+ concentration of 33 +/- 13 mM. On the other hand, unidirectional K^+ fluxes radically change and these changes are extremely early events following DMSO addition. 90% of the unidirectional $^{42}K^+$ influx is ouabain-sensitive in the uninduced cells. While this component of K^+ influx remains unaltered in ouabain sensitivity and in $K_{1/2}$ for extracellular K^+ throughout DMSO-induced differentiation, the V_{max} begins to fall 24-48 hours after DMSO addition, and by 6 days is 1/9 of the uninduced level. A more rapid decrease in unidirectional K^+ efflux is seen. The rate constant for efflux begins to decrease within 2 hours of DMSO addition, and by 6 days is 1/4-1/5 the uninduced rate. This rate is comparable to the rate measured on mature peripheral blood leukocytes under similar conditions. Because these changes in K^+ flux are amoung the earliest events detected following DMSO addition they may play a causal role in the process of differentiation.

0660 LYMPHOEPITHELIAL INTERACTIONS IN THE PATHOGENESIS OF RADIATION LEUKEMIA VIRUS INDUCED THYMIC LYMPHOMAS IN C57BL MICE. J. BONIVER and M.P. HOUBEN DEFRESNE, Institute of Pathology B.23, University of Liege, Belgium.

Radiation Leukemia Virus (RadLV) induces thymic lymphomas in C57BL/Ka mice after a latent period of 3 to 6 months. Target cells belong to a subset of immature thymocytes, corresponding to transitional forms between prothymocytes and the blast cell population of the outer cortex. The two major consequences of target cell infection, i.e. virus replication and induction of "preleukemic" cells require interactions with the thymic microenvironment. Here we demonstrated the involvement of "Thymic Nurse Cells" (TNCs) in this multistage leukemogenic process. Indeed, the first virus producing cells and the first "preleukemic" cells were preferentially located in "TNCs". After one month, TNCs number per thymus drastically decreased and had disappeared almost completely after two months. At that time, "preleukemic" cells were found not associated with TNCs, although they were still thymus-dependent for progression to lymphoma growth. The late preleukemic period was characterized by the appearance of "thymus-independent" preleukemic cells, able to grow in thymectomized hosts.

0661 DIFFERENTIATION OF A SUBSET OF BONE MARROW CELLS HOMING TO THE THYMUS OF ADULT MICE.
F. Lepault and I.L. Weissman: Laboratoire de Cinetique cellulaire INSERM U250, Institut Gustave Roussay, Villejuif, France, and Department of Pathology, Stanford University, Stanford, CA. 94305.

We have recently developed a rapid in vivo assay for thymus-homing bone marrow cells and have provided preliminary evidence that they indeed contain pre-thymocytes. Bone marrow is the major source of thymus-homing cells; these cells represent approximately 0.1% of the total bone marrow cells and co-purify with the lymphoid fraction. The frequency of these cells is increased in the bone marrow of nu/nu mice, and in two cloned in vitro lymphoid cell lines having the phenotype of immature thymocytes. Upon entry into the thymus, the thymus-homing bone marrow cells begin to express Thy-1. We have now used this assay to examine further the state of differentiation of the thymus-homing cells in the bone marrow as well as soon after their immigration into the thymus. It is shown that 1) fractionated bone marrow cells 2-4 fold enriched for pre-B and B cells appear to be 2-10 fold depleted for thymus-homing cells, indicating that pre-B and pre-T cells in bone marrow belong to separate lineages; 2) prior to immigration, thymus-homing bone marrow cells lack specific T cell antigens, but within 3 hours after homing these cells express detectable levels of Thy-1 and Lyt-1 or Lyt-2 antigens. Surprisingly, Lyt antigens appear prior to Thy-1 antigens and immigrants express Lyt-1 or Lyt-2 antigens but not both. TL antigens could not be detected during the assay interval; 3) most of the thymus-homing bone marrow cells are engaged in the cell cycle.