RECENT ADVANCES IN LEUKEMIA AND LYMPHOMA

Robert P. Gale and David W. Golde, Organizer

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Chromosomes, DNA and Leukemia

D 001 MOLECULAR GENETICS OF HUMAN LEUKEMIAS AND LYMPHOMAS, Carlo M. Croce, The Wistar Institute, 3601 Spruce Street, Philadelphia, Pennsylvania 19104

Since certain chromosomal alterations occur in a large fraction of specific human malignancies and since these consistent chromosomal alterations most likely provide a strong selective advantage for the cells that carry them, we reasoned that it should be possible to isolate genes directly involved in the pathogenesis of human cancer by taking advantage of specific chromosome rearrangements. Those reciprocal chromosomal translocations involving the heavy chain locus seemed particularly promising because of the proximity ofthe putative proto-oncogene to genetic elements present in the heavy chain locus capable of activating gene transcription in cis over considerable chromosomal distances. We have used immunoglobulin gene probes to "walk" away from the heavy chain locus and identify genes that could be activated by their juxtaposition to the heavy chain locus. With this approach we have now cloned two genes, bcl-2 and bcl-2, that are involved in many B cell neoplasms. Of particular importance is the finding that the bcl-2 gene is directly involved in the pathogenesis of follicular lymphoma, since this disease is probably the most common human hematopoietic malignancy. Analysis of the breakpoints in B cell neoplasms carrying the t(11;14) and the t(14;18) chromosome translocations has also provided very important information concerning the molecular mechanisms involved in chromosomal translocations in B cell neoplasms. It is of interest that the most common form of Burkitt lymphoma, the African type, is also due to mistakes in immunoglobulin gene rearrangements. The picture resulting from the analysis of T cell neoplasms is remarkably similar. In most cases, the locus for the alpha locus of the T cell receptor seems to play an important and direct role. Thus it should be possible to take advantage of chromosomal translocations involving the genes for the T cell receptor to isolate and characterize genes involved in the pathogenesis of T cell neoplasms. Since the chromosomal rearrangements observed in T cell leukemias and lymphomas in the United States and Europe are observed in HTLV-1 positive Japanese patients from areas where HTLV-1 is endemic, it seems likely that the role of HTLV-1 in the pathogenesis of adult T cell leukemia in Japan may be indirect, by expanding the number of T cells at risk of developing chromosome translocations. Thus the role of HTLV-1 in T cell leukemia may parallel the role of EBV virus in the pathogenesis of African Burkitt lymphoma.

D 002 MOLECULAR ANALYSIS OF THE RECURRING CHROMOSOMAL ABNORMALITIES IN ACUTE LEUKEMIA, Michelle M. Le Beau, University of Chicago, Chicago, IL 60637.

Specific chromosomal rearrangements often are associated with particular subtypes of acute leukemia. Molecular analysis has revealed that these rearrangements result in altered function of the genes located at the breakpoints, thereby playing an important role in the pathogenesis of the corresponding tumor. We have used the techniques of in situ chromosomal hybridization, Southern blot analysis, and pulsed field gel electrophoresis, to identify the genes at or flanking the breakpoints of several recurring abnormalities. For example, we showed that the inv(16)(p13q22) and t(16;16)(p13;q22) in acute myelomonocytic leukemia with abnormal eosinophils splits the metallothionein (MT) gene cluster on 16q. In the critical junction at 16q22, an unknown gene moves near the MTIIA gene, whereas the MTIA gene is relocated to 16p. In the t(9;11)(p22;q23), common in acute monoblastic leukemia, the break in 9p splits the alpha and beta interferon genes with the latter moving to the critical junction on 11q; the Hu-ets-1 oncogene at 11q23 is translocated to 9p. Deletions of chromosome 5 are frequently observed in myeloid leukemia cells, suggesting that the relevant genetic event may be the loss of a critical DNA sequence. Our analysis of these deletions suggests that loss of a gene(s) encoding the hematopoietic growth factors, or their receptors (GM-CSF, CSF-1, FMS) may play a role in abnormal hematopoiesis. We have also studied a t(8;14)(q24;q11) in two leukemia cell lines of T-cell origin, and have shown that the break in 8q is 3' of MYC and that in 14q is within the TCRA gene, suggesting that this translocation is analogous to the variant translocations observed in Burkitt's lymphoma. As more rearrangements are defined, their role in the multistep process of malignant transformation will be clarified.

D 003 MULTIPLE RECURRENT GENOMIC DELETIONS AND DUPLICATIONS ARE COMMON IN LEUKEMIA, LYMPHOMA AND SOLID TUMORS, Jorge J. Yunis, Department of Laboratory Medicine and Pathology, University of Minnesota Medical School, Minneapolis, MN 55455.

In recent years, the finding of a specific reciprocal chromosomal translocation or inversion in leukemias and lymphomas has led to clinical and molecular investigation of the genomic events involved (1). Such rearrangements may generally deregulate a proto-oncogene by placing it in proximity to a regulatory region of an active gene of a differentiated cell. Approximately half of all patients with acute myelogeneous leukemia and the great majority with preleukemia, however, have single or multiple recurrent genomic deletions (2). Also, multiple duplications are common in follicular lymphoma (3) and acute lymphocytic leukemia with hyperdiploidy (1). Most of these genomic rearrangements cannot be explained by direct proto-oncogene alteration since they have variable breakpoints, but they consistently involve a specific chromosomal region or band. In fact, we have now found that most types of cancer in which chromosomes have been analyzed have 5 to 15 of some 40 recurrent deletions and duplications frequently found in malignancies. Such abnormalities appear to be crucial to tumor phenotype and evolution and several are being found to have prognostic importance (2,3). Experimental evidence with various carcinogens suggest that the gain or loss of a specific chromosome segment can be elicited via hypersensitive fragile sites (1,4). General changes in cell growth regulation produced by such genomic alterations could account for altered growth patterns.

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(4) Yunis JJ (In press). Fragile sites as mutagenic sites. Human Genet.

Oncogenes in Leukemia/Lymphoma

D 004 INTERACTIONS OF ONCOGENES WITH HEMATOPOIETIC CELLS, Stuart A. Aaronson, Juan Carlos Lacal, Shiv Srivastava and Alessandra Eva, Laboratory of Cellular and Molecular Biology, National Cancer Institute, Bethesda MD 20892.
Investigations of the genetic alterations that cause normal cells to become malignant have focused on a small set of cellular genes. Acute transforming retroviruses have substituted viral genes necessary for replication with these discrete segments of host genetic information. When incorporated within the retroviral genome, these transduced cellular sequences, termed onc genes, acquire the ability to induce neoplastic transformation. Recent studies have shown that some proto-oncogenes encode growth factors or their receptors. Thus, oncogenes often may act to subvert the normal pathways by which growth factors induce cellular proliferation.

Proto-oncogenes can be activated to become transforming genes in naturally occurring malignancies by mechanisms independent of retrovirus involvement. Genetic changes as small as point mutations, as well as DNA rearrangements such as transpositions and chromosomal translocations, have all been implicated in this process. By use of the NIH/3T3 transfection assay, we have demonstrated the frequent activation of members of the ras family of proto-oncogenes in human tumors. K-ras, H-ras, or N-ras transforming genes are activated in 10-50% of individual hematopoietic tumors of diverse cell types. While the presence of such oncogenes does not appear to be specific to a particular cell type or stage of differentiation, N-ras seems to be preferentially activated in human hematopoietic tumors. Investigations aimed at elucidating the normal functions of ras p21 as well as the biochemical alterations that activate its transforming properties will be presented. A new human oncogene, dbl, was isolated following transfection with DNA of a human diffuse B-cell lymphoma. This gene appears to be distinct from previously identified oncogenes. The detection and characterization of its transforming gene product will be described.

D 005 INVOLVEMENT OF THE bcr AND abl GENES IN HUMAN CHRONIC MYELOID LEUKEMIA. E. Canaani, E. Shtivelman, B. Lifshitz, E. Feinstein, C. Marcelle, R. Amson, O. Dreazen* and R.P. Gale*. Department of Chemical Immunology, The Weizmann Institute of Science Israel, and Dept. of Medicine*, University of California, Los Angeles, CA.
The main event underlying the 9:22 translocation, specific to leukemic cells of patients with chronic myeloid leukemia is the transfer of the abl oncogene from chromosome 9 to within the bcr gene on chromosome 22. This results in formation of a chimeric bcr-abl mRNA translated into a fused protein which possess a high tyrosine kinase activity and is presumably directly involved with the malignant transformation. The structure of the normal abl and bcr genes and of the fused gene , as well as the transcripts and proteins coded by the genes will be described in detail.

D 006 MECHANISMS OF C-MYC AND C-MYB ONCOGENE ACTIVATION IN HUMAN LEUKEMIAS AND LYMPHOMAS. Riccardo Dalla-Favera, Dept. of Pathology, Kaplan Cancer Center, New York University School of Medicine, New York, NY 10016.
Reciprocal chromosomal translocations involving the c-myc and different Immunoglobulin (Ig) loci are consistently found in: i) endemic, African type, Epstein-Bar Virus (EBV) associated Burkitt lymphoma (eBL); ii) sporadic Burkitt lymphomas (sBL); iii) L_3-type acute lymphoblastic leukemias (ALL) and iv) AIDS-associated undifferentiated B-cell lymphomas (AIDS-UBL). Recent findings on the mechanism and role of these translocations will be presented i) Mechanism of chromosomal translocation. The position of chromosomal breakpoints on both chromosome 8 (c-myc) and 14 (Ig_H) varies in eBL vs. sBL. Recombinations between the Ig_HJ region and sequences > 50 Kb upstream to c-myc are found in most eBL, and recombinations between the Ig_H S region and sequences within the c-myc locus are found in most sBL. These different patterns, together with the different stage of differentiation of sBL vs. eBL, suggest that translocations may occur as mistakes of differentiation-stage specific Ig_H gene rearrangements, i.e. mistakes in V-D-J joining in the more immature eBL and mistakes of Ig_H class-switching in the more differentiated sBL. ii) Mechanism of c-myc oncogene activation. Structural alterations of translocated c-myc genes have been found in all BL cases studied, suggesting that they are necessary, if not sufficient, for activation. Truncations of a 5' negative transcriptional regulatory element and/or mutations of the 3' border of exon I appear to be consistently involved. The functional significance of these two regions in normal or translocated c-myc genes will be discussed. iii) Role of c-myc translocation in B-cell transformation. The biological effects of activated c-myc genes have been studied in the putative pathologic target cell of eBL, i.e. EBV-infected B-cells. Transfected c-myc genes, constitutively expressed under the control of different enhancer/promoter elements, cause the malignant transformation of *in vitro* EBV-infected B-cells and *in vivo* EBV-infected B-cells from AIDS patients. The phenotypic expression of this transformation includes changes in morphology, clonogenic properties, serum dependency and *in vivo* tumorigenicity and involves a negative regulation of the endogenous, normal c-myc gene expression. The implications of these findings will be discussed in relationship to the pathogenesis of BL and AIDS-UBL.

Finally, recent data on the possible involvement of the c-myb gene in the $6q^-$ chromosomal aberration present in 25°/° ALL and 30°/° non Hodgkin lymphomas (NHL) will be discussed. *In situ* hybridization experiments indicate that the c-myb locus is located in the proximity of the chromosomal breakpoint of different types of $6q^-$ deletions. Structural alterations (amplification) and functional alterations (overexpression) alterations of the c-myb gene are detectable in all $6q^-$ ALL or NHL tested. The relationship between these findings, the structure of the human c-myb locus and the control of its expression by a differential splicing mechanism will be preliminarily presented.

THE abl ONCOGENE IN HUMAN CHRONIC MYELOGENOUS LEUKEMIA

Owen N. Witte, M.D.

D 007

The Philadelphia chromosome, found in almost all patients with chronic myelogenous leukemia, is known to express a chimeric messenger RNA of 8.5 kb derived from sequences on chromosomes 22 and 9. The chromosome 9 component of the chimeric RNA is derived from a subset of the exons of the abl oncogene. A portion of the exonic sequences of a gene referred to as "bcr" on chromosome 22 make up the amino-terminal portion of this chimeric messenger RNA and gene product. Full-length clones of the 8.5 kb messenger RNA have beeen recently obtained by our laboratory. The sequence analysis of this large messenger RNA reveals an exceptionally G-C rich 5' untranslated region. A complete open reading frame initiating in sequences of the bcr gene and reading through the abl oncogene segment has been determined. The sequence has an extremely high percentage of serine residues in the bcr segment of the chimeric protein.

Viruses and Leukemia/Lymphoma

D 008 **MOLECULAR PATHOGENESIS OF HUMAN T-CELL LEUKEMIA VIRUSES,** Irvin S.Y. Chen, Alan J. Cann, Joseph D. Rosenblatt and William Wachsman, Division of Hematology-Oncology, Department of Medicine, UCLA School of Medicine, Los Angeles, CA 90024.

Human T-cell leukemia viruses, HTLV-I and HTLV-II, are related retroviruses which induce distinct T-cell malignancies in man. We have investigated the pathogenesis of HTLV-II at the molecular level in a recent patient who developed an atypical hairy-cell leukemia associated with HTLV-II infection. Characterization of the virus, its integration sites on leukemic cells, its pattern of viral expression, and cells infected by the virus *in vivo* and *in vitro* provide strong evidence for an etiologic role of HTLV-II in this patient. Molecular cloning of the viral genome demonstrates the virus is related to but not identical to the original isolate of HTLV-II, also obtained from a patient with atypical hairy-cell leukemia.

We have investigated T-cell transformation by HTLV by investigating the function of a gene which is believed to be responsible for transformation by human T-cell leukemia viruses. This gene is termed the *x* gene (also referred to as *lor*, *x-lor*, and *tat-I/II*). One property of the product of this gene is activation of transcription from the HTLV LTR; although both HTLV-I and HTLV-II activate the LTRs, there are differences in their properties. We investigated these differences by constructing mutations in the *x* proteins and constructing recombinants between HTLV-I and HTLV-II *x* proteins, analysing the phenotypes of the resultant molecules in transcriptional activation assays. Our results demonstrate some unusual properties of the *x* proteins which relate to their transcriptional activating properties. The aim of these studies is to gain further insight into the property of the *x* proteins, and ultimately, the role of these proteins in viral replication and T-cell transformation.

Growth Factors and Receptors - I

D 009 **HUMAN GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR,** Judith C. Gasson, Stephen D. Nimer, John F. DiPersio, Gayle Cocita Baldwin, Richard H. Weisbart and David W. Golde, Division of Hematology-Oncology, Department of Medicine, UCLA School of Medicine, Los Angeles, CA 90024.

Human granulocyte-macrophage colony-stimulating factor (GM-CSF) is a 22,000 dalton glycoprotein produced by activated T lymphocytes, as well as fibroblasts and endothelial cells stimulated with various cytokines. This hematopoietic growth factor stimulates the proliferation of myeloid precursors giving rise to mature neutrophils, eosinophils and monocytes. In addition to its effects on proliferation, GM-CSF increases the functional activity of mature effector cells in response to physiologic stimuli. Enhanced chemotaxis, oxidative metabolism, phagocytosis, and antibody-dependent cell-mediated cytotoxicity are observed in neutrophils pre-treated with GM-CSF. All of the biological effects of GM-CSF appear to be mediated by a low number of high affinity (Kd = 10 to 60 pM) receptors on responsive cells including neutrophils, eosinophils, leukemic cell lines, and fresh cells from myeloid leukemias.

GM-CSF is encoded by a gene approximately 2.5 kb in length, localized to the long arm of chromosome 5. We have prepared recombinant constructs linking the 5' flanking sequences of GM-CSF to the bacterial chloramphenicol acetyl transferase (CAT) gene. When transfected into human T-lymphoblast cell lines, expression of the CAT gene is dependent upon stimulation of the cells with lectin, phorbol esters, or monoclonal antibodies. These results suggest that sequences important for regulation of GM-CSF expression in activated T lymphocytes are located upstream from the coding region.

Clinical trials of this important hemopoietin are currently underway to enhance host defense in immunodeficient patients.

D 010 INTERLEUKIN-3 REGULATED GROWTH OF NORMAL AND TRANSFORMED HEMATOPOIETIC STEM CELLS, James N. Ihle*, John L. Cleveland†, Michael Dean*, Yacob Weinstein*, and Ulf R. Rapp†, *NCI-Frederick Cancer Research Facility, BRI-Basic Research Program, Frederick, MD 21701. †Laboratory of Viral Carcinogenesis, National Cancer Institute, Frederick, MD 21701.
IL-3 supports the proliferation and differentiation of hematopoietic stem cells in vitro to a variety of lineages. To define the genes which are involved we have examined the effects of various oncogenes on primary hematopoietic cells or on IL-3 dependent cell lines; alternatively we have associated the rearrangement of proto-oncogenes in leukemia cell lines with alterations of normal functions. A number of oncogenes, including myc, abl, src, and fms, can abrogate the requirement for IL-3 for growth and are speculated to interrupt the normal signal transmission pathway induced by IL-3. In primary cultures several oncogenes can "immortalize" functional mast cells for growth in vitro including abl which also abrogate the requirements for growth factors and ras, mos, and raf which do not alter the requirements for IL-3. This group of oncogenes is speculated to affect functions which regulate the rate of proliferation. None of the above oncogenes affect the differentiation of cells in primary cultures. With both in vivo and in vitro derived cell lines retroviral integrations, and truncation of the c-myb gene are associated with alterations in differentiation and are not associated with alterations in growth factor requirements. The varying effects of oncogenes on growth regulation or differentiation may contribute to the evolution in the transformed phenotypes of hematopoietic tumors.
Research sponsored by the National Cancer Institute, DHHS, under contract NO. N01-CO-23909 with Bionetics Research, Inc.

D 011 TRANSFORMATION POTENTIAL OF THE c-fms PROTO-ONCOGENE. M. F. Roussel[1], T. Dull[2], C.W. Rettenmier[1], P. Ralph[3], A. Ullrich[2] and C.J. Sherr[1]. St. Jude Children's Research Hospital, Memphis, TN[1], Genentech, San Francisco[2] and Cetus Corp[3], Emeryville, CA.

The product of the c-fms proto-oncogene is related and probably identical to the receptor for the macrophage colony stimulating factor, CSF-1 (M-CSF). The v-fms oncogene of the McDonough strain of feline sarcoma virus (SM-FeSV) encodes an analogous glycoprotein that includes the ligand binding domain, transmembrane spanning region, and tyrosine kinase domain of the CSF-1 receptor. The proto-oncogene and oncogene products differ structurally from one another at their extreme C-terminal ends where 40 amino acids of the CSF-1 receptor were replaced by 11 unrelated C-terminal residues in the v-fms product. The unique region of the normal receptor includes a single tyrosine residue (tyr^{969}) whose phosphorylation might negatively regulate the receptor kinase activity.

Although the v-fms gene transforms fibroblasts and produces fibrosarcomas in animals, SM-FeSV has never been shown to induce hematologic malignancy. When the v-fms gene was introduced into an SV40-immortalized, CSF-1 dependent murine macrophage cell line, the cells became factor independent and tumorigenic in nude mice, yielding histocytic sarcomas. The transformed macrophages did not synthesize CSF-1 and coexpressed unaltered levels of CSF-1 receptors which could be rapidly down modulated in response to CSF-1 or phorbol ester. Thus, v-fms transformation was mediated by constitutive kinase activity, and not by autocrine stimulation or by signals which transmodulated the c-fms proto-oncogene product.

Insertion of human c-fms cDNA into mouse NIH-3T3 cells stimulated CSF-1 dependent fibroblast proliferation; receptor transduction was therefore sufficient to enable NIH-3T3 cells to respond to a hematopoietic growth factor. Mutation of wild-type c-fms(tyr^{969}) to c-fms(phe^{969}) did not in itself result in transforming activity. However, cotransfection of c-fms(tyr^{969}) with CSF-1 cDNA into NIH-3T3 cells induced foci of transformed cells at a low efficiency, whereas cotransfection of the c-fms(phe^{969}) and CSF-1 genes generated foci as efficiently as v-fms. The effect of the phe^{969} mutation was therefore unmasked when the mutated receptor was expressed with an endogenous ligand, implying that tyr^{969} is important in regulating the growth-promoting effects of CSF-1 induced signals. In the absence of CSF-1, chimeric v-fms/c-fms(tyr^{969}) genes encoding the unique c-fms C-terminus transformed NIH-3T3 cells inefficiently, whereas identical chimeras containing phe^{969} were as active as the v-fms oncogene in transformation. Thus, complete oncogenic activation of c-fms appears to require two genetic events: one which alters a putative regulatory site at tyr^{969} in the C-terminus, and a second which simulates a ligand-induced conformational change, rendering a CSF-1 independent signal.

D 012 REGULATION OF COLONY STIMULATING FACTOR-1 AND HEMOPOIETIN-1,
E. Richard Stanley, Vincent Praloran and Anna Bartocci, Departments of Microbiology & Immunology and of Cell Biology, Albert Einstein College of Medicine, Bronx, NY 10461.
Colony stimulating factor-1 (CSF-1) is a homodimeric glycoprotein that selectively regulates the survival, proliferation and differentiation of mononuclear phagocytes (CSF-1 dependent precursor → monoblast → promonocyte → monocyte → macrophage). It is produced by fibrolasts and possibly other cell types and occurs in a variety of adult mouse tissues (reviewed in 1). CSF-1 circulates at biologically active concentrations (0.5-1.0 nM) and is selectively cleared from the circulation by macrophages via CSF-1 receptor mediated endocytosis and intracellular destruction. This clearance represents an elegant feedback mechanism by which macrophages selectively control the circulating concentration of the growth factor that regulates their production.
Hemopoietin-1 (H-1) is a 17,000 M_r protein which regulates the most primitive hemopoietic cells yet shown to proliferate and differentiate in vitro (2, 3). H-1 alone has no detectable effect on hemopoietic cell proliferation. Instead, it allows other hemopoietic growth factors to act on more primitive cells than they alone can act on (2, 4). Thus H-1 dramatically increases the plating efficiency of primitive hemopoietic cells cultured with CSF-1 (T.R. Bradley, personal communic.) or IL-3 (4). However, in the presence of CSF-1, the resulting colonies are composed entirely of mononuclear phagocytes, whereas with IL-3 they are composed of small blast cells, megakaryocytes, erythroid precursors, granulocytes and macrophages. Thus H-1 may enable growth factors, such as CSF-1 whose circulating levels are determined by macrophage numbers, to channel multipotent cells into particular cell lineages.

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D 013 ABNORMAL EXPRESSION OF INTRTLEUKIN-3 AND LEUKEMIA,
Ian G. Young, The John Curtin School of Medical Research, The Australian National University, Canberra, Australia.
Disturbances in the normal regulation of haemopoiesis by IL-3 and other haemopoietic growth regulators are one potential mechanism leading to the leukemias. Abnormal expression of a growth factor gene by a cell also expressing a functional receptor for that factor allows a haemopoietic progenitor to escape from normal regulation and may be an important first step leading to the development of tumorigenicity.[1] Our characterization of the murine IL-3 gene[2,3] has facilitated the detection of chances which lead to abnormal gene expression and also has enabled the construction of retroviral factors expressing IL-3.
The murine myelomonocytic leukemia line WEHI-3B produces IL-3 constitutively and thus shows abnormal IL-3 expression. It has been proposed by a number of workers that the genetic change leading to the constitutive synthesis of IL-3 may have been an important step in the development of the original leukemia from which the WEHI-3 cell line was derived. Southern transfer analysis of DNA from WEHI-3B revealed an alteration in the vicinity of the IL-3 gene. We have recently studied this rearrangement in detail and shown that the rearrangement and alteration in gene expression is due to the insertion of an endogenous retroviral element, an intracisternal A particle gemone, near the IL-3 gene promoter[4].
To more directly test the role of autostimulatory loops in leukemogenesis we have initiated a series of experiments[5] using a retroviral expression vector to insert the IL-3 gene into the factor dependent on the continued presence of exogenously-supplied growth factors (IL-3 or GM-CSF). A retroviral shuttle vector (fpGV-IL3) has been constructed which carries the IL-3 gene and has been used to convert FDC-P1 cells into constitutive producers of IL-3. These cells (GDC-P1-IL3) were shown to be capable of independent growth, although their growth was completely inhibited by addition of an anti-IL-3 antibody. These findings indicate that the growth of FDC-P1-IL3 cells is stimulated by endogenously produced IL-3. The FDC-P1-IL3 cells were shown to be leukemogenic, whereas normal bone marrow cells similarly treated with the fpGV-IL3 expression vector were not. Additional experiments are in progress with populations of autocrine-stimulated bone marrow cells to try and determine what additional genetic changes are required for the development of tumorigenicity.

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Growth Factors and Receptors - II

D 014 IN VIVO ACTION OF RECOMBINANT HUMAN G-CSF IN NORMAL, LEUKEMIC OR MYELOSUPPRESSED MICE, Malcolm A.S. Moore, David Warren and Lawrence M. Souza, Lab for Developmental Hematopoiesis, Memorial Sloan-Kettering Cancer Center, New York, N.Y., 10021 and Amgen (LMS), Thousand Oaks, CA 91320.

Recombinant human granulocyte colony stimulating factor (rh-G-CSF) produced a dose dependent sustained neutrophil leukocytosis following twice daily ip injections in C3H/HeJ, Balb/c and $B6D2F_1$ mice. Marked splenomegaly developed, associated with extramedullary erythropoiesis, granulopoiesis and elevated megakaryocyte production. Total CFU-GM responsive to IL-3, GM-CSF, CSF-1 and G-CSF were increased 3-5 fold, and CFU-s 2-3 fold by days 7-14. Following cyclophosphamide treatment (200mg/kg) of C3H/HeJ mice, G-CSF abolished the neutrophil nadir at days 4-5 and promoted an absolute neutrophil leukocytosis 15 fold greater than in mice treated with cyclophosphamide alone by days 7-14. Repeated courses of cyclophosphamide up to 9x200mg/kg over 13 weeks were administered with intermittent G-CSF treatment, resulting in prolongation of survival and persisting neutrophil leucocytosis. Recovery from 5-fluorouracil (150mg/kg) was also accelerated following G-CSF treatment with neutrophils increased 10 fold by D7 and 100 fold by D14. Recovery from graded doses of irradiation (250,500,750 rad) was accelerated by G-CSF treatment with myelopoiesis increased 7 fold within one week and erythropoiesis and BFU-E production increased 7-9 fold in two weeks. CFU-s and CFU-GM populations regenerated more rapidly in G-CSF treated, sublethally irradiated mice given G-CSF. Further evidence that availability of stem cells was not the sole determinant in recovery from irradiation was provided by analysis of lethally irradiated mice transplanted with limiting numbers of syngeneic bone marrow cells. G-CSF therapy doubled the number of hematopoietic cells generated in transplanted mice within two weeks and increased neutrophil numbers 65 fold. Early stem cells (CFU-s,CFU-GEMM) were increased 3 fold, and later progenitors (BFU-E, CFU-GEM, CFU-Meg) 4-12 fold in this time, with the major increase being in the splenic population. In addition to promoting mature neutrophil production and accelerating stem and progenitor cell regeneration in various myelosuppressed states, G-CSF in vitro promotes neutrophil differentiation of the WEHI-3B differentiation-inducible murine myelomonocytic leukemia, limits its self-renewal potential and suppresses its leukemogenic potential upon subsequent transfer to syngeneic Balb/c mice. In vivo treatment of mice injected subcutaneously or intraperitoneally with 10^5 WEHI-3 cells with daily G-CSF prolonged median survival from 19 to 33 days. With lower initial leukemic cell inocula (10^4), long-term cures of 80% of mice were obtained with G-CSF therapy.

D 015 COLONY-STIMULATING FACTOR RECEPTORS AND HEMOPOIETIC CELL DIFFERENTIATION, Nicos A. Nicola, Walter and Eliza Hall Institute of Medical Research, P.O. Royal Melbourne Hospital, Victoria 3052, Australia.

Four murine colony-stimulating factors (CSF's) are known that regulate the production of granulocytes and macrophages - G-CSF, M-CSF (CSF-1), GM-CSF and Multi-CSF (IL-3). The biological specificities of these CSF's are highly complex, overlapping and concentration-dependent. In particular, the CSF's have a hierarchical organization with Multi-CSF and GM-CSF having broad cellular specificities while the actions of G-CSF and M-CSF are essentially lineage-restricted. Iodinated derivatives of each CSF have been prepared and the cellular and molecular specificities of CSF binding interactions have been studied on normal cells, cell lines and leukemic cells. Although each CSF receptor was shown to be unique with no ability to directly bind any other CSF, the CSF's displayed an ability to indirectly down-modulate ('trans-modulate') other CSF receptors on murine bone marrow cells. This pattern of CSF receptor trans-modulation was rapid, dose-dependent and showed the same type of hierarchical organization as observed for the biological activities of the CSF's. It suggested a model of CSF action involving proliferation-inducing receptors (those for Multi-CSF and GM-CSF) and differentiation-inducing receptors (those for G-CSF and M-CSF) where actions are coupled by the process of receptor trans-modulation. This model is consistent with the cellular distribution of CSF receptors and the concentration-dependence of CSF actions. Moreover, it has important implications for the generation of myeloid leukemias and the possibility of using differentiation-inducers to suppress leukemic cell self-renewal.

D 016 GROWTH FACTOR-DEPENDENT PROLIFERATION OF HUMAN LEUKEMIC CELLS, Giovanni Rovera, The Wistar Institute, Philadelphia, Pennsylvania 19104.

Nine permanent cell lines were established from 50 consecutive cases of acute childhood leukemias (18%) during screening to define their *in vitro* growth factor requirements. These included 3/7 T-ALL, 3/13 AML and 3/30 pre-B-ALL; all the established lines had an abnormal karyotype. Only 5 of the 9 grew immediately in Iscove's modified Dulbecco's medium (IMDM) supplemented with FBS; the other lines required growth factors present in the conditioned media (CM) of human tumor cell lines (Mo, JBL-1, 5637, SW879) to maintain growth in early passages.

The great majority of AML (10/13), free of adherent stromal cells, could be cultured for at least 8 weeks in IMDM supplemented with CM. Purification of the active fractions and analysis with blocking antisera indicated that the limited pool expansion of the leukemic cells was due to the presence of GM-CSF, and rhGM-CSF could reproduce the same proliferative response.

At least two other factors produced by solid tumor cell lines were identified that stimulated AML cell proliferation. These factors differed in chromatographic migration properties and in reactivity with antiserum to GM-CSF and to G-CSF.

Proliferation gradually decreased in the GM-CSF-dependent AML culture and the decrease was correlated with terminal differentiation, which in all cases was morphologically and immunophenotypically abnormal. This suggests that the leukemic population requires additional factors in order to remain self-maintaining *in vitro*.

Unexpectedly, 2 highly immature T-ALL required GM-CSF for growth. While one of these cell lines (a biphenotypic T-myelomonocytic) rapidly became growth factor-independent, the other (TALL-101) required the continuous presence of GM-CSF. GM-CSF-induced expression of IL2 receptor on the surface of TALL-101 cells and addition of IL2 to the culture further stimulated the growth of the cells. TALL-101 cells can grow in synthetic medium either in the presence of high concentrations (100 U/ml) of GM-CSF or of lower concentrations of both GM-CSF and IL2. When the cells are kept in medium with FBS at concentrations higher than 5%, a population arises that becomes hemopoietic growth factor-independent, but requires serum for growth. However, this apparent independence is reversed when the cells are again cultured in GM-CSFcontaining medium. The surface phenotype of TALL-101 cells can be modified by a variety of factors but terminal differentiation of these cells cannot be induced.

Inability to efficiently induce terminal differentiation *in vitro* using a variety of physiological and nonphysiological agents was commonly observed in all the established human leukemic lines.

D 017 TRANSFORMING GROWTH FACTOR PRODUCTION BY A HUMAN PRE-B LEUKEMIA, R. Graham Smith, Jerry Zack and Brad Ozanne, Univ. of Texas Health Science Center, Dallas, TX 75235.

Transforming growth factors (TGFs) are a group of proteins isolated from many different tumor cell types, which induce normal cells to grow as if they were transformed. Two classes of TGFs have been described: TGF_{α}, which binds the receptor for epidermal growth factor (EGF); and TGF_{β}, which binds a different receptor. The rat fibroblastoid cell line NRK-49F requires both TGF_{α} and TGF_{β}, acting in concert, to form colonies in soft agar. We have found that the human acute lymphoblastic leukemia cell line SMS-SB produces an activity which promotes the growth of NRK-49F cells in agar. This activity does not require either TGF_{α} or TGF_{β}. Serum-free conditioned medium from SMS-SB cells (SBSFCM) also contains a mitogen for quiescent NRK-49F cells. A panel of other human lymphoid tumor lines have been tested as well; the results show that production of this TGF activity is not a unique property of SMS-SB cells. Chromatographic and functional studies suggest that SBSFCM contains at least two different TGF activities. Both of these activities appear to be unrelated to EGF, TGF_{α}, TGF_{β}, insulin, transferrin, insulin-like growth factor 1, fibroblast growth factor, endothelial cell growth factor, hematopoietic cell colony stimulating factors, B cell growth factors and interleukins 1 or 2. Purification of these factors from SBSFCM should further clarify their relationship, if any, to known mitogens. Experiments are also underway to understand other biologic activities of these TGFs and to ascertain whether they may play any role, direct or indirect, in the transformation of the cells which release them.

D 018 NORMAL AND ABNORMAL REGULATION OF IL-2 RECEPTOR EXPRESSION, Thomas A. Waldmann, Metabolism Branch, National Cancer Institute, NIH, Bethesda, MD 20892.
Antigen or mitogen-induced activation of resting T-cells induces the synthesis of interleukin-2 (IL-2). Resting T-cells do not express IL-2 receptors, but receptors are rapidly expressed on T-cells following activation. Failure of the production of either the growth factor or its receptor results in a failure of the T-cell immune response. Two IL-2 binding peptides a 55kd Tac peptide and an independent 75kd peptide are coexpressed in a multichain receptor on activated T-cells in order to achieve high affinity binding. Anti-Tac, a monoclonal antibody that recognizes the IL-2 receptor has been used to purify the 55kd receptor. The receptor is a 33kd peptide that is post-translationally glycosylated to a 55kd mature form. The amino acid sequence of the IL-2 receptor deduced from cDNA clones indicates that this peptide is composed of 272 amino acids including a signal peptide 21 amino acids in length. There is a single 19 amino acid transmembrane domain and a short intracytoplasmic domain composed of 13 amino acids at the carboxyterminus. In contrast to resting T cells human T-cell lymphotrophic virus I (HTLV-I) associated adult T-cell leukemia cells constitutively express large numbers of IL-2 receptors. To exploit the fact that IL-2 receptors are present on the malignant T-cells but not on normal resting cells clinical trials have been initiated in which patients with adult T-cell leukemia are treated with unmodified anti-Tac or with Pseudomonas toxin conjugates of anti-Tac. These theraputic studies have been extended in Vitro by the demonstration that conjugates of anti-Tac with the alpha-emitting radionuclide bismuth-212 eliminated greater than 98% of the proliferative capacity of HUT 102 cells with only a modest effect on IL-2 receptor negative lines. Finally anti-Tac is being evaluated as a potential therapeutic agent to eliminate activated IL-2 receptor expressing T-cells in other clinical states including certain autoimmune disorders and in protocols involving organ allografts.

Hematopoietic Stem Cells and Differentiation

D 019 MARROW STROMAL CELL REGULATION OF CLONAL HEMATOPOIETIC STEM CELL SELF RENEWAL AND COMMITMENT, Joel S. Greenberger, Pervin Anklesaria, Matsusugu Ohta, T.J. FitzGerald, Valerie Klassen, and Mary Ann Sakakeeny, Dept. of Radiation Oncology, Univ. of Massachusetts Medical Center, Worcester, MA 01605.
Permanent multipotential hematopoietic stem cell line B6SUt proliferates in liquid suspension culture in multi-CSF (IL-3) and differentiates to hemoglobinized macroscopic CFU-GEMM in semisolid culture in IL-3 and erythropoietin. Recombinant GM-CSF, or M-CSF, fail to induce colony formation. The cells "home" in vivo to the spleen or to implanted cellulose acetate membranes. To study regulation of stem cell growth by stromal cells of the marrow microenvironment, permanent stromal cell lines were derived from marrow cultures of C3H/HeJ, Sl/Sl^d, micropthalmic-osteopetrotic mi/mi, and C57BL/6J mice carrying the isoenzyme $GPI\text{-}1^a$ from mus Castaneous (B6.Cast-$GPI\text{-}1^a$). The latter cell line was readily identifiable in vitro by immunoperoxidase staining with antisera to $GPI\text{-}1^a$. In the absence of detectable IL-3 poly-A messenger RNA in extracts from clonal C3H/HeJ marrow stromal cell line D2XRII, proliferation of B6SUtA cells was stimulated by cocultivation. Adherence and proliferation was further stimulated by x-irradiation of plateau phase D2XRII cells. Several weeks cocultivation of IL-3 dependent hematopoietic progenitor cells with x-irradiated cloned stromal cell lines induced factor-independent subclonal lines that formed monomyeloid tumors in vivo. Sl/Sl^d cell lines did not support B6SUt cells in vitro. Both uncloned populations of fresh marrow stromal cells and cloned permanent stromal cell lines supported engrafted pooled fresh hematopoietic stem cells in vitro for 7 to 16 weeks. Engraftment in vivo of permanent cloned bone marrow stromal cell line GPIa to total-body-irradiated C57BL/6 mice resulted in successful seeding of marrow cavities and stimulation of recovery of hematopoiesis in vivo. Thus, bone marrow stromal cell lines stimulate homing and self renewal of both uncloned and clonal IL-3 dependent hematopoietic progenitor cell lines in the absence of detectable IL-3.

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D 020 TUMOR NECROSIS FACTORα (TNFα) AND HEMATOPOIESIS,
H. Phillip Koeffler, Andreas Tobler, John Ranyard, Donald Johnson, Division of Hematology/Oncology, University of California, Los Angeles, CA 90024; Reinhold Munker, University Hospital Grosshadern, Munich, West Germany.

Recombinant (rec.) TNFα potently inhibits the clonogenic cells from 6 of 9 human myeloid leukemia cell lines with a 50% inhibitory concentration (ED_{50}) that ranged between 6 and 150 U/ml. A decrease in DNA-, RNA- and protein synthesis and in cloning efficiency occurred within 3 hours of exposure of HL-60 promyelocytes to TNFα. Fresh leukemia cells from 12 of 15 patients with myeloid leukemia were sensitive to TNFα in the clonogenic assay for leukemic cells, with an ED_{50} in the range of 20-2,500 U/ml. TNFα also suppressed normal myeloid progenitors from 9 normal donors, however, the degree of suppression strongly depended on the source of colony stimulating factor used in the assay; the TNFα ED_{50} varied between 100 U/ml in the presence of rec. G-CSF and 50,000 U/ml in the presence of rec. GM-CSF. Part of the action of TNFα on myeloid leukemia cells probably can be explained by induction of terminal differentiation (~30% HL-60 at 20 U/ml TNFα). No correlation exists between the receptor number for TNFα (range 600-1900 per cell, with K_d 30-90 pM) and the biologic effect on myeloid cells with the exception that trans-retinoic acid (10^{-8}M) increased the TNFα receptors two-fold on HL-60 cells and synergistically enhanced the ability of TNFα to inhibit HL-60 clonal growth. Northern blot analysis and transcriptional run-on experiments showed that TNFα rapidly decreased levels of c-myc transcription in HL-60 cells and this transcriptional control did not require synthesis of another protein. Transcriptional rates and mRNA levels of β-actin did not change in HL-60 cells cultured with TNFα under similar conditions. Transcription of c-myc was not affected by TNFα in HL-60 variant cells which are resistant to the cytotoxic effects of TNFα. Some of the cytotoxicity of TNFα may be explained by a direct effect of TNFα on c-myc transcription. Further studies showed that TNFα, but not TNFβ, is a strong stimulant to the accumulation of G- and GM-CSF protein and mRNA in fibroblast cells. GM- and G-CSF mRNA levels increased within 1 and 4 hrs, respectively of exposure to TNFα (200 U/ml). The GM-CSF synthesized by fibroblasts cultured with TNFα is heterogenously glycosylated and has a similar molecular weight as observed in activated T-lymphocytes (15-30 kD); the mRNA from TNFα-stimulated fibroblasts has a half-life similar to phorbol diester stimulated T-lymphocytes (>6 hrs) and the start site is the same as that observed in PHA-activated T-lymphocytes. Stimulation of detectable levels of G-CSF mRNA by TNFα is inhibited by cycloheximide, and further studies suggest that levels of the G- and GM-CSF mRNA can be controlled at the posttranslational level. Taken together, the data suggests that TNFα can have profound effects on normal and abnormal hematopoiesis.

D 021 INDUCED DIFFERENTIATION OF TRANSFORMED CELLS: MODULATION OF GENE EXPRESSION

P.A. Marks, R. Ramsay, K. Ikeda, R.A. Rifkind, Memorial Sloan-Kettering Cancer Center New York 10021.

Hexamethylene bisacetamide (HMBA) is an effective inducer of erythroleukemia (MEL) cell differentiation. Studies with these cells and various other systems demonstrate that transformation does not necessarily destroy the potential of cells to express differentiated phenotype. These studies focus on HMBA induced modulation of gene expression in MEL cells, in particular, genes for the erythroid phenotype, α^1 and β^{maj} globins; for general metabolic function, rRNA, and genes possibly related to regulation of cell proliferation, c-myc, c-myb, c-fos and p53. HMBA induced MEL differentiation is a multi-step process. During the early period, when cells remain uncommitted as indicated by the fact that upon removal from culture with inducer to fresh medium, cells will continue to proliferate and not differentiate, HMBA causes a decrease in c-myc and c-myb and an increase in c-fos mRNA levels and a decrease in p53 protein within 4 to 6 hr. Commitment is detectable by 12 to 18 hrs., c-myc in mRNA level returns to that of uninduced cells by 12 hrs., but c-myb remains depressed. Dexamethasone inhibits HMBA induced MEL cell differentiation and inhibits continued suppression of c-myb mRNA levels but does not affect the early changes in proto-oncogene expression. Hemin, which induces MEL cells to accumulate globin mRNAs but not commitment to terminal cell division, does not suppress c-myb expression. Thus, the early transient alterations in proto-oncogene expression may be important to HMBA induced commitment, but continued suppression of c-myb appears to be a characteristic of terminal cell differentiation.

HMBA induces a 20-30x increase in transcription of α^1 and β^{maj}-globin genes. In uninduced cells certain chromatin structural changes in the globin gene domains associated with the configuration of actively transcribed genes exists, e.g., partial nucleosome disruption, increase in DNase sensitivity and hypomethylation, but the genes are not actively transcribed. HMBA causes further nucleosome destruction, development of DNase I HSS 5' to the α and β-globin cap sites, and active transcription. HMBA modulates expression of a number of other DNA sequences in relation to commitment to terminal cell division.

D 022 PHYSIOLOGY OF HEMOPOIETIC STEM CELLS STUDIED IN SEMI-SOLID CULTURE, Makio Ogawa, VA Medical Center and Medical University of South Carolina, Charleston, SC.
The main feature of the hemopoietic system is its very stable, life-long process of cellular turnover. This process is supported by stem cells which possess self-renewal capacity and the ability to generate progenitors committed to differentiation in individual hemopoietic lineages. During the past two decades, clonal culture assays provided the means to study the mechanisms of stem cell functions. Most recently, murine blast cell colonies identified in our laboratory provided a unique tool to study stem cells in vitro. Micromanipulation of individual blast cells and their paired progenies provided experimental data which are consistent with the stochastic model of stem cell differentiation. Our studies also suggested that interleukin-3 (IL-3) provides a permissive environment for the sustained proliferation of early hemopoietic progenitors but it does not trigger proliferation of stem cells.
There is growing evidence suggesting that factors such as interleukin-1 and tumor-necrosis factor stimulate a variety of cells which in turn release colony-stimulating factors (CSF's). In addition, these factors appear to overlap in their target progenitors. In order to study the precise mechanisms of CSF actions, it is necessary to examine the effects of purified factors on purified progenitors in serum-free cultures. We recently developed a consistent human blast cell colony assay. We observed that human IL-3 is capable of supporting the formation of blast cell colonies and is more effective than human GM-CSF in the support of multilineage colonies. Finally, I will also discuss our efforts to develop a serum-free culture for purified human progenitors.

Immunology of Leukemia and Lymphoma

D 023 LINEAGE ASSOCIATION OF CHROMOSOMAL TRANSLOCATION, Stanley J. Korsmeyer, Masao Seto, Winfried Graninger, Richard Hockett, John Wright and Ajay Bakhshi, Washington University School of Medicine, St. Louis, MO 63110 and National Institutes of Health, Bethesda, MD 20892.
The chromosomal translocations of B cell neoplasms characteristically involve the immunoglobulin (Ig) gene loci. The most frequent of these is the t(14;18)(q32;q21) found in over 80% of follicular lymphomas. Molecular analysis of both chromosome 14q32 and 18q21 germline substrates and both reciprocal products, derivative (der) 14 and der 18 revealed insights into the mechanism of translocation. The t(14;18) is not fully reciprocal as the der 14 breakpoint juxtaposes 18q21 with the 5' end of J_H while the der 18 juncture is with the 3' end of D_H segments. Furthermore, extranucleotides resembling "N" segments are found at the chromosomal junctures indicating that the break on 14 is mediated by Ig recombinase at a pre B cell stage. The breaks at 18q21 also cluster within a 2.8Kb major breakpoint region (mbr) (70%) and most fall within but 150bp. However, no Ig-like rearrangement signals are present, but instead the mechanism of breakage on 18 appears to be a random, staggered dsDNA break. A functional basis for the focused breaks at 18q21 results from the interruption of a 3' exon of a B cell associated gene (BCl-2) at 18q21. cDNAs of translocated and germline products reveal a fusion transcript in t(14;18) lymphomas. This gene is normally highly expressed in pre B cells (time of translocation) and down regulated in mature B cells; however t(14;18) mature B lymphomas have a deregulated gene. Thus, the t(14;18) is B lineage-associated because it translocates a pre B cell stage gene (BCl-2) into Ig recombinase cleaved D_H and J_H regions on 14. Yet, 10% of T cells have D_H/J_H Ig rearrangement and we have noted that some J_H rearrangements in T cell neoplasms are classic 14;18 translocations. However, the most highly associated translocations in T cells involve the T cell receptor loci. These findings raise important issues as to whether the lineage-association of specific translocations is a primary or secondary event. An inherited constitutional t(9;14)(p12;q11) has been found in the germline of a family that develops T cell lymphoma. Thus, a translocation near the α TCR locus (14q11) appears to have predetermined the lineage of the subsequent neoplasm. The antigen receptor genes of Ig and TCR genes that normally rearrange to generate diversity also mediate illegitimate chromosomal translocations providing means to identify new transforming genes.

D 024 IMMUNOLOGICAL APPROACHES TO LYMPHOMA TREATMENT, Dan L. Longo, Jonathan Ashwell, and Sandra Bridges, Biological Response Modifiers Program, Division of Cancer Treatment, National Cancer Institute-FCRF, Frederick, MD 21701
With the advent of biotechnology, a number of new biological treatment approaches have become available. Several have been tested against human lymphoma and others are still in preclinical development. Recombinant cytokines like interferons, IL 2 and tumor necrosis factor, adoptive cellular therapy with LAK cells plus IL 2, and monoclonal antibodies (both native and conjugated to isotopes and toxins) directed at differentiation antigens, immunoglobulin idiotypic determinants, and growth factor receptors have been used to treat patients with lymphoid malignancies. In general, only transient partial responses have been obtained to date, but much has been learned that may improve the outcome in subsequent studies. We have been studying three preclinical models of lymphoma treatment, all of which have led to the cure of animals bearing lymphoma of aggressive natural history. We have compared the efficacy of unconjugated anti-idiotypic antibodies to those conjugated with intact ricin or recombinant ricin A chain in the guinea pig L2C B cell leukemia. The intact ricin conjugate is dramatically more effective at prolonging survival than comparable molar amounts of the A chain conjugate but is also more toxic to the animal. The A chain conjugate can be administered safely at much higher doses than the intact ricin conjugate and can be escalated to a level that is equally effective to the maximum tolerated dose of the intact ricin conjugate. Rapidly growing B cell tumors appear to alter their idiotype less commonly than has been seen in patients with indolent lymphoma. We have used unconjugated antibodies against class II MHC products in mice bearing the L10.A lymphoma. Fifty percent of animals can be cured with multiple injections of anti-I-A^d antibodies. The capacity of the antibody to cure the lymphoma appears to be dependent on antibody-dependent cellular cytotoxicity and is achievable by virtue of the fact that the tumor Ia antigens do not modulate as readily as their analogues on normal immune cells. We have also examined the effect of stimulation through the T cell antigen receptor on the growth of T cell tumors in vitro and in vivo. Antigen stimulation of normal T cells results in proliferation and the production of lymphokines. In T cell hybridomas, antigen stimulation also elicits lymphokine secretion, but, paradoxically, irreversible growth arrest in G1 phase of the cell cycle. In vivo administration of antigen to mice bearing hybridomas results in cure of established tumors and the development of long-term immunity to the tumor by a T cell-mediated mechanism. This suggests that peripheral T cell tumors may be vulnerable to growth arrest by stimuli transduced through the antigen receptor complex.

D 025
IDIOTYPIC NETWORKS IN EXPERIMENTAL LEUKEMIA, Robert S. Schwartz, Blair Ardman, Susan Burdette and Ruth Maron, Hematology-Oncology Division, New England Medical Center, Boston MA 02111.
Our work concerns the receptors for recombinant leukemogenic retroviruses and the role such receptors might have in the pathogenesis of leukemia in AKR mice. We have used anti-idiotypes to define cellular receptors for recombinant retroviral gp 70. The principal behind the method is that an anti-idiotype against the combining site of an anti-gp 70 antibody could be an "internal image" of gp 70, and thus bind to the receptor for this glycoprotein. Starting with a monoclonal antibody specific for the gp 70 of a recombinant leukemogenic retrovirus, a rabbit anti-idiotype was prepared and affinity purified on the original idiotype. The interaction of the idiotype with the anti-idiotype was specifically blocked by the recombinant retrovirus, indicating the combining site-specificity of the anti-idiotype. The affinity purified anti-idiotype was shown to bind to retrovirus-induced leukemia cells of T-cell, B-cell and erythroid origin. Infection of _M. dauni_ fibroblasts with a recombinant retrovirus inhibited their binding to the anti-idiotype, whereas infection of the cells with an ecotropic virus did not. We term the receptor epitope detected by the anti-idiotype AVID (anti-viral idiotype). Immunoprecipitates of radiolabeled membranes of a mouse erythroleukemia cell line (PC4) with the anti-idiotype produced a single band with an apparent Mr of 86 Kd (SDS PAGE). In the PC4 line, AVID is confined to undifferentiated cells: its expression declines sharply within 48 hours after initiating differentiation with DMSO or HMBA. To examine the possible biological role of AVID in leukemogenesis, we sought auto-anti-idiotypes in pre-leukemic AKR mice. Hybridomas derived from such animals produced monoclonal antibodies with specificity for the combining site of a monoclonal anti-recombinant gp 70 antibody. Other hybridomas produced monoclonal antibodies with specificity for recombinant retroviral gp 70. The spontaneously produced AKR anti-idiotypes bind to AKR leukemia cell lines and to some fresh AKR thymomas. Similar anti-idiotypes were found in AKR serum. Some monoclonal AKR auto-antiidiotypes stimulate proliferation of normal T-cells _in vitro_. Thus, during the pre-leukemic period, AKR mice produce antibodies against neoantigens of recombinant gp 70 as well as auto-anti-idiotypes which bind to receptor-like determinants on AKR leukemia cells. The results suggest the idiotype networks may participate in the pathogenesis of retroviral leukemia.

Gene Transfer into Hematopoietic Stem Cells

D 026 INTRODUCTION OF NEW GENES INTO HEMATOPOIETIC STEM CELLS, John Dick[1], Robert Phillips[2], and Alan Bernstein[2], Research Institute of the Hospital for Sick Children and the [2]Mount Sinai Hospital Research Institute, Toronto, Canada. The introduction of new genes into the pluripotent stem cells of the hematopoietic system provides a new approach to addressing questions concerning the role of various genes in stem cell behaviour and correcting genetic defects in mice and men. To this end, we and others have developed retroviruses as high efficiency gene transfer vectors. In this presentation, data presented will be demonstrating gene transfer into a wide variety of committed and pluripotent stem cells. We have examined retrovirus vectors with different transcription regulatory regions for their ability to sustain long term expression of transduced genes *in vivo*. Biologically significant levels of expression with vectors containing the herpes *tk* promoter, but not the SV40 early region promoter on the Moloney LTR, were observed. Equivalent results were obtained with both mouse and human cells. In collaboration with T. Caskey's laboratory, we have inserted a functional cDNA to the human gene for adenosine deaminase into both mouse and human hematopoietic progenitor cells. Finally, experiments will be described in which a variety of activated *onc* and growth factor genes have been transferred into the defective stem cells of *W/W^v* mice. The properties of these cells will be discussed. (Supported by grants from the MRC and NCI of Canada.)

D 027 TRANSFER OF GENES INTO HEMATOPOIETIC CELLS USING RETROVIRAL VECTORS, Arthur W. Nienhuis, Stefan Karlsson, Peter M.C. Wong, Jeffrey Holt, and Robert Redner. Clinical Hematology Branch, National Heart, Lung, and Blood Institute, Bethesda, MD 20892 Recombinant retroviral vectors provide a high efficiency means to transfer genes into hematopoietic stem, progenitor, and precursor cells. We have constructed and utilized such vectors to investigate several aspects of gene expression and hematopoietic cell function. A human genomic gamma/beta fusion globin gene was inserted in the reverse transcriptional orientation into the N2 retroviral vector. A titer retroviral stock was obtained using the amphotrophic packaging line, P317. Transfer of the globin gene into mouse erythroleukemia cells yielded cell clones that expressed the human globin gene at a high level with appropriate regulation during induced maturation. Despite the high level of globin mRNA, protein synthesis was low apparently because of instability of the composite gamma beta protein and "anti-sense" inhibition of the globin transcript by that driven by the retroviral LTR. To overcome this problem, retroviral vectors containing the natural human beta globin gene have been inserted into a self inactivating retroviral vector. In a second series of experiments, the mouse IL-3 gene has been inserted into the N2 vector. A high titer ecotropic stock was prepared and used to infect primary mouse hematopoietic cells. Endogenous production of IL-3 from the transfered gene supported differentiation of hematopoietic progenitors of several lineages. Immortalized mast cell lines were derived from colonies formed from multi-potential and mast cell progenitors. These were factor independent but were not tumorigenic in nude mice. In contrast, established factor dependent cell lines were rendered factor independent and were tumorigenic in nude mice. A third application of retroviral vectors involves transfer of anti-sense transcription units. We had shown, using conventional DNA transfer techniques, that segments of the c-fos and c-myc genes when placed in the reverse transcription orientation downstream from the inducible MMTV promoter, caused reversible growth inhibition of mouse 3T3 cells. To facilitate transfer of these transcriptional units into hematopoietic cells, we are constructing appropriate retroviral vectors to be used to study the role of the c-myc and c-fos genes in normal hematopoietic cells and established cell lines.

Therapy

D 028 CHILDHOOD NON-HODKGIN'S LYMPHOMA AND LYMPHOMA-LEUKEMIA: CONCEPTS OF THERAPY FROM CHILDRENS CANCER STUDY GROUP (CCSG) STUDIES, W. Archie Bleyer, Richard Sposto, Stuart E. Siegel, Anna T. Meadows, John H. Kersey, Peter G. Steinherz, Robert R. Chilcote, R. Derek T. Jenkin, and G. Denman Hammond, CCSG, Pasadena CA 91101.

Between April 1977 and September 1986, the CCSG entered 1,444 patients under 21 years of age with newly-diagnosed non-Hodgkins's lymphoma (NHL) and lymphoma-leukemia onto five therapy studies. The CCG-551 study [642 patients] included all stages and histologies. The subsequent studies divided patients according to stage and histology:
CCG-501 [108 patients] - localized NHL regardless of histology; CCG-503 [235 patients] - nonlocalized non-lymphoblastic (non-LB) NHL with or without marrow involvement, and Burkitt's leukemia; CCG-502 [150 patients] - nonlocalized lymphoblastic (LB) NHL with <25% blast cells in the bone marrow; and CCG-123 [309 patients] - LB NHL with >25% blast cells in the bone marrow ("lymphoma-leukemia").

Six major conclusions regarding the treatment of diffuse NHL in children have been derived from the CCG-500 series of studies. 1) The efficacy of therapy is histology dependent: LB NHL is more effectively treated with the 10-drug LSA2L2 program than with the 4-drug COMP regimen, whereas for non-LB NHL the reverse is true. 2) Among the histologic subtypes of non-LB NHL (undifferentiated, pleomorphic, Burkitts, histiocytic, but excluding Burkitt's leukemia), the efficacy of COMP is similar. 3) The efficacy of chemotherapy is more dependent on stage in non-LB than in LB NHL. Localized non-LB NHL does not need more than six months of COMP, whereas 18 months of COMP is inadequate therapy for stage IV non-LB NHL with either marrow or CNS involvement. 4) In LB NHL, the optimal duration of therapy is more than 6 months, regardless of stage. 5) Regardless of histology, cranial irradiation is unnecessary for CNS prophylaxis if <25% blasts in the bone marrow and if intrathecal chemotherapy is administered during induction and maintenance. 6) With rare exception, the prognosis of recurrent NHL after either LSA2L2 or COMP therapy is extremely poor.

The CCG-123 lymphoma-leukemia study is evaluating the subgroup of patients who present with lymphomatous masses and >25% blast cells in the bone marrow, who may be regarded at diagnosis as having either NHL with extensive marrow involvement or leukemia with a lymphomatous pattern of bulk disease. As characterized by CCSG criteria, this interface between LB leukemia and NHL represents 15% of children with acute lymphoblastic leukemia. The primary therapeutic objectives of the study are to determine 1) whether this subgroup is best treated with one of two leukemia programs ("BFM" or "NY") or with CCSG's conventional LB lymphoma therapy (LSA2L2 chemotherapy and radiotherapy to bulk disease), and 2) whether cranial irradiation is necessary for optimum CNS prophylaxis.

D 029 ADVANCES IN BONE MARROW TRANSPLANTATION FOR TREATMENT OF LEUKEMIA. R. Champlin, M.D. Division Hematology/Oncology, UCLA Center for Health Sciences, Los Angeles, CA 90024

Bone marrow transplantation is an effective treatment for acute and chronic leukemias. Results are largely dependent on histocompatibility of donor and recipient, patient age and remission status of the leukemia. Bone marrow transplants are most successful from an HLA-identical sibling donor. 40-70% of good prognosis patients with AML in first remission, ALL in 1st or 2nd remission, CML in chronic phase and 20-30% of patients with more advanced disease have achieved prolonged disease free survival. Major problems are graft-versus-host disease (GVHD), interstitial pneumonitis and recurrent leukemia. There is little controversy that bone marrow transplantation is more effective than alternative therapies for CML or acute leukemia in $\geq$ second remission. There is controversy, however, whether patients in first remission should receive bone marrow transplants or conventional chemotherapy.

Graft-versus-host disease results from reactivity of T-lymphocytes present in the donor marrow against host tissues. There is considerable recent interest in depleting T-cells from the donor bone marrow as a means to prevent GVHD. This approach is successful in dramatically reducing the incidence and severity of GVHD but graft rejection and leukemia relapse are increased, possibly by abrogation of the allogeneic graft-versus-leukemia effect.

The rate of graft rejection and graft-versus-host disease increases with use of HLA-nonidentical donors and even with T-cell depletion techniques, results of HLA-haploidentical transplants have been relatively poor.

High dose chemotherapy, irradiation and bone marrow tranpslantation is an effective treatment for many forms of leukemia, producing a much greater antileukemic effect than alternative therapies. Bone marrow transplantation is associated with a high risk of complications which are fatal in 20-35% of patients. Improvement in treatment results requires more effective means to prevent the immunologic and infectious complications of transplantation as well as more effective pretransplant chemotherapy and irradiation regimens to reduce the risk of recurrent leukemia.

D 030 LEUKEMIA TREATMENT-1987, Robert Peter Gale, M.D., Ph.D., UCLA School of Medicine, Los Angeles, CA 90024.

In this lecture, I review the current status of the treatment of acute and chronic leukemia in humans. In some instances, such as acute lymphocytic leukemia (ALL) and acute myelogenous leukemia (AML), the therapeutic objective is curative. In other instances, such as chronic myelogenous leukemia (CML) and chronic lymphocytic leukemia (CLL), the objective is to prolong survival. In adult ALL and in AML, brief intensive therapy with cytotoxic drugs is the most effective approach. Prolonged maintenance chemotherapy using low doses of cytotoxic drugs and immunotherapy are not useful in most instances. The role of CNS prophylaxis is likewise limited. Achievement of remission typically results from temporary or permanent eradication of the leukemic clone with regrowth of normal hematopoesis. Attempts to achieve remission by inducing maturation of leukemic cells is usually not successful. Modern, intensive therapy results in 5-year leukemia-free survival (LFS) in 30-50% of adults with ALL and 25-35% of those with AML. When chemotherapy is combined with bone marrow transplantation, the cure rate can be increased by an additional 10-30%. In CML, the objective is to prolong duration of the chronic phase. This is typically attempted with cytotoxic drugs, or, recently, interferon. There are no convincing data that any of these approaches are successful in improving survival beyond a median of 4 years. Recently bone marrow transplantation in chronic phase CML has produced >50% LFS at 5 years. Therapy of CLL is usually palliative since only 10-40% of individuals die of their leukemia. Intensive treatment with cytotoxic drugs and radiation have not convincingly improved survival. The role of biologic agents such as interferon and monoclonal antibodies also appears limited. In summary, these data indicate substantial progress in the treatment of ALL, AML, and CML in the past decade. Most progress has been achieved with cytotoxic drugs or by transplantation but these may be near the limit of toxicity. New drugs and new approaches are required.

D 031 THERAPEUTIC DISSECTION OF THE BIOLOGIC HETEROGENEITY OF CHILDHOOD LEUKEMIAS AND LYMPHOMAS, Sharon B. Murphy, Joseph Mirro, Dorothy Williams, Susana Raimondi, A. Thomas Look, Geoffrey Kitchingman, David Kalwinsky, Fred Behm, Ching-Hon Pui, Paula Roberson, Stephen George, and Gaston K. Rivera, St. Jude Children's Research Hospital, Memphis, TN 38101.

Acute lymphoid leukemias (ALL) and diffuse high-grade aggressive non-Hodgkin's lymphomas (NHL) of childhood, once considered fairly homogeneous disorders, are now known to actually consist of quite distinct clinico-pathologic subtypes distinguished on the basis of their immunophenotype (lineage and level of differentiation), genetics (molecular and karyotypic), growth rate, patterns of tumoral infiltration, and typical total body burden and distribution of neoplastic cells. Leukemias and lymphomas are model neoplasms for studies of biology and therapy. Application of uniform therapies is curative in the majority of cases of ALL and NHL in children, yet reveals a range of therapeutic responsiveness useful for study of biologic determinants of response. Our combined studies of the biology and therapeutic responsiveness of lymphoid neoplasms have had dual aims: firstly, to optimize the risk: benefit ratio of modern multidrug therapy by adjustment of therapy to fit the initial clinical and biologic risk features present at diagnosis, and secondly, to use clonally expanded populations of neoplastic lymphoid cells as models for study of normal lymphoid differentiation and ontogeny. In our experience with treatment of over 250 pediatric NHL cases seen at our institution from 1975-1985, we have recognized significant differences in the relapse hazard related to the primary site, stage, and immunopathologic subtype. Successive improvement in outcome of treatments for NHL has resulted from adoption of treatment strategies which are both stage and histology specific, in particular distinguishing lymphoblastic (T-cell derived) from non-lymphoblastic (B-cell derived) in advanced stages III-IV. We have also analyzed over 650 ALL cases seen here from 1979 until the present, who were treated uniformly and studied with a coordinated morphologic, cytochemical, flow cytometric, karyotypic, and immunologic approach and we have concluded: 1) that the blast cell genetic features (ie, DNA content and presence or absence of translocations) are powerful independent prognostic factors which significantly add to a better definition of risk of treatment failure in ALL when combined with traditional clinical features (age, white blood cell count, race), and 2) that there is a remarkable and hitherto unappreciated degree of heterogeneity between and within neoplastic lymphoid cell populations, including mixed-lineage leukemias (20-25% of cases), clonal evolution, and even lineage switches (ALL --> ANLL).

Genetics

D 100 CYTOGENETIC ANALYSIS AND TRANSFORMING CAPACITY OF DNA IN MURINE MULTIPLE MYELOMA
M.H.C. Bakkus, Y.A. Punt, J.W. Croese, J. Radl, Th.W. van den Akker
Dept. Cell Biology, Immunology and Genetics, Erasmus University, Rotterdam, The Netherlands

To study the genetic alterations involved in the neoplastic transformation of plasma cells in multiple myeloma we investigated a unique model of spontaneous origin for this disease which is being offered in the aging C57BL/KaLwRij mouse. So far, we have established five stable in vivo mouse multiple myeloma lines. Typical myeloma cells are found in the bone marrow of these mice together with bone lesions. This in contrast with induced plasmacytomas which grow only locally without involvement of bone and bone marrow.

Cytogenetic studies of bone marrow cells from one multiple myeloma line (5T2) showed a triploid number of chromosomes and many chromosomal aberrations (60, XY, +1, $4q^+$, +6, mar7, mar8, mar8, +10, +14, mar15, +17, +18, +19, +X, + not fully identified markers: +m1 (2 copies), +m2, +m3, +m4 (4 copies), +m5 (4 copies)).

In order to identify possible transforming gene(s) in this murine myeloma model we transfected DNA from bone marrow of 5T2 and another multiple myeloma (5T14) bearing mice into NIH/3T3 cells. Transformed cells were obtained and injected into nude mice. Tumors arose within 3 weeks while mice injected with untransformed NIH/3T3 cells did not develop tumors within 4 weeks. Second transfection rounds with DNA from these tumors and Southern blot analysis with the different ras-probes are in progress.
Supported by the Netherlands Cancer Foundation ('Koningin Wilhelmina Fonds').

D 101 NOVEL TRANSFORMING SEQUENCES IN HUMAN LEUKEMIAS, Claus R. Bartram, Ada C.M. Steenvoorden and Johannes W.G. Janssen, University of Ulm, D-7900 Ulm, FRG.
DNAs of three AML cell lines (U937, HEL and ML-1) were tested in the tumorigenicity assay based on the cotransfection of NIH/3T3 cells into nude mice. All three cell lines were positive in the latter assay, but negative in the 3T3 focus assay. In five experiments two independent tumors were obtained from U937 DNA (U937 T_1 and U937 T_2). Southern blot analyses of DNAs from secondary and tertiary tumors showed human repetitive sequences. All secondary tumors derived from the same primary tumor DNA contained a common set of human alu-fragments. However, repetitive sequences differed among secondary tumors derived from all four different primary tumors (U937 T_1, U937 T_2, HEL T_1 and ML-1 T_2). DNAs from all four transforming genes exhibit no homology with c-Ha-ras, c-Ki-ras, N-ras, c-raf and c-fms sequences; moreover, U937 T_1 DNA lacks homology with v-rel, B-lym, c-myc, N-myc, c-src, v-src, v-fes, p53, c-myb, mcf-2, mcf-3, c-sis and met sequences.
We currently try to clone these putative oncogenes. To that end we screened a cosmid library prepared from DNA of a tertiary U937 T_1 tumor and obtained several alu-positive clones. Since none of these cosmid clones contained biologically active sequences as indicated in DNA transfection analyses, we presently clone cDNAs complementary to U937 T_1 mRNA.
Moreover, we will report on DNA transfection analyses of 10 primary juvenile AMLs. We observed activated Ki-ras (1/10) and N-ras (5/10) genes mainly due to point mutations affecting codon 12 and 61 as indicated by oligonucleotid hybridization analyses.

D 102 DETECTION OF C-REL RELATED TRANSCRIPTS IN MOUSE HEMATOPOIETIC TISSUES, FRACTIONATED LYMPHOCYTE POPULATIONS, AND CELL LINES, Elise Brownell[1], Bonnie Mathieson[2], Howard A. Young[3], Jonathan Keller[4] James N. Ihle[5], and Nancy R. Rice[1], [1]BRI-Basic Research Program Laboratory of Molecular Virology & Carcinogenesis, NCI-FCRF, [2]BRMP, Laboratory of Experimental Immunology, NCI-FCRF, [3] BRMP, Laboratory of Molecular Immunoregulation, NCI-FCRF, [4]Program Resources, Inc., Laboratory of Molecular Immunoregulation, NCI-FCRF, [5]BRI-Basic Research Program, Laboratory of Molecular Mechanisms of Carcinogenesis, NCI-FCRF, Frederick, MD. 21701
A portion of the human cellular homolog of v-rel, the transforming gene of the leukemogenic retrovirus reticuloendotheliosis virus, strain T, has been used to survey RNAs from several mouse tissues, selected lymphocyte populations, and hematopoietic cell lines for c-rel expression. Relatively high levels of a high molecular weight transcript are observed in peripheral B- and T-cells, while lower levels are detectable in functionally immature thymocytes. These results suggest that, unlike c-myb, the c-rel protooncogene plays a role in later stages of lymphocyte differentiation.
(Research sponsored by the National Cancer Institute, DHHS, under contract NO. NO1-CO-23909 with Bionetics Research, Inc.)

D 103 A NOVEL abl PROTEIN IS EXPRESSED IN PHILADELPHIA CHROMOSOME POSITIVE ACUTE LYMPHOBLASTIC LEUKAEMIA WHICH DOES NOT INVOLVE THE "BREAKPOINT CLUSTER REGION",
Li C. Chan, Kimmo K. Karhi, Sydonia I. Rayter*, John Groffen**, Melvyn F. Greaves and Leanne M. Wiedemann, Leukaemia Research Fund Centre at the Institute of Cancer Research, London SW3 6JB, U.K.; *National Institute of Medical Research, Mill Hill, London, U.K.; **Oncogene Sciences, Inc., Long Island, New York.
In chronic granulocytic leukaemia (CGL) with the Philadelphia (Ph) chromosome the proto-oncogene c-abl is translocated from chromosome 9 band q34 to a 5.8kb region on chromosome 22 band q11 designated the breakpoint cluster region (bcr). This leads to the formation of a fusion gene which transcribes an 8.7kb mRNA comprising sequences from the 5' region of bcr and 3' region of c-abl. The product of this hybrid message is an abnormal 210kDa protein (p210) with enhanced in vitro protein-tyrosine kinase (PTK) activity compared with the normal p145 c-abl protein. A proportion of acute lymphoblastic leukaemias (ALL) appear to mimic lymphoid blast crisis of CGL by possessing the t(9;22)(q34;q11) karyotypic marker and a similar immunophenotype, but the underlying molecular mechanisms appear to be variable, some cases having no bcr rearrangement. We have analysed the abl protein in Ph^+ ALL. In one patient with rearranged bcr (bcr^+), we observed a c-abl protein of M_r=210kDa with elevated PTK activity. In three other cases, however, a novel c-abl protein was detected (M_r=190kDa) that also has elevated in vitro PTK activity. This new abl protein does not appear to involve sequences derived from the bcr (bcr^-). Our results demonstrate that at least two distinct molecular mechanisms can result in the activation of the c-abl PTK activity.

D 104 HL-60 NUCLEAR PROTEINS WITH PREFERENTIAL BINDING TO DNA FRAGMENTS CONTAINING 5'-END PUTATIVE REGULATORY SEQUENCES OF C-MYC PROTO-ONCOGENES,
Robin H. Chou and Judy R. Churchill, Hahnemann University School of Medicine, Philadelphia, PA 19102.
The proto-oncogene c-myc is amplified in the human promyelocytic leukemia cell line, HL-60 where it expresses at high levels. When cells are induced to differentiate chemically, the gene is transcriptionally down regulated. We have identified myc gene sequence-specific DNA-binding proteins from HL-60. Nuclear proteins were separated by SDS-PAGE, blotted to nitrocellulose filters and probed with an equimolar mixture of [^{32}P]-labeled restriction fragments from a plasmid bearing an 8.2 kb insert of the human c-myc gene. Protein-bound DNA fragments were dissociated from the nitrocellulose and analyzed on agarose gels to detect preferential retention of individual fragments. A 25,000 Dalton protein (P25) demonstrated preferential retention of the 1.3 kb Cla I/Xho I DNA fragment from the immediate upstream region of the gene. Histone H1 and H1' preferentially retained the 1.3 kb fragment and a 1.05 kb Hind III/Cla I fragment which represents the adjacent 5' region of the c-myc gene. Each DNA fragment bears sequences expected to bind trans-acting regulatory factors either near DNase I hypersensitive sites or promoter sequences.

D 105 DIRECT EVIDENCE THAT MULTIPOTENT STEM CELLS ARE TARGETS FOR ABELSON VIRUS (A-MuLV).
Siu-Wah Chung[1], Peter MC Wong[2], Arthur W Nienhuis[2], Sandra Ruscetti[1]. [1]Lab of Genetics, NCI, and [2]Clinical Hematology Branch, NHLBI, NIH, Bethesda, MD 20892.
It has been shown that under a number of experimental conditions, A-MuLV can infect various hemopoietic cells, including pre-B lymphoid cells, mast cells, macrophages and erythroid cells. However, there is no direct evidence that the multipotent hemopoietic cells are involved. We thus generated helper free virus stock by transfecting P160 A-MuLV proviral DNA into ψ2 cells. It was then used to infect blast colonies known to contain high numbers of pluripotent and committed progenitors of high proliferative potential. A high correlation between the generation of cell lines containing the integrated P160 A-MuLV proviral DNA and the frequency of mixed clolony formation in replating strongly suggested that the primitive multipotential progenitors may be targets for A-MuLV. In another series of experiments, yolk sac cells as well as peripheral blood cells from 8 and 10 day gestation embryos were infected with A-MuLV and plated in a clonogenic methylcellulose assay in the absence of any exogenous growth factors such as interleukin 3 (IL3) and erythropoietin (Epo). No colonies were observed in cultures without viral infection whereas factor-independent colonies were consistantly observed with virus-infected cultures. Aside from the presence of pure erythroid and granulocyte-macrophage(GM) colonies, erythroid-mixed colonies consisting of erythroblasts as well as macrophages, granulocytes or mast cells could be observed. Individual colonies were then picked and carried in the presence or absence of an irradiated 3T3 feeder layers for 1-2 months. Continuous mast cell lines could be generated only from erythroid-mixed colonies in the presence of a feeder layer. These data indicate that primitive multipotent stem cells can be infected and transformed by A-MuLV.

D 106 ISOLATION AND ANALYSIS OF THE ACUTE MYELOGENOUS LEUKEMIA (AML) 3;3 TRANSLOCATION,
H.A. Drabkin[1,2], M.M. LeBeau[3], J.D. Rowley[3], J. Carrino[3] and M.O. Diaz[3]
[1]University of Colorado Medical Center; [2]Eleanor Roosevelt Institute for Cancer Research Denver Co. 80262; [3]University of Chicago Medical Center, Chicago, IL 60612.
AML cells containing either an inversion inv(3)(q21,q26), or a translocation, t(3;3)(q21;q26), involving the long arm of chromosome 3, is characteristically associated with a marked elevation in the platelet count. This suggests that a gene(s) located at either band q21 or q26 is altered by chromosome rearrangement and contributes to the malignant transformation. Two genes of potential interest, transferrin (TF) and the transferrin receptor (TFR) have been localized to bands 3q21 and 3q26 respectively. We have isolated both derivitive chromosomes in somatic cell hybrids. TF(3q21) DNA sequences are absent in the 3q-chromosome and present in the 3q+ chromosome with no evidence of rearrangement. Some DNA sequences homologous to the 3' untranslated portion of the TFR are absent in the 3q- chromosome and present in the 3q+ chromosome, although no rearrangement has been detected. The relevance of this finding is not clear at the present time. Pulsed-field experiments are underway to further characterize this translocation.

D 107 ALTERED EXPRESSION OF TRANSPLANTED LEUKEMIA IN FISCHER RATS PRETREATED WITH PYRIDINE (PYR). J.E. FRENCH, M.P. DIETER, AND S.A.STEFANSKI. NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SCIENCES, RESEARCH TRIANGLE PARK, NC 27709 A transplant model for F344 rat mononuclear cell leukemia in young rats demonstrated expression of this tumor in 3 months that usually occurs after 18 - 24 months of age in 30 - 35% of the rats. We have studied the potential of this short term test model to detect chemical effects on the development of F344 rat leukemia. Male F344 rats were given 0, 0.5 or 1.0 mg/ml PYR in drinking water for 90 days. One-half then received leukemic spleen mononuclear cells (2 x 10E7 cells/rat, SC) from syngeneic donors. Sixty-five days after transplantation the effects of 155 days of PYR treatment on the expression of leukemia were evaluated. PYR alone was toxic at these dose levels as indicated by increases in spleen and liver to body weight ratios (S or L/BW) and changes in hematology and serum chemistry relative to controls without leukemia. After 65 days post-transplantation the S/BW and L/BW were greater in the PYR treated leukemia recipients than the untreated leukemia recipients. Changes in hematology and serum chemistry indicative of leukemia progression were greater in PYR treated leukemia recipients than either leukemia recipients not treated with PYR or the PYR treated animals without leukemia. Evaluation of selected tumor cell marker enzymes in spleen and blood mononuclear cells also verified that PYR exacerbated the pattern of biochemical responses indicative of leukemia progression. These indices, diagnostic in this tumor transplant model for a pre-leukemic state, were confirmed by histopathological examination. Data for the chemical effects of 2-ethoxyethanol on tumor regression are being presented jointly for comparison.

D 108 A REARRANGED bcr IN A Ph' NEGATIVE CML PATIENT WITH A COMPLEX TRANSLOCATION. Hans Grünwald, Alan Shanske, Perry Cook, Nora Heisterkamp and John Groffen, Queens Hospital Center Affil. of Long Island Jewish Medical Center, Jamaica, New York 11432 and Oncogene Science, Inc., Mineola, New York 11501.
Recent evidence indicates that the break of chromosome 9 at q24 may be the critical event in the evolution of CML, and its functional significance is related to the translocation of the c-abl oncogene from 9q to 22q. We recently found a complex translocation involving 9, 12 and 15 in a 42 year-old Haitian male with clinical findings of CML. Complex translocations have been shown to result in masking of the Ph' chromosome. In order to test this hypothesis in our patient, a mixture of two bcr-specific DNA probes were used for Southern blot analysis. Both of these have been previously used to detect rearrangements in bcr specific for the Ph' translocation (Groffen et al, 1984). High molecular weight DNA was digested with the restriction enzymes Bgl II, Bam HI and Hind III. The Bgl II digestion revealed the presence of 2 abnormal fragments of 3.9 and 3.0 kb and one Bam HI fragment of 15 kb was found. No abnormal fragments were visible upon digestion with Hind III, and a relatively weakly hybridizing normal Bam HI fragment of 3.2 kb was visible. These data tentatively locate a breakpoint in region 2 of bcr. The identification of this breakpoint confirms our hypothesis that a Ph' translocation occurred in the leukemic cells of our patient. A second translocation involving chromosomes 12 and 15 has hidden the effects of this translocation. Combined cytogenetic and molecular investigation establish the karyotype as 46, XY, t(9;12;15;22)(q34;q12;q21;q11).

D 109 METHYLATION OF DNA CONTAINING FRAUDULENT AND MISMATCHED NUCLEOTIDES, Thomas A. Hardy, David J. Baker and Steven S. Smith, Divisions of Surgery and Biology, City of Hope National Medical Center, Duarte CA 91010.

Heteroduplex M13 DNA in which 5-bromodeoxyuridine (BrdUrd) is uniformly substituted for dThd in the minus strand is selectively methylated on the plus strand by human DNA (cytosine-5-)methyltransferase. Oligodeoxynucleotide heteroduplex DNA in which dThd is mismatched with dGuo in a d(pCG) pair is preferred by the enzyme over the fully complementary duplex. The data suggest that BrdUrd and dThd promote de novo methylation of d(pCG) dimers when mismatched with dGuo residues at these sites. As a consequence, mismatches of this type may induce the clonal silencing of genes by DNA methylation. Silencing of the thymidine kinase locus by DNA methylation in response to BrdUrd exposure is well known. BrdUrd • dGuo mismatch induced methylation may provide an epigenetic mechanism for the genesis of this form of resistance to the drug. In addition, our results suggest a new role for mammalian DNA methylation patterns in the formation of a clonally inheritable record of mismatch copying errors and certain other forms of DNA damage.

D 110 THE ROLE OF THE c-abl ONCOGENE IN PHILADELPHIA CHROMOSOME POSITIVE CHRONIC MYELOGENOUS LEUKEMIA, André B.C. Hermans, Annelies de Klein, Gerard C. Grosveld, Dirk Bootsma, Nora Heisterkamp[a], Kees Stam[a] and John Groffen, Dept. of Cell Biology and Genetics, Erasmus University Rotterdam, The Netherlands, (a) Oncogene Science Inc., Minola, N.Y. U.S.A.

Chronic myelogenous leukemia (CML) is characterized by the presence of the Philadelphia (Ph^1) chromosome in the leukemic cells of 96% of all CML patients. The Ph^1 chromosome (22q-) is the result of a reciprocal translocation between chromosome 22 and chromosome 9, t(9q34,22q11). Previously we described the localization of the human c-abl oncogene on chromosome 9 and demonstrated its translocation to the Ph^1 chromosome in CML patients. The cloning and analysis of breakpoint fragments revealed that the breakpoints on chromosome 22 all cluster in a very limited area, the breakpoint cluster region, bcr. Breakpoints on chromosome 9, however, are scattered over a large area which may vary from zero to more as 100 kb upstream of the v-abl homologous sequences of the c-abl gene. A unique 8.5 kb chimeric bcr-abl RNA is detected in the leukemic cells of all CML patients. Cloning of chimeric c-DNA's (5' bcr and 3' abl) from a CML derived cell line strongly indicates that bcr and c-abl coding sequences are linked in frame by RNA splicing, independent from the highly variable distance between these two genes on the Ph^1 chromosome. The specific presence of the chimeric bcr-abl RNA (and protein) in CML cells suggests the involvement of this hybrid product in the development of CML. Recent cloning of full-length hybrid c-DNA's will help to test this hypothesis.

D 111 CYTOGENETIC AND MOLECULAR CHARACTERIZATION OF TUMORS IN NUDE MICE DERIVED FROM MULTIDRUG (MDR) RESISTANT HUMAN LEUKEMIA CELL LINES, Anna B. Hill, William T. Beck* and Jeffrey M. Trent, Department of Radiation Oncology, Cancer Center Division, University of Arizona, Tucson AZ 85724; *Department of Biochemical and Clinical Pharmacology, St. Jude Children's Research Hospital, Memphis TN 38101

In this report, we have evaluated whether CEM/VLB100 cells which express the MDR phenotype (via amplification of the P-glycoprotein gene) retain their tumorigenicity. CEM/VLB100 has been shown to have a 10-fold amplification of the P-glycoprotein gene. *In situ* hybridization to metaphase chromosomes localized the amplified sequences to an abnormal banding region (ABR). In order to assess the relationship (if any) of the acquisition of the MDR phenotype to tumorigenicity, 10^7 cells/mouse were injected from velban sensitive and resistant CEM cells. The results showed that 4/4 drug sensitive and 4/5 drug resistant cell lines formed tumors in nude mice within 5 or 6 weeks. Cells were then isolated from tumors derived from the drug resistant and drug sensitive cells and examined for P-glycoprotein gene amplification and tumor karyotype. None of the 4 sensitive tumors demonstrated the amplification of P-glycoprotein (analyzed by Southern blot) or the presence of the ABR marker chromosome. In contrast, 3/4 drug resistant tumors contained both amplified P-glycoprotein genes and the ABR marker chromosome. The remaining drug resistant tumor lacked both the P-glycoprotein gene amplification and the ABR marker chromosome. These studies show conclusively that overexpression of P-glycoprotein does not alter tumorigenicity in CEM leukemia cells. A.B.H. (Special Fellow) and J.M.T. (Scholar) are supported by the Leukemia Society of America.

D 112 DNA SEGMENT CONTAINING $C\beta_1$ GENE OF T-CELL RECEPTOR β CHAIN, NORMALLY DELETED IN THE PROCESS OF $D\beta_1$-$J\beta_2$ JOINING, WAS INSERTED INTO CHROMOSOME 6 IN A HUMAN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA CELL, M.Hirano[1], T.Ino[1,3], M.Yoshida[2] and Y.Kurosawa[3], Dept of Med[1] and Institute of Comprehensive Medical Science, Fujita-Gakuen Health Univ, School of Med, Toyoake, Aichi, and Dept of Science[2], Hokkaido Univ, Sapporo, Japan.

DNA rearrangements in T cell receptor β chain gene loci were examined in human acute T-cell lymphoblastic leukemia cells. Dβ gene, Jβ gene cluster and Cβ gene are tandem duplicated on chromosome 7. It was observed that in leukemia cells from a patient, $D\beta_1$-$J\beta_{2\cdot3}$ joining occurred on one chromosome and the other chromosome kept germline configuration. If Dβ-Jβ joining occurred by deletion mechanism, $C\beta_1$ gene which is located between $D\beta_1$ and $J\beta_{2\cdot3}$ had to disappear after DNA rearrangement. In the cells, however, the $C\beta_1$ gene was detected as a rearranged band. This $C\beta_1$ gene was inserted into chromosome 6. This indicates the presence of a new pathway to induce non-regulated expression of an oncogene in lymphocytes.

D 113 ISOLATION AND CHARACTERIZATION OF PHOSPHOTYROSYL PROTEINS FROM CHRONIC MYELOGENOUS LEUKEMIA CELL LINES AND PERIPHERAL BLOOD LEUKOCYTES, Richard D. Huhn and A. Raymond Frackelton, Roger Williams Cancer Center, Brown University, Providence, RI 02908.
The tyrosine kinase-encoding *abl* oncogene is translocated from chromosome 9 to chromosome 22 in Philadelphia chromosome-positive (Ph[1]) chronic myelogenous leukemia (CML) cells. The 3'-*abl* sequence becomes fused to the 5'-sequence of the *bcr* gene of chromosome 22, resulting in the synthesis of a chimeric 210 kd *bcr-abl* phosphoprotein ($P210^{bcr\text{-}abl}$). The constitutive tyrosine kinase activity of $P210^{bcr\text{-}abl}$ is thought to be involved in the pathogenesis of CML, but physiologically relevant substrates of the *abl* kinase are as yet unknown. To address this problem, we have utilized a highly specific monoclonal antibody to phosphotyrosine to isolate and affinity-purify phosphotyrosyl proteins from the CML cell lines RWLeu4, K562, and BV173 and from peripheral blood leukocytes of a patient in the acute phase of CML. In addition to the tyrosine-phosphorylated $P210^{bcr\text{-}abl}$, prominent phosphotyrosyl proteins of M_r 185 kd, 150 kd, 120 kd, 105 kd, 63 kd, 56 kd, 36 kd, and 32 kd, as well as several less prominent proteins, were isolated from Ph[1]-positive CML cell lines but not Ph[1]-negative control cell lines. Despite the expected genetic diversity of the patients from whom the cell lines were derived, the electrophoretic patterns of the isolated phosphotyrosyl proteins were essentially identical among cell lines. With but one exception, proteolytic peptide mapping confirmed that proteins of the same molecular weights were identical among cell lines. Most of the same proteins were also seen in peripheral blood leukocytes obtained directly from a patient in the acute phase of CML, demonstrating that they are actually part of the neoplastic phenotype in this disease.

D 114 GENETICALLY DETERMINED RESISTANCE TO MCA-INDUCED T CELL LYMPHOMA IS EXPRESSED AT THE LEVEL OF BONE MARROW-DERIVED CELLS. Sally T. Ishizaka and Frank Lilly, Albert Einstein College of Medicine, Bronx NY 10461
Inbred mouse strains differ in the frequency with which they develop T cell lymphomas after exposure to 3-methylcholanthrene (MCA). In strains which are arylhydrocarbon hydroxylase noninducible, and hence do not develop skin tumors after cutaneous application of MCA, lymphoma susceptibility ranges from 20% for the ST/bN strain to 90% for the RF/J strain. Genetic studies show that lymphoma resistance is due to a single dominant gene.

The studies described here were designed to determine whether susceptibility and resistance are inherent characteristics of bone marrow-derived cells, or whether either can be conferred on marrow cells by the environment in which they mature. Reciprocal bone marrow transfers were done between (ST x RF) F_1 (resistant) and RF (susceptible) mice. Chimeras are designated by placing the name of the marrow donor strain before an arrow and the name of the recipient strain after it, e.g. (ST x RF)→ RF. RF→ (ST x RF) mice, like both normal RF and syngeneic RF→ RF chimeras showed a lymphoma incidence of around 90% after MCA treatment. In contrast, lymphoma incidences in (ST x RF), (ST x RF)→ (ST x RF), and (ST x RF)→ RF lay in the 30-50% range. Pure strain ST/bN marrow also showed a low lymphoma incidence (7-15%) whether developing in a normal unmanipulated animal, or an RF recipient. Immunofluorescence typing of thymuses and lymphomas from chimeric animals indicated that 80% were composed of cells derived from donor marrow. We conclude that lymphoma resistance is a trait of marrow-derived cells, and that stromal cells cannot influence lymphoma incidence in this system.

D 115 CHARACTERIZATION OF cDNA AND GENOMIC CLONES FOR HUMAN MYELOPEROXIDASE, Keith R. Johnson, William M. Nauseef, Margaret J. Wheelock, Alessandra Care, H. Phillip Koeffler*, Michael Selsted*, Carl Miller* and Giovanni Rovera, Wistar Institute, Philadelphia, PA 19104 and *UCLA School of Medicine, Los Angeles, CA 90024.

Myeloperoxidase (MPO) is an enzyme synthesized by cells of the myelomonocytic lineage with a window of expression from the promyelocyte to the granulocyte. The mature enzyme is stored in the primary granules of neutrophils. HL-60 acute promyelocytic leukemia cells have been shown to express MPO. Utilizing a specific antiserum, we have isolated cDNA clones for MPO from an HL-60 expression library. Hybridization-selection experiments verified the identity of the clones. This was further confirmed by sequencing data that identified the N-terminal protein sequences of both the heavy and light subunits of mature MPO. The light subunit is coded by sequences 5' of those coding for the heavy subunit. At least two mRNAs for MPO (4.0 and 3.3 kb) are evident in both HL-60 cells and normal human bone marrow. We have isolated two classes of cDNAs (3.3 and 3.1 kb are the longest clones) that are very similar over their 3' ends but differ at their 5' ends. These cDNAs probably represent both types of mRNA and suggest that alternative splicing is involved in the generation of the messages. We have isolated genomic clones that hybridize to both 5' and 3' cDNA probes and suggest that the MPO gene spans 15-20 kb of DNA. Sequencing of the cDNA clones and additional characterization of the genomic clones will be presented.

D 116 USE OF Q-BAND HETEROMORPHISMS AND IN SITU HYBRIDIZATION WITH Y-SPECIFIC DNA IN STUDIES TO MONITOR ENGRAFTMENT IN PATIENTS WITH AML RECEIVING MARROW FROM LIKE AND UNLIKE SEX DONORS, M.T. Khokhar, Dept of Genetics, I.C.R. Royal Marsden Hospital, Sutton, Surrey, UK

Bone marrow transplant in (BMT) is becoming an effective treatment for leukaemia under specific conditions. Documentation of both the dividing and non-dividing cells is important to monitor graft establishment. When the donor and patient were of like sex, autosomal heteromorphisms were found useful in 85% of cases treated at the Leukaemia Unit of the Royal Marsden Hospital. Among five cases followed for up to a year BMT using these heteromorphisms donor metaphases could be detected in the marrow within two weeks of BMT when the graft was successful. Chimerism was dectected in lymphocytes even when the graft persisted.

In cases of graft failure donor cells did not persist in marrow and the lymphocyte population did not convert to donor type. These studies confirm results of a previous study using sex chromosomes as cellular marker (Lawler et. al. 1984). In cases where the donor and patient were of unlike sex, in situ hybridization with a Y-specific DNA probe was used to detect origin of the non-dividing cells.

D 117 MOLECULAR HETEROGENEITY OF ADULT PH+ ALL. D. Leibowitz, K. Schaefer-Rego, Z.Arlin, L. Shapiro, J.G. Mears, A. Bank. Department of Medicine, Columbia University College of Physicians and Surgeons, N.Y., N.Y., and Division of Neoplastic Diseases, New York Medical College, Valhalla,N.Y.

The 9,22 translocation creating the Ph chromosome (ch) combines sequence from the abl oncogene on ch 9 with sequence from the bcr gene on ch 22 to generate a new fusion gene. In patients with CML the ch 22 translocation breakpoint characteristically lies within the 5.8kb breakpoint cluster region (bcr). Up to 20% of adult patients who present with ALL are subsequently found to have a Ph ch, and some of these patients may have had CML, with a clinically silent chronic phase. Restriction analysis and Southern blotting has been performed on 9 adults with Ph+ ALL. Only 5 of the 9 Ph+ patients had a breakpoint within the bcr. The fact that 4 of the 9 Ph+ ALL patients do not have evidence of a bcr rearrangement suggests that the Ph ch may play a different role in these ALL patients when compared to CML patients. It is possible that the leukemogenic process in these patients does not include activation of the abl oncogene on the Ph ch. The Ph ch in these and other ALL patients may either indicate a different activation of the abl oncogene, or a process not involving abl or bcr. These data suggest that Ph+ ALL is a heterogeneous disease at the DNA level, and that this heterogeneity may correlate with different clinical characteristics.

D 118 ONCOGENES AND CHROMOSOME ABERRATIONS IN K-562 SUBLINES, C.B. Lozzio, D. Johnson, E. Bamberger, University of Tennessee Memorial Research Center, Knoxville, TN 37920
The K-562 stem cell leukemia cell line was established at our laboratory in 1970, and has been maintained in cultures for 830 passages. The cells were hypodiploid until passage 60 and have been near triploid for the next 770 passages. At passage 798 the original line had 18 chromosome markers resulting from breaks at the locus of 7 oncogenes, 3 growth factors, 12 fragile sites and 14 breakpoints of other neoplasias. Many sublines derived from samples of cells frozen at various passages have also undergone clonal evolution. The "de novo" markers which characterize each subline involved breaks at similar locus. Four markers have been present in all sublines. One of them is a complex Ph^1 chromosome M_2 with 5 duplicated bands. The M_3 marker with four bands was derived from M_2 in the near triploid cells. Amplified c-abl and the lambda genes were localized in the M_3 marker by Seldon et al (Proc. Natl. Acad. Sci. USA 80:7289,1983) Using "in situ" hybridization we have localized c-abl, c-sis and bcr in both M_2 and M_3. Neither the original line nor the sublines of K-562 have a 9q+ marker. Thus, M_2 may be a retranslocation of 9q+ with c-sis to the 22q-Ph^1 and the c-abl already translocated to the bcr region became close to c-sis. The "de novo" marker M_7 is a t(1;18) (p22;21) in which N-ras is translocated to the region of the YES1 and the BCL2 locus. Other "de novo" translocations including a break at 1q21 involves the SKI oncogene, the adenovirus 12 modification site 3, and the break of malignant lymphomas. It is concluded that oncogene activation at the site of chromosomal translocation plays an important roll in tumor progression and in explaining differences among sublines derived from the same original line.

D 119 QUANTITATION OF AMPLIFIED ONCOGENES FROM TUMOR CELL LINES BY A SANDWICH HYBRIDIZATION TECHNIQUE. Kenneth Lundström, Jukka Tenhunen, Marjut Ranki and Hans Söderlund, Orion Genetic Engineering Laboratory, Valimotie 7, 00380 Helsinki, Finland and Kari Alitalo, Department of Virology, Helsinki, Finland.
The aggressiveness of tumors is suggested to be related to the degree of genomic amplification of certain oncogenes. Detection and quantitation of specific oncogene amplifications can therefore be of clinical importance. We have applied the sandwich hybridization technique to detect and quantitate the amplification of oncogenes in human tumor cell lines. The method is based on the use of two non-overlapping but adjacent DNA regions derived from the gene to be identified. The method is rapid and sensitive. Biopsy specimens can be directly assayed.
We have used the second exon of the human N-myc gene and its flanking sequences to quantitate its amplification in human tumor cell lines. Increased levels of N-myc was measured by comparing to the DNA amount of the human α-2 (I) collagen gene, which is known to exist stably as a single copy in the human genome. At present attention is directed to the use of the technique to measure the level of oncogene mRNA and its correlation to DNA amplification.

D 120 MOLECULAR ANALYSIS OF THE TRANSLOCATION 14;19 IN CHRONIC LYMPHOCYTIC LEUKEMIA, Timothy W. McKeithan, Manuel O. Diaz, Michelle M. Le Beau, and Janet D. Rowley, Department of Medicine, University of Chicago, Chicago IL 60637.
Our laboratory has recently identified the t(14;19)(q32;q13) as a recurring abnormality in chronic lymphocytic leukemia. The breakpoint in chromosome 14 occurs at the site of the immunoglobulin heavy chain (IGH) locus; no oncogenes have so far been localized to the site of the breakpoint on chromosome 19. Using Southern blot analysis and genomic cloning in bacteriophage lambda, we have analyzed DNA from two cases with this translocation. In both cases, the translocation was part of a three-way translocation with loss of one of the derivative chromosomes, containing the distal end of chromosome 14. By Southern blot analysis using probes for the IGH locus, both cases showed a number of DNA rearrangements and deletions; some of these rearrangements presumably were due to the normal DNA splicing events which B-cells undergo and others to the translocation. One case showed extensive deletions, including both alleles of the μ constant region (Cμ), one of the two alleles of J_H, all but one of the ten Cγ alleles (two alleles of four gene segments and one pseudogene), and probably two of the four Cα alleles (two Cα1's and two Cα2's); one rearranged Cα, a rearrranged Cγ, and one unrearranged Cα2 remain. Both the rearranged C α and the rearranged Cγ were cloned. From the Cγ clone, a HindIII band of abnormal size was identified. This band was subcloned (pγ5.5H) and used as a probe in in situ hybridization to normal metaphase chromosomes. Significant labeling was found on both chromosomes 14 and 19, suggesting that pγ5.5H contains the breakpoint junction of the t(14;19) translocation. Confirmation of this result by Southern blot analysis of appropriate somatic cell hybrids is pending.

D 121 ANALYSIS OF THE APL BREAKPOINT REGION ON CHROMOSOME 17.
G Moore, W Xu, J Shipley, S Rider, D Sheer, E Solomon.
Imperial Cancer Research Fund, Lincoln's Inn Field, London, England.
Leukaemic cells from patients with acute promyelocytic leukaemia (M3) contain a reciprocal translocation between the long arms of chromosome 15 and 17, t(15:17) (q22; q12-21). Sequences present at the breakpoints need to be identified as they may be involved in promyelocyte transformation. Unlike some other leukaemias in which breakpoints have been localised within known genes (i.e. the oncogene c-abl in chronic myeloid leukaemia), no known genes or random probes map the breakpoint in APL. However in APL two oncogenes c-erbAl and c-neu have been mapped in the region above the breakpoint on chromosome 17. It is not clear whether these oncogenes are involved in cell transformation. We have approached the problem by screening for probes which map in the region of the breakpoint on chromosome 17. We have isolated a cosmid containing sequences homologous to c-erbA which maps in the region below the breakpoint and also contains a sequence which is repeated in the breakpoint region. We have mapped such a probe onto short fragments of chromosome 17 generated by chromosome mediated transfer, and compared them to a hybrid containing 15q+, in order to obtain the shortest chromosome fragment still containing the breakpoint region. This type of fragment is currently being used to further identify the gene present at the breakpoint.

D 122 IN VIVO EXPRESSION OF A NON SELECTED GENE TRANSFERRED INTO MURINE HEMOTOPOIETIC STEM CELLS BY ELECTROPORATION. Ramaswamy Narayanan, Malgorzata M. Jastreboff, Chang-Fang Chiu, Joseph R. Bertino. Department of Pharmacology, Yale University School of Medicine, New Haven, Connecticut

The successful introduction of DNA into mouse bone marrow cells by electric-field mediated transfer (electroporation) was demonstrated by detection of transient CAT (chloramphenicol acetyl transferase EC23.1.28) activity in marrow cell extracts, electroporated with RSVCAT plasmid. When the bone marrow cells were cotransfected with RSVCAT and SV_2NEO plasmids (1:1 molar ratio) by electroporation, CAT activity was detected in extracts of granulocyte-macrophage colonies (CFU_{GM}) resistant to G-418, an analogue of neomycin. The G-418 resistant CFU_{GM} showed expression of the NEO gene and were morphologically indistinguishable from the control bone marrow CFU_{GM}. RSVCAT electroporated bone marrow cells were injected into the tail vein of lethally irradiated mice and fourteen days later, mice were sacrificed. Extracts from hematopoietic tissues, but not non-hematopoietic tissues, showed CAT expression. Integrated sequences of RSVCAT were detected in the hematopoietic tissues. Electroporation offers the opportunity to transfer genes effectively into hematopoietic progenitor cells and avoids some of the inherent disadvantages associated with other methods of gene transfer.

D 123 Nucleotide Sequence Analysis of Mouse Testis-Derived c-abl Complementary DNA Clones.
Cristina Oppi and E. Premkumar Reddy, Department of Molecular Oncology, Roche Institute of Molecular Biology, Nutley, NJ 07110, U.S.A.
V-abl is the oncogene of the Abelson murine leukemia virus (A-MuLV) which induces B cell lymphomas in vivo and transforms both lymphoid and fibroblastic cells in vitro. C-abl, the normal cellular homolog of v-abl, is expressed in most murine cell types as two distinct mRNA species of 6.5 kb and 5.5 kb. Ben-Neriah et al. (Cell 44:577-586, 1986) have recently demonstrated that these mRNAs are generated by differential splicing of their 5' exons. Higher levels of c-abl expression are found in the mouse testis where there is an additional post-meiosis-specific mRNA species which is 4 kb long. To understand the mechanism by which the testis-specific mRNA is generated, we have undertaken the cDNA cloning of c-abl mRNA found in mouse testis. A total of 14 cDNA clones were examined. One of these cDNA clones, which was apparently derived from the 4.0 kb mRNA, was subjected to nucleotide sequence analysis which revealed that the 5' end of this DNA has an identical sequence as that of type 1 cDNA described by Ben-Neriah et al. However, this cDNA lacked approximately 1.1 kb of the 3' sequence present in the longer mRNAs. Polyadenylation of the mRNA was found to occur in an unusual position which generates the truncated mRNA. It, therefore, appears that heterogeneity in c-abl mRNA could be generated by differential splicing and/or transcriptional termination at the 5' and 3' ends.

D 124 EXPRESSION OF bcl-2 PROTO-ONCOGENE DURING NORMAL HUMAN LYMPHOCYTE PROLIFERATION. J. Reed, Y. Tsujimoto*, J. Alpers, C. Croce*, P. Nowell. Dept. Pathol., Univ. of Penna.; *Wistar Institute, Phila. PA

Chromosome translocations in common B cell neoplasms bring the bcl-2 and c-myc genes under control of the Ig gene locus, resulting in high levels of constitutive expression. To understand the factors controlling expression of these proto-oncogenes, we examined expression of bcl-2 and c-myc in normal human peripheral blood or tonsil lymphocytes fractionated into purified T and B cell populations. Stimulation with mitogens (PHA or *Staph. aureus*) showed that steady-state levels of accumulated mRNAs for c-myc, bcl-2, and histones rose from undetectable to maximal within 1, 8, and 48 hr respectively; levels of constitutively produced mRNA for c-fgr were unchanged. Nuclear transcription assays showed marked elevations in bcl-2 transcription rate, peaking 6 hr post-stimulation; only slight increases in c-myc transcription were seen (max. at 1 hr), suggesting its expression is regulated mainly by post-transcriptional mechanisms. Because cycloheximide (CHX) augmented c-myc and bcl-2 mRNA levels, we determined bcl-2 and c-myc mRNA degradation rates in the presence or absence of CHX. Stability of c-myc mRNA increased markedly in CHX-treated cells, but little effect was seen for bcl-2 and none for c-fgr. Our data show marked differences in the mechanisms regulating bcl-2 and c-myc genes, and suggest a role for these proto-oncogenes in the control of normal lymphocyte growth.

D 125 EXPRESSION OF p53 IN HUMAN LEUKEMIA AND LYMPHOMA. V. Rotter and M. Prokocimer, Department of Cell Biology, The Weizmann Institute of Science, Rehovot, 76100, Israel.

Analysis of fresh human tumors have indicated that patients with B type lymphoproliferative diseases and the majority of patients with acute lymphoblastic leukemia (ALL) express elevated levels of p53 production. It is suggested that in these human malignancies, p53 may provide a novel tool for monitoring cancer activity. Conversely, p53 is not expressed in acute myeloid leukemias, myeloproliferative diseases, or myeloid leukemic cell lines. Analysis of the p53 gene structure indicated the existence of similar patterns of p53 restriction fragments in producer and nonproducer cells, which suggests that the p53 gene is not altered in the latter. However, in one case of acute promyelocytic leukemia (APL), we have observed a rearrangement in the p53 gene. Karyotype analysis has indicated that these APL cells do not contain the typical 15:17 translocation. In other APL patients who exhibit a 15:17 translocation we found no genomic changes of the p53, suggesting that the p53 gene, which was recently mapped to the short arm of chromosome 17 in the human, is not structurally related to the typical chromosomal break point found in the long arm of chromosome 17 or APL patients.

D 126 DELETION OF 5'-CODING SEQUENCES OF THE CELLULAR p53 GENE IN MOUSE ERYTHROLEUKEMIA: A NOVEL MECHANISM OF ONCOGENE REGULATION, Benjamin Rovinski[1], Donald Munroe[1], Jim Peacock[1], Michael Mowat[2], Alan Bernstein[3] and Samuel Benchimol[1], The Ontario Cancer Institute, Toronto, Ontario, M4X 1K9, Canada[1], Manitoba Institute of Cell Biology, Winnipeg, Manitoba, R3E 0V9, Canada[2], and Mount Sinai Research Hospital, Toronto, Ontario, M5G 1X5, Canada[3].

A rearrangement of the p53 gene in an erythroleukemic cell line (DP15-2) transformed by Friend retrovirus resulted in expression of a 44,000 dalton protein (p44). Epitope mapping studies indicated that amino acid residues at the extreme amino-terminus of p53 are missing in p44. In addition, p44 appears to have undergone a change in conformation since it is recognized by PAb246, a monoclonal antibody that does not recognize p53 in other erythroleukemic cells. PAb246 may recognize a conformation-dependent epitope on p53. A quantitative radioimmunological phase assay indicated that the amount of p44 was ≈ 100-fold higher in DP15-2 cells than the amount of p53 in other erythroleukemic cells. Pulse chase studies demonstrated that p53 has a half-life ($t_{1/2}$) of ≈ 2 hr in erythroleukemic cells; in contrast, p44 has a $t_{1/2} >$ 9.5 hr. Hence, the increased stability of p44 may account for its elevated steady-state levels in DP15-2 cells. Southern blot analysis and genomic cloning revealed that DP15-2 cells contain a ≈ 3-kb deletion of nucleotide sequences in the p53 gene that removes exon 2 coding sequences. In summary, these results demonstrate that deletion of 5'-coding sequences in the p53 gene provides a novel mechanism for increasing p53 protein levels *in vivo*.

D 127 MOLECULAR HETEROGENEITY OF CML IN ITALIAN POPULATION.
Giuseppe Saglio 'Angela Tassinari, 'Alfonso Zaccaria, 'Bomina Celso, 'Nicoletta Testoni, 'Sante Tura, Giovanna Rege Cambrin, Luigi Pegoraro, GianCarlo Avanzi, Anna Serra, Umberto Mazza and Felice Gavosto. Dip. di Scienze Biomediche e Oncologia Umana, Universita' di Torino, Torino, Italy - 'Istituto di Ematologia "L&A Seragnoli" Universita' di Bologna, Bologna, Italy.

A rearrangement between the bcr and the c-abl genes has been established to represent the molecular lesion caused by the Philadelphia chromosome (Ph') translocation t(9;22) in CML. While the breakpoint position on chromosome 9 is quite variable, mapping over more than 150 Kb, on chromosome 22 the rearrangement involves a restricted area within a Bgl II DNA fragment of 5 Kb, easely detectable by Southern analysis. The molecular study of a large series of CML patients of Italian origin has revealed that some exceptions to this general rule may exist: sporadic cases of classical Ph' + CML are lacking a bcr rearrangement in spite of the clear presence of a Ph' chromosome and others are showing a variant bcr rearrangement with a breakpoint position more 5' within the bcr gene than that usually present in CML. The same spectrum of molecular defects has been found in Ph + acute leukemias, but in a much higher percentage of cases.

D 128 CLONAL SILENCING OF GENES BY ERROR-INDUCED DNA METHYLATION AT d(pCG) SITES,
Steven S. Smith, Divisions of Surgery and Biology, City of Hope National Medical Center, Duarte CA 91010.

Several lines of evidence suggest that the heritible methylation of cytosine at d(pCG) dimers in DNA is induced by errors in DNA replication and by certain forms of damage to DNA. Other evidence suggests that the accumulation of methylation in response to this damage will result in the clonal silencing of affected genes. These findings suggest a new and unique role for DNA methylation in the clonal shut-off of genes that have become extensively damaged and whose expression is thus potentially deleterious. Organisms whose complexity and life cycle require the extensive somatic cell division of a large genome are most likely to require a damage control mechanism of this type. This mechanism may be of particular relevance in carcinogenesis. Error-induced methylation of activated (i.e. extensively damaged) cellular oncogenes may provide one means by which these deleterious genes are held in check in affected somatic cells. This suggestion is consistent with the capacity of DNA methylation to silence the proviral DNA of certain oncogenic retroviruses, with the capacity of certain tumor promoters and ultimate carcinogens to inhibit DNA methylation, and with the widely documented hypomethylation of tumor DNA relative to normal DNA. Some of the implications of this role for DNA methylation in the molecular biology of longevity, carcinogenesis and development will be discussed.

D 129 DIRECT PARTICIPATION OF c-myc PROTEIN IN DNA SYNTHESIS OF HL 60 CELLS,
George P. Studzinski, Zamir S. Brelvi, Susan C. Feldman and Rosemary A. Watt, UMDNJ-New Jersey Medical School, Newark NJ 07103, and Smith Kline and French Laboratory, Philadelphia PA 19101

Abnormal expression of the oncogene c-myc is believed to result in the uncontrolled proliferation of HL 60 cells and other neoplastic cells. Since the mechanism for this action is not understood, we have tested whether the c-myc protein is required for DNA synthesis. Nuclei isolated from HL 60 cells were incubated under conditions known to permit the elongation of the DNA chains that were initiated in the intact cells. Addition of a monoclonal or a polyclonal affinity purified antibody to the human c-myc protein markedly inhibited DNA synthesis, but not RNA synthesis, which also takes place in isolated nuclei under appropriate conditions. Following the incubation of the nuclei with the antibody, its presence in the nuclei was shown by immunofluorescence. In control experiments an antibody to DNA polymerase alpha inhibited DNA synthesis in a similar manner, while an antibody to RNA polymerase II inhibited RNA, but not DNA, synthesis. Antibodies to non-nuclear constituents of the cell inhibited neither DNA nor RNA synthesis, showing the specificity of the inhibitiory effects. The inhibition of DNA synthesis by the antibody to c-myc protein was blocked by the addition of the c-myc protein. We conclude that the functions of c-myc protein include participation in DNA synthesis. Thus, increased or inappropriate expression of c-myc gene may contribute to the growth advantage of neoplastic cells by facilitating the replication of DNA. (Supported by grant #385-028 from NJ State Commission on Cancer).

D 130 ORGANIZATION AND EXPRESSION OF IMMUNOGLOBULIN AND T CELL RECEPTOR GENES IN HODGKIN CELL LINES, H. Tesch, M. Falk, H. Stein, D. Jones, V. Diehl and G. Bornkamm, 1. Med. Klinik, University of Cologne, FRG, Institute for Virology, Freiburg, FRG and Dept. of Pathology, Southampton, GB

To investigate the origin of Hodgkin- and Sternberg-Reed cells we analyzed the organization and expression of immunoglobulin (Ig) and T cell receptor (TCR) genes in four Hodgkin's diesease derived cell lines. Our experiments revealed that two of these lines show various rearrangements of Ig genes and express Ig specific mRNAs (Cy specific transcripts in 428 cells, Ca, Ck and Cl specific transcripts in 591 cells). The remaining two lines have rearranged TCR ß and y genes and express either TCRß specific transcripts (Cole) or TCRa specific transcripts (540) but not both. These data demonstrate that the Hodgkin derived cell lines can be classified as lymphoid cells and resemble different differentiation stages within the B or T lineage.

D 131 THE TRANSLOCATION BREAKPOINT IN PHILADELPHIA CHROMOSOME-POSITIVE (PH^1+) ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) IS LINKED TO BCR, Carol A. Westbrook, Charles M. Rubin, Arthur L. Hooberman, Timothy A. Geiger & Janet D. Rowley, Univ. of Chicago, Chicago IL 60637.

The Ph^1 arises from a chromosomal 9;22 translocation, and occurs in the malignant cells of virtually all patients (pts) with chronic myelogenous leukemia (CML) and 10% of pts with ALL. In CML, the protooncogene ABL is translocated from chromosome 9 to a breakpoint cluster region (bcr) within the PHL gene on No. 22, producing a fusion gene which gives rise to a hybrid 8.5 kb bcr-ABL mRNA. In a subgroup of Ph^1+ALL, the t(9;22) differs from that of CML at the molecular level; the breakpoint lies 5' (centromeric) of bcr, which is unrearranged, and there is no 8.5 kb bcr-ABL mRNA. Thus, it is unclear whether PHL or ABL are involved. To identify the breakpoint on No. 22, we first made a restriction map of the normal chromosome using pulsed field gel eletrophoresis (PFGE); we show that bcr and the immunoglobulin lambda gene (IGL) lie on one ≈1600 kb NotI restriction fragment, though a polymorphic NotI site divides the two loci in some alleles. We then studied two pts with Ph^1+ALL and one cell line (SUP-B13) derived from a third pt. Using conventional Southern blot anlysis and a bcr probe, no rearrangement was detected up to 20 kb 5' of bcr; also, IGL was germline in one sample studied with a constant region probe. However, hybridization of the bcr probe to NotI digests fractionated by PFGE revealed rearranged bands of 300-350 kb in each sample, as well as germline fragments of ≈1600 or 450 kb. We conclude that the breakpoint junctions lie between 20 and 350 kb 5' of bcr in Ph^1+ALL. Whether 5' elements of the PHL gene are involved is unknown. To identify the breakpoint on No. 9, we have made a 575 kb restriction map of the ABL locus and are currently analyzing this region for rearrangement.

D 132 SUBTRACTION cDNA CLONING IN MONOCYTIC DIFFERENTIATION: A TRANSCRIPT DOWN REGULATED BY BOTH PMA AND VITAMIN D_3 IN HL-60 CELLS, Marie-Cecile Wetzel-Raynal, Alan J. Dayan and Yvon Cayre, Memorial Sloan-Kettering Cancer Center, New York, NY 10021

A double resistant variant cell line called 1F10 was derived from the human promyelocytic leukemia cell line HL-60 by culturing the cells successively in presence of increasing doses of one of the two available monocytic inducers - Phorbol 12-myristate 13-acetate (PMA) and $1,25(OH)_2$ Vitamin D_3 (Vit D_3). Vit D_3 maintained cells and PMA-maintained cells are arrested at discrete steps in the differentiation process, while when the two inducers are applied concomitantly, 1F10 cells acquire all the characteristics of fully differentiated monocytes. A cDNA library specific for Vit D_3 induction was constructed by subtraction of cDNA from Vit D_3-maintained 1F10 cells with mRNA from PMA-maintained 1F10 cells. One cDNA clone (pD_3-120) isolated by differential screening corresponded to a 1.3 kb transcript down regulated in the course of monocytic differentiation. In 1F10 cells, pD_3-120 mRNA is expressed prior to any induction, increased in presence of Vit D_3, and strongly decreased in presence of PMA. When the two inducers are applied concomitantly, pD_3-120 transcript becomes undetectable. In HL-60 cells, pD_3-120 expression is down regulated to an undetectable level after 24 hrs of induction with either PMA or Vit D_3. In addition, this transcript is not expressed in myeloid leukemia cells blocked at an earlier blast-like stage of maturation (KG-1 and K562). These results suggest that pD_3-120 mRNA expression is specific of the promyelocytic/promonocytic differentiation stage.

D 133 MODULATION OF C-FMS AND C-FGR EXPRESSION IN NORMAL MONOCYTIC CELLS STIMULATED TO PROLIFERATE WITH THE CSF-1 MONOCYTIC COLONY-STIMULATING FACTOR. Cheryl L. Willman, Carleton C. Stewart, Hsiu-san Lin, and Thomas B. Tomasi, University of New Mexico, Albuquerque, NM 87131; Los Alamos National Laboratory, Los Alamos, New Mexico 87545; Washington University, St. Louis, MO 63110; and Roswell Park Memorial Institute, Buffalo, NY 14263.

The cellular role of proteins encoded by proto-oncogenes highly related to the src oncogene of Rous avian sarcoma virus remain elusive. The expression the protein encoded by c-src in fully differentiated, post-mitotic cells has suggested that this protein has a differentiation-related, rather than a proliferation-related, function in normal cells. However, we have determined that the closely related proto-oncogene c-fgr may have a proliferation-related function in normal monocytic cells. Normal murine monocytic cells were derived from short-term bone marrow cultures grown in the presence of the monocytic lineage-specific growth factor CSF-1 (M-CSF). The fully differentiated monocytic cells derived from these cultures expressed high levels of c-fms RNA (encoding the CSF-1 receptor). When these cells were rendered quiescent by the removal of CSF-1, the c-fms mRNA levels increased 6-fold. When these cells were stimulated to proliferate by the re-addition of CSF-1 to the culture medium, c-fms mRNA levels were slowly down-modulated over an 8 hour time period. The re-addition of CSF-1 to the culture medium induced the expression of c-fgr mRNA. A major c-fgr transcript of 3.0kb, as well as minor transcripts of 3.5 and 4.7kb, were detected 4 hours after growth factor addition, peaked at 8 hours, and were no longer detectable by 12 hours. At 12 hours, the monocytic cells have just begun to enter S phase of the cell cycle. suggesting that c-fgr expression may regulate cell cycle progression.

D 134 A NEW TRANSFORMING VIRUS ISOLATED FROM A CULTURED FeLV-NEGATIVE FELINE LYMPHOSARCOMA CELL LINE. J.H. Wolfe, E.E. Zuckerman, W.S. Hayward, W.D. Hardy, Jr. Sloan-Kettering Institute for Cancer Research, New York, NY 10021.

Thirty percent of cats with lymphosarcomas (LSA) show no evidence of FeLV infection in LSA or normal tissues. However, FeLV appears to induce most of these virus-negative (VN)-LSAs because they arise subsequent to FeLV-exposure and latent FeLV can be activated from bone marrow cells, but not from LSA cells, of such cats. We have established a lymphoid suspension cell line, with feline fibroblast feeders, derived from an intestinal VN-LSA (cell line FL-3754L). After several months in tissue culture, transformed adherent cells (growth in nude mice and soft agar) were isolated as a subline (3754B) from the FL-3754L cultures. The 3754B cells produce no viral particles, but do express FeLV antigens. A transforming virus was rescued from 3754B cells on feline fibroblasts (FEA cells) with FeLV-AB helper virus and on mink fibroblasts with amphotropic MuLV. The 3754B cells express a transformation-specific protein (87kd) that is precipitated by anti-FeLV antiserum. Experiments, including molecular cloning of the 3754B virus, are in progress to identify the viral transforming gene. The 3754B virus does not appear to contain myc because, unlike the myc-containing FeLVs, it transforms FEA cells. The 3754B virus rapidly induces fibroid-type sarcomas in newborn kittens at the inoculation site and in the thymus. Tumors from the subcutaneous lesions and the thymuses of two kittens have been established as cell lines and grown in nude mice. In cats, FeSVs do not induce thymic tumors and FeLVs do not induce sarcomas at the inoculation site. Thus, the 3754B virus (or mixture of viruses) appears to have different biological properties than any previously described FeLV or FeSV.

D 135 High frequency of Clonal Immunoglobulin or T-cell Receptor Gene Rearrangements in Acute Myelogenous Leukemia (AML) Expressing Terminal Deoxy-transferase (TdT). S. Seremetis, P.-G. Pelicci, A. Tabilio, F. Grignani, R. Winchester, D.K. Knowles, and R. Dalla-Favera. New York University School of Medicine, New York, NY 10016, and University of Perugia, Italy.

Immunoglobulin (Ig) and T-cell receptor (TCR) rearrangements have been used as irreversible markers of lineage and clonality in the study of B- and T-lymphoid populations. However, Ig or TCR rearrangements are sporadically reported in AMLs. TdT expression, also regarded as lymphoid lineage specific, is reported in 10% of AMLs. We tested the hypothesis that these two uncommon events might be associated in AMLs and represent activation, albeit abortive or transient, of a program of lymphoid initiation expressed prior to irreversible commitment. We analyzed TCR (TβC) and Ig (J_H) gene configurations in 25 TdT-negative AMLs, 13 TdT-positive AMLs, and 4 TdT-positive undifferentiated leukemias. While only 2/25 of the TdT-negative AMLs displayed clonal rearrangements, 8/13 of TdT-positive AMLs displayed rearrangements (>60%), including 3 J_H rearrangements, 4 TβC rearrangements and one leukemia displaying rearrangement at both loci. All 4 TdT-positive undifferentiated leukemias displayed rearrangements at both loci. These data demonstrate a significant association between TdT expression and Ig or Tβ gene rearrangements even outside the lymphoid lineage, further supporting a role for TdT in Ig and TCR gene assembly. These data also indicate that a coordinated program of lymphoid gene expression involving TdT expression and Ig/Tβ rearrangements can be activated prior to myeloid commitment. Whether activation of this program represents a normal, albeit rare, event in early myelopoiesis or a transformation related event present only in leukemic cells remains to be determined.

Viruses

D 200 MURINE THYMIC LYMPHOSARCOMAS AFTER INFECTION WITH A B-ECOTROPIC MURINE LEUKEMIA VIRUS AND/OR X-IRRADIATION : PROVIRAL ORGANIZATION AND RNA EXPRESSION. T. Astier-Gin, M. Galiay, E. Legrand, D. Moynet, N. Rebeyrotte, A. Artus, B. Guillemain and J.F. Duplan. INSERM U. 117, 229 cours de l'Argonne, 33076 BORDEAUX Cédex - France

The role of retroviruses in murine radioleukemogenesis was reinvestigated with a protocol associating the injection of a non-pathogenic retrovirus (T1223/B virus) and a subleukemogenic dose of X-radiation (2 x 1.75 Gy). Using blotting technics we studied MuLV proviral organization and RNA expression in thymomas induced by the combined effect of virus and irradiation or irradiation alone. A recombinant provirus was detected in the chromosomal DNA of every tumor induced by associating virus and radiation whereas it was unconstantly found in radio-induced tumors. In every instance, the provirus was randomly integrated. No relationship was observed between viral RNA expression and tumor induction. Trisomy 15 was observed in all metaphases irrespective of the protocol of tumor induction. An extraband on chromosome 6 was observed in several thymomas induced by irradiation and T1223/B virus injection.

D 201 DIFFERENTIAL EXPRESSION OF MURINE CELLULAR PROTO-ONCOGENES IN MYELOID LEUKEMIA LINES, H. G. Bedigian, Jackson Laboratory, Bar Harbor, ME 04609.

Recently we have described a recombinant inbred (RI) mouse strain (BXH-2) that has a high incidence of spontaneous leukemia (89% by 8 months of age) associated with the expression of ecotropic type C Murine leukemia virus (MuLV). The BXH-2 strain is derived from crossing two strains, C57BL/6J and C3H/HeJ, which both have a low incidence of neoplastic disease. Results obtained from pathological studies as well as cytochemical and immunological assays suggest that the majority of tumors in BXH-2 mice are of myeloid origin. The ecotropic MuLV isolate of BXH-2 mice induced tumors by a year of age in another BXH strain. The range of neoplasms induced by the virus paralleled that occurring spontaneously in BXH-2 mice. Also, tumor cells obtained from BXH-2 mice retained their neoplastic properties on transplantation into isogenic mice. Several cell lines have been established from both primary and transplantable tumors. The cell lines show cytochemical and morphological properties characteristic of cells of the myeloid lineage. The cell lines show various degrees of competence for differentiation by regulators of the hematopoietic system. The majority of the cell lines are malignant and cannot be made to differentiate. Proto-oncogenes comprise a class of cellular genes that may regulate growth and differentiation and if abnormally expressed may establish or maintain the malignant properties of the cell lines. We are presently examining mRNA levels of several known proto-oncogenes to determine their involvement in BXH-2 myeloid leukemic development and how oncogene expression might be altered by treatment of the cell lines with chemical inducers. The results of these experiments will be presented.

D 202 A NEW B-LYMPHOTROPIC VIRUS POSSIBLY ASSOCIATED WITH LYMPHOPROLIFERATIVE DISEASES, Biberfeld, P.[1], Salahuddin, Z.[2], Ablashi, D.V.[2], Josephs, S.F.[2], Kramarsky, B.[2], Wong-Staal, F.[2] & Gallo, R.C.[2]. (1) Dept. of Pathology, Karolinska Institute, Sweden (2) Lab. Tumor Cell Biology, N.C.I., Bethesda.

We will report on a B-lymphotropic virus which was isolated from AIDS-patients with lymphoproliferative disorders. (1) The ultrastructure of this virus had features similar to those of the Herpes family. However, Its molecular biology, host-cell range, and immunology showed no relatedness to known primate Herpes virus. Thus, this virus seems to represent a new member of the Herpes virus family, possibly associated with human disease.

(1) Salahuddin, Z. et al; Science; in press.

D 203 DIFFERENTIATION DEPENDENT TRANSFORMATION OF CELLS IN THE MONOCYTE LINEAGE BY V-MYB. David Boettiger and Margaret Olsen, University of Pennsylvania, Philadelphia, PA 19104

The v-myb gene of avian myeloblastosis virus is restricted in its transforming potential to cells in the monocyte lineage. In the embryonic yolk sac the myeloid progenitors are unable to mature beyond the immature blast stage. The monocyte progenitors which compose about 5% of this population express normally high levels of c-myb (200-400 m-RNA/cell) and are not susectpible to transformation by AMV in vivo. Infected cells in other hematopoietic organs which do not restrict monocyte differentiation contain numorous transformed cells, and the infected YSC transform in 24 hours in vitro. In vitro studies demonstrate a different factor requirement for transformation of YSC in comparison to cells from other organs and to YSC allowed to differentiate in vitro. Hence cells which normally express high levels of c-myb appear to be refractory to transformation by v-myb. When these cells differentiate either in vivo or in vitro, c-myb expression rapidlt declines, and the cells become susceptible to transformation by v-myb. This suggests that infection of the monocyte progenitors by AMV produces an effective failure to shut off myb during normal differentiation which leads to their transformation. We suggest that myb plays a role in normal monocyte differentiation to suppress the production of the differentiated cell products during clonal expansion and that v-myb represents a charature of this function in the more mature cells.

D 204 ANALYSIS OF LYMPHOMAGENESIS IN THE AVIAN LEUKOSIS SYSTEM, Donald L. Ewert, Charles Goldstein and David Urquhart, The Wistar Institute, Philadelphia, PA 19104.

The first detectable phase of avian lymphoid leukosis virus induced lymphomagenesis is the appearance of hyperplastic follicles in the bursa of Fabricius. These follicles are typically pyroninophilic and number between 20 and 100 per bursa. Individual hyperplastic follicles were analyzed for viral protein expression and for the presence of viral-myc oncogene junction fragments in the cellular DNA. Failure to detect viral protein synthesis in a large proportion of the hyperplastic follicles indicates that viral synthesis is not required to sustain the hyperplastic state. Although most of the hyperplastic follicles have detectable virus-Myc junction fragments, no such fragments could be detected in DNA from about 6% of the hyperplastic follicles.

The next phase involves the progression and eventual metastasis of 1-2 bursal lymphomas. The effect of the immune system on tumor progression was assessed in chickens tollerized to the viral antigens. A similar frequency of primary bursal and metastatic lymphomas developed in chickens lacking detectable antibodies to viral antigens as those having high titers of antibody. Furthermore metastastic lymphoma cells sustained a higher level of growth in vitro than either primar bursal lymphoma cells or normal bursal cells. Taken together these results suggest that progression of bursal lymphomas may depend on changes intrinsic to the cell.

D 205 USE OF INFECTIOUS SIMIAN VIRUS 40 (SV-40) TO GENERATE HUMAN MARROW STROMAL CELL LINES, Louis A. Gaboury, Allen C. Eaves and Connie J. Eaves, Terry Fox Laboratory, B.C. Cancer Research Centre, Vancouver, B.C. V5Z 1L3, Canada.

We have previously shown that pluripotent and other primitive, but committed, human hemopoietic progenitors can be maintained for several weeks in cultures containing a heterogeneous adherent cell population of marrow origin including various mesenchymal phenotypes. Moreover, recent data indicate that in such cultures primitive hemopoietic progenitor proliferation is subject to extrinsic regulation, as shown by their cyclic variation between an active and a quiescent state following each medium change. Because of the phenotypic heterogeneity of marrow adherent cell layers, analysis of molecular mechanism(s) mediating these positive and negative signals await the isolation of pure cell types. Since normal human mesenchymal cells senesce within a few generations in vitro, some immortalization procedure is required. Transformation with SV-40 virus has been used previously for this purpose, without loss of the differentiated state expressed prior to transformation in some cases. To utilize this approach, we generated high titre virus stock (10^{10} pfu/ml) by standard procedures and then infected (at 100 pfu/cell) a variety of cell types including long-term human marrow culture adherent monolayers, normal human skin fibroblasts, human embryonic lung fibroblasts and mouse 3T3 fibroblasts. Twenty four hours after infection the SV-40 large "T" antigen was detected in most cells in the first two types of culture and infected 3T3 cells yielded transformed foci at a frequency of 2.7×10^6 per ml. Experiments to isolate human mesenchymal cell transformants and to characterize them both functionally (support and regulatory action on hemopoietic precursors) and phenotypically are in progress.

D 206 SELECTIVE ACTIVATION OF ENDOGENOUS ECOTROPIC RETROVIRUS IN TISSUES OF B6C3F1 MICE DURING THE PRELEUKEMIC PHASE OF 1,3-BUTADIENE EXPOSURE, Richard D. Irons, Wayne S. Stillman and Rekha S. Shah, Chemical Industry Institute of Toxicology, Research Triangle Park, NC 27709, and Miles W. Cloyd, Duke University School of Medicine, Durham, NC 27710.
1,3-Butadiene (BD) is a colorless gas used as a monomer in the production of styrene-butadiene or "synthetic" rubber. Epidemiologic studies to assess potential health hazards associated with occupational exposure to BD, while inconclusive, have raised concern over the potential for increased risks of certain cancers, including hematopoietic or lymphoid neoplasms. Chronic exposure of B6C3F1 mice to BD results in a high incidence of thymic lymphoma/leukemia. These are of T cell origin and exhibit elevated expression of endogenous retroviruses. We found that chronic exposure to BD (1250 ppm) 6 hr/day 5 days/wk for 3 to 21 weeks increased the quantity of ecotropic virus recoverable from bone marrow, thymus and spleen. However, expression of other types of endogenous retroviruses (xenotropic, MCF-ERV) was not enhanced. The mechanism of this increase in ecotropic retrovirus was shown to be de novo activation in greater numbers of cells and not related to changes in virus Fv-1 tropism or host Fv-1 restriction, which would enable virus spread throughout the mouse. Viruses isolated from tumor lines derived from BD-induced lymphomas were heterogeneous as defined by a panel of env-specific monoclonal antibodies but none appeared to be standard MCF-ERV-type recombinants. The role of endogenous retroviruses in this leukemogenesis model is currently under study.

D 207 CALCIUM DEPENDENT PROTEIN ASSOCIATED WITH RETROVIRUSES, Mark G. Lewis, Jason Y. Chang,, Richard H. Fertel and Richard G. Olsen, Ohio State University, 1925 Coffey Road, Columbus Ohio, 43210.
Disrupted gradient purified retroviruses were found to contain a calcium dependent activity which has characteristics similar to that of calmodulin. The virus associated activity was able to activate both cyclic AMP phosphodesterase (PDE) and calcium dependent ATPase activity between 2 and 4 fold. The activity was heat stable, with heating at 100°C for 10 minutes having no effect, and not associated with lipid presence. This activation is calcium dependent as shown by its inhibition in the presence of EGTA and return with the addition of and exogenous Ca^{2+}. The PDE activation by the retroviruses was inhibited by two calmodulin specific inhibitors, R24571 and W7, and by a group of anti-psychotic drugs, such as trifluoperazine (TFP), known to be calmodulin antagonists. A calmodulin specific RIA found immunoreactivity in all retrovirus isolates tested, indicating a binding of between 10 to 25 ug/mg protein. Treatment of infectious feline leukemia virus with 25mM TFP caused a significant decrease in infectious particles after a 30 minute pre-incubation. These results indicate that retroviruses contain a calcium dependent substance, which is similar to calmodulin both physically and enzymatically.

*Supported by NIH-NCI grant CA-31547 and American Cancer Society grant IN-16Y

D 208 THE EFFECT OF VIRUS DOSE AND TRANSMISSION ROUTE ON FELINE RETROVIRUS INDUCED DISEASE, Lawrence E. Mathes, Phyllis Polas and Richard G. Olsen, The Ohio State University, Columbus OH 43210.

Pooled plasma from feline leukemia virus (FeLV) infected cats was determined to be a potent source of infectious virus. Ten microlites of infectious plasma induced FeLV disease in 4 of 6 cats. The route of exposure to FeLV influenced the rate of disease induction, disease severity and survival time. The intravenous route produced the most acute disease form characterized by viremia, severe immunosuppression, and short survival time (10 weeks). Challenge by the intraperitoneal route was intermediate in pathogenesis, characterized by viremia and a mean survival time of 16 weeks. Oronasal exposure was the least effective route of viral challenge producing fewer animals with viremia, longer survival times (22 weeks) and a more chronic disease form.

The outcome of these studies demonstrates the importance of route of exposure to retrovirus and may have relevance to the AIDS disease in humans.

D 209 COLONY ASSAY SYSTEM FOR THE GROWTH AND ISOLATION OF CELLS FROM SEVERAL STAGES OF MURINE THYMIC LEUKEMOGENESIS. Glenn Miller, Nancy Famulari and Paul O'Donnell. Memorial Sloan-Kettering Cancer Center, New York, NY 10021.

The isolation and characterization of cell lines derived from thymic leukemias accelerated with Mink Cell Focus-inducing murine leukemia virus(MCF) is of importance to the understanding of the discrete stages of leukemogenesis seen in this disease. Techniques used previously to isolate such lines involved either serial in vivo passage or outgrowth of leukemic cells on a feeder layer of thymic epithelial cells(TEC). A semi-solid colony assay system is presented that functions to reproducibly grow primary leukemias without the use of TEC or prior selection by serial in vivo passage. Thymocytes taken from AKR/J mice demonstrating signs of frank leukemia demonstrated colonial growth in 30/62 instances in the presence of either PMA and PHA or PMA alone. Individual colonies were harvested and placed in liquid culture with 35 established cell lines resulting. All of the lines tested thus far are transplantable. Cell surface marker analysis of several of these lines demonstrated 2 major categories of cell types grouped according to thymic maturation. Flow cytometric analysis of thymocytes at various time points following intrathymic injection of MCF virus demonstrates several discrete stages of thymic leukemogenesis. Colony assays completed at various stages demonstrated that thymocytes from 7/18 early stage and 6/12 late stage mice could form colonies. Southern blot analysis of proviral integrations and rearrangement of known oncogenes and insertion site genes will be presented.

D 210 HTLV-I INFECTED T LYMPHOCYTES CONTAIN FACTORS THAT BIND SPECIFICALLY TO THE HTLV-I PROVIRAL LTR. Jennifer K. Nyborg, William S. Dynan, Irvin S. Y. Chen* and William Wachsman*, University of Colorado, Boulder, *University of California, Los Angeles.

The human T Cell Leukemia Viruses (HTLV) type I and II are retroviruses associated with specific leukemias in humans. In addition to the characteristic gag, pol and env genes, both viruses contain a novel fourth gene termed X or lor. Recent studies by several investigators have demonstrated that the product of the X gene is necessary for efficient transcription and replication of the viral genomes. DNA sequences in the long terminal repeats (LTRs) of the integrated provirus have been shown to be important for efficient promoter activity. HTLV-I and -II share limited homology within their LTRs, primarily consisting of three imperfect 21 bp repeated elements located upstream of the RNA start site.

Using DNase I footprinting, we have identified two major regions in the HTLV-I proviral LTR that are protected from DNase I digestion by proteins present in extracts of HTLV-I infected T lymphocytes (SLB-1 cells). These regions of protection map to the first (most 5') and second (middle) 21 bp repeat in the proviral LTR. Proteins in extracts of uninfected HeLa cells also bind specifically to the proviral LTR. Protection in the first repeat is similar to that with SLB-1, but protection in the second repeat is both weaker and altered in pattern. We have studied binding in several additional cell lines. Extracts prepared from an HTLV-II infected T lymphocyte cell line (Mo-T) produce a DNase I protection pattern similar to SLB-I cells. Extracts from an uninfected T lymphocyte cell line (Jurkat) produce a pattern of protection similar to HeLa cells.

These studies demonstrate that factors are present in both infected and uninfected cells that specifically bind to sequences in the HTLV-I proviral DNA. These binding sites are located at the first and second repeats in the proviral LTR. Comparison of DNase I protection patterns with nuclear extracts prepared from various cell lines reveals a consistent difference and suggests that infected cells contain an additional factor that specifically binds or modulates binding at the second (middle) 21 bp repeat.

D 211 EXPRESSION OF HTLV-I RELATED ANTIGENS AND THEIR RELEASE FROM MT-2 CELLS, Richard G. Olsen, Devraj J. Pillay, Laura Y. Huff, Mark G. Lewis, Lawrence E. Mathes and James R. Blakeslee, Jr.

This investigation addresses the possibility of obtaining HTLV-I or related proteins from MT-2 cells (HTLV-I positive human T-cell line). These proteins will then be characterized, purified and tested for immunogenicity and protection against HTLV-I infection. In this study antigen expression was correlated with cell cycle and HTLV-I antigen release. Antigen expression was most pronounced during the G_2M phase. To determine the conditions for the release of HTLV-I associated proteins The MT-2 cells were cultured in RPMI 1640 medium supplemented with HB101 or HB102. Cell and virus free supernatants were collected on day 4, lyophilized and concentrated up to 100 times. The proteins in these supernatants were characterized using SDS-PAGE and tested for reactivity against rabbit anti-HTLV-I sera and human ATL (Adult T-cell leukemia) sera by Western-Blot analysis. Preliminary results from the Western-Blot analysis indicate that the supernatants obtained from MT-2 cells grown in HB102 supplemented medium contained detectable amounts of proteins which reacted with human ATL and rabbit anti-HTLV-I sera. The molecular weights of these proteins appeared to be around 68kd, 46kd and 20kd. The fast protein liquid chromatography (FPLC) separation of the proteins in the supernatant using a sephacryl-300 column resulted in five distinct peaks. Further analysis revealed that peak number 2 contained major bands of 68kd, 15kd and peak number 4 contained major bands of 46kd, 25kd, 20kd and 15kd. In addition, rabbits injected with MT-2/HB102 supernatants were successfully immunized against HTLV-I related antigens.

D 212 EXPRESSION OF HTLV-I RELATED ANTIGENS AND THEIR RELEASE FROM MT-2 CELLS, Devraj J. Pillay, Mark G. Lewis, Lawrence E. Mathes and Richard G. Olsen.
This investigation addresses the possibility of obtaining HTLV-I or HTLV-I-related proteins from MT-2 cells (HTLV-I positive human T-cell line). These proteins will then be characterized, purified and tested for immunogenicity and protection against HTLV-I infection using in vitro and in vivo assays. In this study antigen expression was correlated with cell cycle and HTLV-I antigen release. Antigen expression was most pronounced during the G_2M phase. To determine the conditions for the release of HTLV-I associated proteins The MT-2 cells were cultured in RPMI 1640 medium supplemented with HB101 or HB102. Cell and virus free supernatants were collected on day 4, lyophilized and concentrated up to 100 times. The proteins in these supernatants were characterized using SDS-PAGE and tested for reactivity against rabbit anti-HTLV-I sera and human ATL (Adult T-cell leukemia) sera by Western-Blot analysis. Preliminary results from the Western-Blot analysis indicate that the supernatants obtained from MT-2 cells grown in HB102 supplemented medium contained detectable amounts of proteins which reacted with human ATL and rabbit anti-HTLV-I sera. The molecular weights of these proteins appeared to be around 68kd, 46kd and 20kd. The fast protein liquid chromatography (FPLC) separation of the proteins in the supernatant using a sephacryl-300 column resulted in five distinct peaks. Further Western-Blot analysis of these peaks revealed that peak number 2 contained major bands of 68kd, 15kd and peak number 4 contained major bands of 46kd, 25kd, 20kd and 15kd. In addition, rabbits injected with MT-2/HB102 supernatants were successfully immunized against HTLV-I related antigens as shown by Western-Blot analysis.

D 213 EXPRESSION OF BOVINE LEUKEMIA VIRUS EARLY AFTER INFECTION OF SHEEP. K. Radke, D. Grossman, L. Adamson and D. Lagarias. Department of Avian Sciences, University of California, Davis, California, 95616.

Bovine leukemia virus (BLV) is an oncogenic, lymphotropic retrovirus that is closely related to human T-cell leukemia virus I. BLV causes tumors in cattle, its natural host, with long latency after infection, but sheep are susceptible to experimental infection and tumorigenesis by BLV. We have monitored the progress of viral infection in peripheral blood cells of lambs to understand events surrounding the establishment of infection. The presence of virus-infected cells among peripheral blood mononuclear cells was determined twice weekly by a syncytium induction assay on indicator cells. Expression of viral RNA by individual cells was monitored by **in situ** hybridization with radioactive, asymmetric RNA probes. Sera were assessed in parallel for their content of precipitating and neutralizing antibodies specific for BLV.

Several patterns of response to viral infection were displayed among ten infected lambs. In five animals, a sharp peak of virus-infected peripheral blood cells occurred within 7 weeks of infection. The serum of most of these contained precipitating antibody and neutralizing antibody preceeding the peak appearance of infected cells in the blood. Three animals showed less marked peaks of virus-infected blood cells and in two, infection was not apparent. Transcription of viral genes is thought to be repressed **in vivo** in lymphocytes of animals that have well-established infections. However, our analysis by **in situ** hybridization has revealed that a small percentage of mononuclear cells obtained directly from the blood of the infected lambs contain significant amounts of viral RNA.

D 214 **OLIGOCLONAL INTEGRATION OF HTLV-II IN OKT8⁺ T-CELLS IN A PATIENT WITH "ATYPICAL" HAIRY-CELL LEUKEMIA: EVIDENCE FOR TWO MALIGNANCIES** by Joseph D. Rosenblatt, Janis V. Giorgi, John Glaspy, Phil Bierman*, Ira Smalberg, Mordecai Aboud, David W. Golde and Irvin S.Y. Chen. Division of Hematology-Oncology and Department of Microbiology and Immunology, UCLA School of Medicine, Los Angeles, CA 90024; *Department of Hematology, City of Hope National Medical Center, Duarte, CA 91010.

Human T-cell leukemia virus type II (HTLV-II), has been moleculary cloned in our laboratory from a second patient (NRA) with atypical hairy-cell leukemia. The patient's illness was characterized for most of its course by a predominance of circulating Tac^- $OKT8^+$ cells. Recently, however, the patient expired, following the appearance in the peripheral blood of a rising percentage (~25%) of Tac^+ $TRAP^+$ B-cells. The B-cells were clonal, as demonstrated by an immunoglobulin gene rearrangement. Oligoclonally integrated HTLV-II was detected in DNA extracted from peripheral blood mononuclear cells obtained on two dates one year apart. To study the role of HTLV-II in this patient, we separated peripheral blood mononuclear cells obtained at autopsy into four enriched subpopulations: a T-cell fraction, a non-T-cell (B-cell and monocyte) fraction, an $OKT8^+$ T-cell-enriched fraction, and an $OKT4^+$-T-cell-enriched fraction. Oligoclonally integrated HTLV-II was preferentially detected in DNA from the T-cell and $OKT8^+$-T-cell fraction, poorly detectable in the $OKT4^+$-T-cell fractions, but not detected in the non-T-cell fraction. We conclude that this patient harboured two lymphoproliferative disorders: a $TRAP^+$, Tac^+ B-cell malignancy which did not contain integrated HTLV-II, and a Tac^- $OKT8^+$ lymphoproliferative process with oligoclonally integrated HTLV-II.

D 215 Cis-ACTING REGULATORY SEQUENCE FOR TRANSCRIPTION IN HTLV-I AND HTLV-II, Kunitada Shimotohno, Masako Takano, Toshiyuki Teruuchi, Masanao Miwa and Takashi Sugimura, National Cancer Center Research Institute, Tokyo, 104, Japan.

The cis-acting regulatory sequence of transcription from long terminal repeats(LTRs) of human T-cell leukemia virus type I and type II (HTLV-I and HTLV-II), which is essential for action of the virally encoded trans-acting transcriptional factor(s) in HTLV-I and HTLV-II was identified.

Comparison of the LTRs of HTLV-I and HTLV-II revealed the presence of conserved nucleotide sequence stretches in the "TATA" region, cap site, poly(A) site and U3 region. The conserved nucleotide sequence in the U3 region consists of a 21 nucleotide sequencs that is repeated three times with some distance. The location of the 21 nucleotide sequence suggests that this sequence function as enhancer for transcription.

Deletion of most of the U3 region resulted in loss of transcriptional activity. However, when the chemically synthesized 21 nucleotide was inserted into the U3 deleted LTR, transcriptional activity from the LTR was restored. The extent of restoration of the activity was proportional to the number of copies of the sequence inserted (up to 5 copies of the insert was tested). The restoration of the activity is not depend on the orientation of the 21 nucleotide. Increase of the activity by insertion of the 21 nucleotide seems not only depend on number of copies of the insert but also depend on distance between the two 21 nucleotide sequences inserted.

This 21 nucleotide sequence functions as cis-acting element for transcription only in HTLV-I or HTLV-II infected cells or pX of HTLV-I or HTLV-II expressing cells.

D 216 SITE-MODIFICATION OF F-SFFV GLYCOPROTEINS. Ranga V. Srinivas, David Kilpatrick and Richard W. Compans. Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL. 35294

The leukemogenic gp52 glycoprotein encoded by the Friend strain of Spleen Focus-Forming Virus has been identified as a recombinant envelope glycoprotein derived by a series of changes involving substitutions, deletions and insertions in the envelope gene of ecotropic F-MuLV. Unlike MuLV glycoproteins, SFFV gp52 is defective in its transport to the cell surface. In an attempt to determine which of the specific changes undergone by SFFV is responsible for its leukemogenicity, as well as its defective intracellular transport we have generated a series of SFFV mutants by site-specific modification of the envelope gene. The different mutants that have been generated include: 1. A chimeric molecule in which the 3' end of the SFFV envelope gene, which codes for the hydrophobic membrane-spanning domain and the cytoplasmic domain (if any) of gp52 has been substituted by a corresponding gene segment from the F-MuLV envelope gene. 2. A MuLV envelope glycoprotein in which domains coding for the membrane-spanning and cytoplasmic domain have been substituted by a corresponding gene segment from the SFFV envelope gene. 3. A unique Avr2-Asu2 restriction fragment which borders the boundary of deletion found in SFFV gp52 has been substituted with a corresponding domain from the F-MCF envelope gene. 4. The Avr2-Asu2 fragment in the F-MCF envelope gene has been substituted by a similar fragment derived from the SFFV envelope gene. 5. The SFFV envelope gene has been reconstructed such that the amino terminal MCF-specific sequences are substituted by ecotropic F-MuLV derived sequences. 6. A stop codon has been introduced 5' to the region which codes for the membrane anchor domain of SFFV gp52 to generate a truncated molecule which is expected to be secreted from the cells. Results on the transport and leukemogenic potential of these mutant molecules will be presented.

D 217 AUTOCRINE FACTOR SECRETION TRIGGERS TUMORIGENIC PROGRESSION IN MYELOID CELL LINES, C.Stocking, C.Laker, C.Löliger, M.Kawai and W.Ostertag, Heinrich-Pette-Institut, D-2000 Hamburg 20, F.R.G.

We have examined several myeloid cell lines that have become independent of exogenous growth factor due to endogenous expression of colony-stimulating factor (CSF). These cell lines were established either after infection of FD-CP1 or FD-CP2 with a MPSV retrovirus-based vector carrying the gene for GM-CSF or Multi-CSF or alternatively by screening for spontaneous mutants of D35, a promyelocytic cell line established after virus infection of bone marrow cells. Two out of four independent cell lines obtained by the latter procedure showed rearrangement and expression of a GM-CSF allele. The cell lines are tumorigenic when introduced into nude mice and their supernatants support growth of factor dependent cell lines. However, these cells grow linearly in relationship to cell density, suggesting that secretion of GM-CSF is not necessary for continued factor independent growth. To analyze the relationship of factor production and autonomous proliferation, the degree of density dependent growth and growth inhibition by GM-CSF antibody was determined for cell lines held for only a short time in culture after infection with the GM-CSF transducing vector or after sub-cloning from the D35 cell line. We present evidence to show that autocrine production of CSF increases the rate of a second mutation that abrogates the requirement of external factor.

D 218 The effect of HIV infection on the production of T cell proliferating factor (TPF) by immature human malignant T cell lines. Yoshiki Ueno, Parunag G. Nishanian and Christel H. Uittenbogaart. Department of Microbiology and Immunology, UCLA School of Medicine, Los Angeles, CA 90024.

We have previously shown that the immature T cell lines, MOLT-4f, CCRF-CEM and CCRF-HSB-2, produce two non-IL2 T cell growth factors; an autostimulatory growth factor, Leukemia Derived Growth Factor (LDGF), and a growth factor for normal T cells with a higher molecular weight (40-65K) than LDGF. This growth factor, termed T cell Proliferating Factor (TPF), promotes the growth of T lymphocytes in the presence of accessory cells, but not of thymocytes. TPF is clearly different from IL2 in its molecular weight and biological activity. HIV infection of the immature T cell lines can enhance the activity of TPF produced by these cell lines. In contrast to the lack of growth promoting activity by TPF from non-infected cells, the supernatants from HIV-infected cells do promote the growth of activated thymocytes. This effect is additive to that of recombinant IL2 and only partially reduced by the monoclonal anti-Tac antibody. Although TPF from non-infected cell lines does not significantly induce the IL2 receptor on T cells, the supernatants from HIV-infected cells with TPF activity increase IL2 receptors on activated T cells, but not on thymocytes. These results indicate that IL2 receptor expression is not essential for the growth promoting effect of TPF and TPF-like activity detected in the supernatants from HIV-infected cell lines. Our data suggest that HIV infection of immature T cell lines can change their production of lymphokines.

D 219 RETROVIRAL VECTORS WITH RECOMBINANT LTRs, D. Valerio, C.L. Li, P.M. Wamsley, D. St. Louis and I.M. Verma, The Salk Institute, San Diego, CA 92138

Retroviruses have been used as an efficient gene delivery system in tissue culture cells as well as in normal bone marrow cells. It has been observed, however, that the expression of genes introduced into normal bone marrow cells by retroviral vectors is very inefficient. To investigate whether this observed low level of expression is due to negative influences of the enhancer element present in the Moloney Leukemia Virus (MLV) LTR, we have constructed a retroviral vector in which the MLV enhancer has been replaced by the enhancer of a polyoma virus that has been selected to grow on F9 cells (ΔMo+Py*) (Linney, E. et al. (1984) Nature 308, 470). Helper free retroviruses were generated in which the neo^R gene is under the transcriptional control of either the normal MLV-LTR (Mo-neo), or the ΔMo+Py*-LTR (ΔMo+Py*-neo).

Our study compared the expression obtained with these viruses in: 3T3 cells, F9 cells, MEL cells and normal bone marrow cells. In all cases we found enhanced expression of the neo^R gene when it was introduced with the ΔMo+Py*-neo virus. Efficacy of such retroviruses with recombinant LTRs for expression in a variety of cell types will be discussed.

D 220 ANALYSIS OF THE TRANSFORMING PROPERTIES OF p56*lck*, A NOVEL TYROSINE PROTEIN KINASE. Anna F. Voronova and Bartholomew M. Sefton. The Salk Institute and Department of Biology, UCSD.

We have resently isolated cDNA clones encoding a novel cellular tyrosine protein kinase, p56*lck*, from the LSTRA Moloney murine leukemia virus-induced thymoma cell line (Voronova and Sefton, 1986, Nature **319**:682-685). Nucleotide sequence analysis shows that p56*lck* is encoded in LSTRA cells by a hybrid mRNA; approximately 200 nucleotides at the 5' untranslated region of the mRNA are identical to the 5' end of the genome of Moloney virus, up to the splice donor site for the subgenomic envelope mRNA of the virus. The 3 to 9 fold transcriptional activation of the gene therefore results from retroviral promoter insertion. Comparison of the deduced amino acid sequence of p56*lck* from LSTRA cells with that from normal thymus (Marth et al., 1985, Cell 43:393-404) shows that the proteins are identical. It is possible therefore that the elevated level of p56*lck* contributes to the malignant properties of LSTRA cells. In order to address the question whether p56*lck* can function as an oncogene the complete coding region of the p56*lck* cDNA clone was inserted into the pSV2*neo* and p*neo*MLV expression vectors. Antibiotic G-418-resistant colonies of transfected NIH 3T3 cells have been optained and their properties are now being tested.

D 221 RETROVIRAL MEDIATED TRANSFER OF IL3 AND GM-CSF INTO HEMOPOIETIC CELLS: EFFECTS ON DIFFERENTIATION, FACTOR INDEPENDENCE AND TUMORIGENICITY,
Peter MC Wong[1], Siu-Wah Chung[2], Timothy M Browder[1], Sandra Ruscetti[2] and Arthur W Nienhuis[1], [1]Clinical Hematology Branch, NHLBI, and [2]Lab of Genetics, NCI, NIH, Bethesda, MD 20892.
Recombinant retroviruses containing either the IL3 or GM-CSF coding sequences have been constructed in the N2 vector which contains the neomycin resistance gene (Neo^r) and packaged to yield high titer, helper-free virus stocks in the ψ2 cell line. Infection of fresh day 12 fetal liver cells with either the IL3 or GM-CSF virus but not the parental N2 virus resulted in the formation of various types of G418 resistant colonies including the erythroid-mixed type. These colonies could be detected in the absence of the usually essential IL3 containing mitrogen-stimulated spleen cell conditioned medium (SCM). These data clearly indicate that successful infection of hemopoietic stem cells with the recombinant growth factor (GF)-retroviruses resulted in G418 resistance as well as factor-independent proliferation and differentiation. Colonies from IL3-virus but not GM-CSF-virus infected cultures were able to give rise to immortalized mast cell lines growing in a factor independent manner. These data suggest that the differentiation program of stem cells is influenced by the presence of a partiuclar growth factor. These cell lines were shown to contain the IL3 proviral DNA, to express the viral IL3 mRNA and release IL3 into the medium. Injection of these cell lines into nude mice did not give rise to tumors even 5 months later. In another series of experiemnts factor independent cell lines could also be obtained after infection of secondary immortalized IL3-dependent 32D cells with the IL3 retrovirus. In contrast to the cell lines derived from infection of the primary fetal liver cells, these cell lines are tumorigenic when injected into nude mice. Our data clearly indicate that factor independence, immortalization as well as tumorigenicity are discrete and separable events during the multi-steps process of carcinogenesis. In addition, they may not occur in an orderly manner.

Growth Factors and Receptors

D 300 INDUCTION OF INTERLEUKIN-2 RECEPTORS ON U937 CELLS BY GAMMA INTERFERON: ROLE OF THE HIGH AFFINITY p72 Fc RECEPTOR.
C.N. Abboud, J.L. Liesveld, D.H. Ryan, C.N. Frantz, R.J. Looney, University of Rochester School of Medicine, Rochester, NY 14642.
Recently, gamma interferon (IFN) has been reported to induce interleukin 2 receptors (IL-2R) in myeloid cell lines and peripheral blood monocytes. We and others have reported that murine IgG2a antibodies bind readily to the p72 high affinity FcR identified by the monoclonal antibody mab32, and this receptor is induced by IFN treatment of U937 cells. We investigated the induction of IL-2 receptors on these cells in relation to the expression of the high affinity p72 FcR. Two MoAb to the IL-2 receptor were used: one anti-IL2R (Coulter) is of the IgG2a subclass, and the other is an IgGl (BD) anti-IL2 receptor antibody. U937 cells grown in log phase increased their binding of the IgG2a anti-IL2R antibody when exposed to (1000u/ml) recombinant gamma interferon over 48hrs. This increase was not seen with the IgGl anti-IL2 receptor antibody. The magnitude of the fluorescence increase was similar for control IgG2a FITC antibody as for the IgG2a anti-IL2R. Excess purified RPC5 IgG2a antibody not known to react with U937 completely reversed the increased fluorescence of the control and anti-IL2R IgG2a MoAbs in IFN treated cells and controls. These findings suggested that the IgG2a anti-IL2R MoAb was binding via the p72 high affinity FcR, recognized by the mab32. Similar results were obtained in monocytes. We conclude that the flow cytometric immunofluorescent measurement of the induction of IL2 receptors in U937 cells by gamma interferon, may reflect the increased expression of p72 high affinity FcR and not IL2 binding sites.

D 301 ABROGATION OF INTERLEUKIN-3 DEPENDENCE BY V-FMS IN MYELOID CELLS, David S. Askew*, Esther F. Wheeler†, James N. Ihle*, and Charles J. Sherr†, *NCI-Frederick Cancer Research Facility, BRI-Basic Research Program, Frederick, MD 21701. †St. Jude Children's Research Hospital, Memphis, TN 38105.

The feline oncogene v-*fms* is highly related or identical to the macrophage lineage-specific receptor for CSF-1 (c-*fms*). Since transformation of hematopoietic cells often involves alterations of growth factor requirements, we determined whether v-*fms* could abrogate the factor requirements of myeloid cells which do not express c-*fms*. In these studies an IL-3 dependent myeloid cell line, FDC-P1, was infected with a retrovirus containing v-*fms*, together with the *neo* gene. Cells that were selected only for G418 resistance contained a single copy of the provirus, expressed very low levels of v-*fms*, were non-tumorigenic and were IL-3 dependent for growth. When cells were selected for IL-3 independence, clones were obtained which contained 10-20 copies of proviral sequences, expressed high levels of v-*fms* detectable by Northern or FACS analysis, were tumorigenic and no longer required IL-3 for growth. The IL-3 independent cells continued to express IL-3 receptors at levels comparable to FDC-P1 cells, and they did not produce any growth factors mitogenic for the parental line. Factor independent derivatives maintained the undifferentiated phenotype of FDC-P1 cells with the exception of Thy-1 which was lost. These results demonstrate that high levels of v-*fms* expression can abrogate the requirements of early myeloid cells for their normal growth factors without affecting differentiation.

Research sponsored by the National Cancer Institute, DHHS, under contract NO. N01-CO-23909 with Bionetics Research, Inc.

D 302 IDENTIFICATION AND CHARACTERIZATION OF THE HUMAN G-CSF RECEPTOR, Belinda R. Avalos, David W. Golde and Judith C. Gasson, UCLA School of Medicine, Los Angeles, CA 90024.

Human granulocyte colony-stimulating factor(G-CSF) is a regulatory glycoprotein that stimulates neutrophilic granulocyte colony formation from committed precursor cells in semi-solid medium, as well as induces terminal differentiation with suppression of stem cell renewal in the murine myelomonocytic leukemic cell line WEHI-3B. In addition, G-CSF modulates multiple differentiated functions of mature neutrophils. Using biologically active radioiodinated biosynthetic (recombinant) human G-CSF (generously provided by F. Takaku) we have demonstrated the presence of cell surface receptors for this factor on human neutrophils. We are currently examining the presence and properties of the human G-CSF receptor on acute and chronic myeloid leukemic cells and a variety of established human myeloid cell lines. Studies are also in progress to assess the interaction of G-CSF with GM-CSF at the receptor level, as well as characterize modulation of the human G-CSF receptor by chemotactant ligands and phorbol esters.

D 303 COLONY-STIMULATING FACTORS ENHANCE NEUTROPHIL CYTOTOXICITY TOWARDS HUMAN LEUKEMIA AND VIRUS-INFECTED TARGET CELLS, Gayle Cocita Baldwin, Ina Fabian, Judith C. Gasson and David W. Golde, UCLA School of Medicine, Los Angeles CA 90024.

Purified biosynthetic granulocytic macrophage (GM-CSF) and granulocyte (G-CSF) colony-stimulating factors enhance antibody-dependent cell-mediated cytotoxicity (ADCC) of human neutrophils toward human promyelocytic leukemia cells (HL-60) and HTLV-II-infected human B-lymphoblastoid cells. The stimulation of ADCC is rapid (less than one hour), requires the binding of specific antibody to target cells, occurs at picomolar concentrations of the CSF, but does not require the presence of CSF during killing. We therefore conclude that neutrophils may be targeted by antibody and their killing activity enhanced by CSF in vitro, suggesting a therapeutic role for CSFs in anti-cancer therapy. Currently clinical trials of GM-CSF in AIDS patients are underway. Early data show that neutrophil cytotoxicity is enhanced by GM-CSF in vitro using cells from AIDS patients. In addition, in vitro studies in progress indicate CSFs may also be effective in enhancing the cytotoxicity of DMSO-treated HL-60 and U937 cells towards a variety of neoplastic target cells, including T-cell leukemia, virus-infected, and solid tumor cell lines.

D 304 IN VIVO RECONSTITUTION OF HEMATOPOIESIS IN BABOONS USING 12.8-POSITIVE MARROW CELLS ISOLATED BY AVIDIN-BIOTIN IMMUNOADSORPTION, R.J. Berenson, R.G. Andrews, W.I. Bensinger, D. Kalamasz, G. Knitter, I.D. Bernstein, Fred Hutchinson Cancer Research Center and University of Washington Regional Primate Center, Seattle, WA 98104.
The monoclonal antibody 12.8 recognizes a Mr 115,00 antigen on human and primate hematopoietic progenitors detectable in colony forming assays and long term marrow cultures. Our purpose was to test whether this antigen is expressed by hematopoietic stem cells that can reconstitute hematopoiesis in vivo. In baboons the antibody reacts with 1-4% of marrow cells including blast cells, promyelocytes, immature monocytic cells, and colony forming cells. We used avidin-biotin immunoadsorption chromatography to isolate 12.8+ cells for infusion into lethally irradiated (9.2Gy) autologous recipient baboons. A total of 0.8 - 1.5 x 10^9 marrow cells were labeled successively with 12.8, biotinylated goat antisera to mouse IgM and passed over a column of avi<din-Biogel. We recovered and infused 20 - 27 x 10^6 column adherent cells (representing 1.6 - 2.5% of the starting nucleated cell number) that were 65.0 - 80.3% 12.8+ in three animals. In a fourth animal recovered column adherent cells were further separated by fluorescence activated cell sorting prior to infusion. The four animals achieved granulocyte counts >500/cm^3 and platelet counts >20 x 10^3/cm^3 at a range of 13-22 days following the infusion of 3.9 - 5.0 x 10^6 12.8+ enriched cells/kg. All animals have developed complete hematologic recovery as documented by normal peripheral blood parameters and bone marrow biopsies. We conclude that stem cells which reconstitute hematopoiesis in vivo can be isolated using monoclonal antibody 12.8 and avidin-biotin immunoadsorption. This aproach may be useful for isolating stem cells for marrow transplantation in humans.

D 305 THE INHIBITORY EFFECT OF DIMETHYLSULFOXIDE (DMSO) ON HL-60 GROWTH IS SPECIFICALLY REVERSED BY GRANULOCYTE-MACROPHAGE COLONY STIMULATING FACTOR (GM-CSF). J.K. Brennan, K.S Lee, C. Erickson-Miller, and C.N. Abboud, University of Rochester School of Medicine, Rochester, NY
We have previously found that DMSO inhibits the spontaneous growth of highly passaged HL-60 cells and that GCT cell conditioned medium and a fraction of GCT CM containing both GM and G CSFs restores growth in DMSO-inhibited cultures (Blood 66, S1:126a, 1984). In further studies we have compared the ability of GCT-, Mo-, HL-60-, and RPMI 1788-CMS; highly purified GM-, G-, and M-CSFs from GCT CM, recombinant human GM-CSF (rhGM-CSF) derived from COS cells transfected with the Mo cell GM CSF gene; and recombinant human G-CSF (rhG-CSF) derived from E-coli transfected with the G-CSF gene from 5637 cells, to reverse DMSO inhibition of HL-60 growth in liquid culture. Only sources of GM CSF (GCT CM, Mo CM, GCT GM-CSF, rhGM CSF) reversed inhibition; mediums not containing CSFs (HL-60, RPMI 1788), GCT derived G- and M-CSFs, and rhG-CSF were ineffective by themselves and did not interact with GM CSF. We conclude that DMSO exposure specifically increases the growth requirement of HL-60 cells for GM CSF, a phenomenon which may be useful in studies of GM-CSF-HL-60 interactions.

D 306 IN VIVO INTERLEUKIN-2 RECEPTOR EXPRESSION FOLLOWING IMMUNIZATION-MODULATION BY IMMUNOTHERAPEUTIC AND PHARMACOLOGIC AGENTS, Larry D. Butler, Pamela E. DeRiso, Philip Marder and Maurice E. Scheetz, Lilly Research Laboratories, Indianapolis, IN 46285.
The correlation between Interleukin-2 (IL2) production, Interleukin-2 Receptor (IL2R) expression and T cell proliferation has been well documented with in vitro studies using human or non-human T lymphocytes. However, the normal in vivo physiology of the IL2 system and the relationship to in vivo T cell proliferation is largely not understood. We have developed a murine model system to examine the interleukin 2 system in vivo following antigen sensitization. In this study, we have focussed on IL2R expression and the relationship to T cell proliferation. Following immunization, IL2R expression was examined by cytometry as well as radioligand binding. We have established a kinetic relationship between IL2R expression and T cell proliferation as monitored by ^{125}IUdR uptake. Draining lymph nodes displayed a range of 3-10% IL2R positive cells by cytometric analysis. Treatment of mice with anti-T cell antibodies or an anti-IL2R antibody significantly reduced IL2 receptor expression with differential effects on IUdR uptake. Antibodies with different isotypes are now being tested. Induction of immunologic tolerance specifically reduced IL2R expression relative to the tolerizing antigen. Immunosuppressive agents (cyclosporin A, cyclophosphamide and dexamethasone), all affected ^{125}IUdR uptake and IL2R expression. Our system will now allow examination of IL2R expression in various states of immunoregulatory dysfunction and provide an in vivo test system for specific modulation of immune responses targeted to the IL2R system.

D 307 A RECOMBINANT HUMAN COLONY STIMULATING FACTOR-1 (CSF-1) IS ACTIVE ON BONE MARROW CELLS OF MURINE ORIGIN BUT NOT OF HUMAN ORIGIN. D.P. Cerretti, W. Clevenger, V. Price, J. Wignal, R.J. Tushinksi, D.Y. Mochizuki, H.M. Sassenfeld, D. Urdal, S. Gillis, and D. Cosman. Immunex Corp., 51 University St., Seattle, Wa. 98101.
Colony stimulating factor (CSF-1) is a subclass of colony stimulating factor that is required for the proliferation and differentiation of macrophages from immature hematopoietic progenitor cells. Recently, a cDNA clone, isolated from a library prepared from the human pancreatic tumor cell line, MIA-PaCa-2, was shown to encode CSF-1 by its ability to stimulate murine bone marrow proliferation and macrophage colony formations (Kawasaki, et al. 1985, Science 230:291-296). The CSF-1 cDNA encodes a protein with a predicted molecular weight of 26,000 daltons that is larger than the deglycosylated monomer subunit molecular weight (14,500 daltons) previously attributed to CSF-1 (Das and Stanley 1982, J. Biol. Chem. 254:6226-6228). It was suggested that this size discrepancy was due to the synthesis of CSF-1 as a membrane bound precursor that is post-translationaly cleaved to a smaller mature form. Using the DNA sequence of this CSF-1 cDNA clone, we constructed expression vectors designed to secrete both a full-length form and a truncated form (lacking the transmembrane region) of CSF-1 from yeast cells or COS-7 monkey kidney cells. The recombinant CSF-1 protein secreted by these cells was biologically active as judged by assay on murine bone marrow cells, but surprisingly was inactive when assayed on human bone marrow cells for proliferation and monocytic colony formation. These results indicate that the human CSF-1 cDNA isolated by Kawasaki et al. does not contain all of the information necessary for activity on human bone marrow cells.

D 308 CONFIGURATION AND EXPRESSION OF GENES OF GM-CSF AND G-CSF IN BLAST CELLS FROM PATIENTS WITH ACUTE MYELOBLASTIC LEUKEMIA, G.Y.M. Cheng, C. Kelleher, J. Miyauchi, C. Wang, G. Wong, S. Clark, E.A. McCulloch, M.D. Minden, The Ontario Cancer Institute, Toronto, Ontario, Canada, M4X 1K9
The hemopoietic growth factors GM-CSF and G-CSF, available as recombinant products, stimulate the growth in culture of blasts from patients with Acute Myeloblastic Leukemia (AML). We used a cDNA probe for each gene to study their genomic organization and expression. DNA was examined from the blasts of 22 patients and RNA from the blasts of 18 patients. Abnormalities were found in 4, 2 for G-CSF and 2 for GM-CSF. For the former, abnormal patterns were seen in Southern blots using both Bam H1 and Eco R1 restriction enzymes. For the latter two abnormal bands were seen only after treatment with Bam H1. Fifteen of 18 patients showed no expression of either growth factor on analysis of RNA using Northern blots. Both patients with GM-CSF abnormalities by Southern analysis expressed an abnormally large GM-CSF message but no G-CSF messages. One patient with an abnormal Southern pattern with G-CSF expressed normal sized G-CSF and GM-CSF messages. The biological significance of these findings remain to be determined. Nonetheless, the abnormal Southern patterns may prove to be useful clonal markers in the study of AML.

D 309 HUMAN INTERLEUKINS-1α AND β: IN VITRO EXPRESSION AND MUTAGENESIS. D. Cosman, P. Conlon, A. Larsen, C. Grubin, S. Gillis, S. Dower, and B. Mosley. Immunex Corp., 51 University St., Seattle, Wa. 98101.
The cytokines, interleukin-1α and β, mediate a diverse series of biological phenomena including recently recognized effects on the hematopoietic system, namely radioprotection and hemopoietin 1 activity. Human IL-1α and IL-1β have only 26% amino acid homology, yet bind to the same receptor. It will be of importance to define the active site(s) of the IL-1 molecules in order to understand whether the multiple biological functions are due to multiple structural domains. We have initiated mutagenesis of IL-1α and IL-1β, using an in vitro expression system in which IL-1 cDNAs are cloned downstream of the SP6 promoter. Transcription of these plasmids with SP6 polymerase generates large quantities of IL-1 specific RNA, which is translated in a rabbit reticulocyte lysate system to yield the appropriate IL-1 protein, and assayed for ability to stimulate IL-2 production by the murine T lymphoma cell line LBRM-33-1A5. We have used this system to demonstrate that: 1) full length (30kd) IL-1β is biologically inactive and does not bind to the IL-1 receptor, 2) full length (30kd) IL-1α is fully active and does bind to the IL-1 receptor, 3) core sequences from amino acids 127 to 266 for IL-1α and from amino acids 120 to 264 for IL-1β must be left intact to retain full biological activity. These data suggest that reports of IL-1 activities having molecular weights greater than 20kd are probably attributable to IL-1α rather than IL-1β. It is also apparent that the sizes of the biologically active "cores" of IL-1α and IL-1β are similar, and that further deletions of N or C terminal amino acid sequences result in a major loss of biological activity.

D 310 HUMAN ERYTHROBLASTIC PROGENITORS ADHERE ON EXTRACELLULAR MATRIX COMPONENTS. L. Coulombel, M.H. Vuillet, C. Leroy, G. Tchernia. Laboratoire d'Hématologie, Hôpital Bicêtre, Paris, France.

Extracellular matrix (ECM), a major mediator of adhesion processes, is secreted in marrow long-term cultures (LTC) and has been implicated in hematopoïesis maintenance. In order to investigate if the preferential location of human progenitors in human LTC adherent layer is mediated by ECM components, we tested the ability of human CFU-E, BFU-E and CFU-C to adhere onto ECM prepared from confluent monolayers of purified marrow fibroblasts or umbilical vein endothelial cells. Light density marrow cells were incubated undisturbed on ECM-coated or uncoated dishes in x medium + 5 % serum. After 2 hours at 37° C, cells strongly adherent on ECM or plastic were trypsinised and plated in methylcellulose colony assays. Aliquots from the non-adherent fraction, and from the initial cell suspension were similarly assessed to get reliable counts of CFU-E, mature (day 13) and immature (day 16) BFU-E, and CFU-C. In each of 17 experiments, CFU-E were the most adherent precursors with 26 ± 3 % of initial CFU-E found in the adherent fraction. In the BFU-E category, the high proportion of ECM-adherent mature BFU-E (23 ± 2 %) contrasted with the low adhesiveness of immature BFU-E (10 ± 2 %), thus indicating stage specific expression of precursor adhesion. Moreover, the low proportion of ECM-adherent CFU-C (12 ± 1 %) as compared to CFU-E revealed another type of specificity linked to the cell lineage. Maximum expression of this property by precursor cells was dependent on the temperature, the time of incubation and the presence of divalent cations and serum. No adhesion occured on plastic alone. These results show that human precursor cells are able to selectively adhere on components of the microenvironment. The lineage-and stage-specific expression of this property suggests that it may be an important regulator of hematopoietic cells interactions during differentiation.

D 311 CHARATERIZATION OF THE HUMAN GM-CSF RECEPTOR AND PATHWAYS INVOLVED IN SIGNAL TRANSDUCTION.
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Division of Hematology-Oncology, UCLA, Los Angeles, California 90024.

Human GM-CSF is a 22,000 dalton glycoprotein which induces the growth of myelomonocytic stem cells in vitro and a multitude of more differentiated functions of mature granulocytes including chemotaxis, superoxide generation, bacterial and parasitic killing and tumoricidal activity. The actions of GM-CSF are mediated through a single class of high affinity binding sites on responsive cells (K_D 40pm, 10-1000 sites/cells). Affinity labelling of the human GM-CSF Receptor yielded a complex pf 84k from normal neutrophils and a complex of 155kd from a patient with chronic myeloid leukemia. Binding of GM-CSF to responsive cells does not result in any change in intracellular concentrations of free Ca+, activation or altered cellular distribution of protein Kinase C, changes in cellular phosphorylation, or c AMP. Cross-linking of GM-CSF to its receptors does not result in receptor autophosphorylation. GM-CSF binding up-regulated the expression of high affinity f-met-leu-phe receptors on human neutrophils where as binding of f-met-leu-phe down-regulates the expression of GM-CSF receptors on these same cells. The intramembrane association of the FMLP receptor, the GM-CSF receptor and G-binding proteins is currently under investigation.

D 312 ALTERED EXPRESSION OF TRANSPLANTED LEUKEMIA IN FISCHER RATS PRETREATED WITH 2-ETHOXY ETHANOL (2-EE). M.P. Dieter, J.E. French and S.A. Stefanski. National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709

An in vivo cell transplant model that we developed in Fischer rats results in the expression of leukemogenesis in 95d. The morphology and biochemistry of the leukemic mononuclear cells from blood and spleen were characterized. We have tested the capability of this transplant model to detect negative trends for chemical leukemogenesis using 2-EE. Data for positive leukemogenesis using pyridine is being presented jointly for comparison. Male F344 rats were given 0, 2.5, or 5.0 mg/ml 2-EE in drinking water for 90d. One-half were then injected with leukemic spleen mononuclear cells from syngeneic donors. Dosage was continued, and at 65d the effects of chemical pretreatment on the expression of leukemia were evaluated. There was no mortality, and 2-EE per se, caused no effects in rats without leukemic transplants. At 65d post-transplantation of leukemic cells the average spleen weight of the recipients increased 341% above controls; pretreatment with the low dose of 2-EE reduced this to 102%, while the high dose completely ameliorated the effect. Mononuclear cell counts from spleen and blood corroborated these results. Evaluation of selected tumor marker enzymes, e.g., acetylcholinesterase, glucose-6-phosphate dehydrogenase, and malate dehydrogenase, in spleen and blood mononuclear cells verified that 2-EE also reversed the pattern of biochemical responses typically expressed by these neoplastically transformed cells. These morphological and biochemical indices diagnostic for pre-leukemia were confirmed by histopathological examination of blood, spleen, and liver from each transplant case. The progression of these changes will be evaluated again at 95d and compared to earlier responses.

D 313 PROMOTIONAL PROCESSES AND REPAIR PROCESSES ARE INDEPENDENT IN RADIATION-INDUCED NEOPLASTIC TRANSFORMATION, M. M. Elkind and C. K. Hill, Colorado State University, Fort Collins CO 80523, and University of Southern California, Los Angeles CA 90015.
The phorbol ester, tetradecanoyl-phorbol-13-acetate (TPA), is known to promote neoplastic transformation _in vitro_ following initiation with ionizing radiation [e.g., A.Han and M.M. Elkind, _Cancer Res._, _42_, 477 (1982)] or chemicals. However, some agents effect both processes. We have used mouse C3H 10T½ cells to inquire if the promotional property of TPA is influenced by cellular events which result in the repair of radiation damage. Cells were irradiated at either a high (100 cGy/min) or a low (0.1 cGy/min) dose rate in order to allow for the repair of sublethal damage as well as for the repair of pretransformational damage [A.Han, C.K.Hill, and M.M.Elkind, _Cancer Res._, _40_, 3328 (1980); C.K.Hill, A.Han, and M.M.Elkind, _Carcinogenesis_, _5_, 193 (1984)]. The reduced dose rate resulted in a substantial increase in survival which was accompanied by a reduction in the rate of transformation by some 0.4-fold. TPA added 24 hours after irradiation did not influence the survival of cells but it increased the rate of transformation by some 12-fold after both high and low dose-rate exposures. Thus, the linear dose dependencies of the frequencies of transformation without TPA remained linear with TPA but were some 12 times steeper. These results show a clear separation between initiating events, which are thought to be nuclear, and promotional processes which very likely start at receptor sites on the membrane of the cell. (Work supported by a grant from the U.S. Public Health Service, Department of Health and Human Services, National Cancer Institute, CA 29940, and by a contract from the Department of Energy, No. W-31-109-Eng-38.)

D 314 **INDUCTION OF DIFFERENTIATION OF THE HUMAN LEUKEMIA CELL LINE, KU812,** **Steven A. Fischkoff, John Hakimi, Randall M. Rossi, Michel C. Hoessly, James A. Hoxie, and Kenji Kishi. Hematology/Oncology Section, University of Pennsylvania, Philadelphia, Pennsylvania, Hoffman-LaRoche Research Laboratories, Nutley, New Jersey, and Niigata University, Niigata, Japan.**
The KU812 cell line, isolated from a patient with chronic myelogenous leukemia in blast crisis, is notable for its ability to differentiate to basophils. To attempt to enhance this ability, we treated actively growing KU812 cells with a variety of concentrations of the following compounds: dimethylsulfoxide, retinoic acid, butyric acid, actinomycin D, deazauridine, hypoxanthine, tetradecanoylphorbol acetate, and 1,25-(OH)$_2$-Vitamin D$_3$. After 5 days of culture with butyric acid and also with actinomycin D, the cells became nonproliferative and developed pale cytoplasm and a segmented nucleus with less conspicuous nucleoli. The minimum and optimum concentrations to see this effect were: butyric acid, 0.75 mM and 1.5 mM; and actinomycin D, 0.5 nM and 1.0 nM. In addition, they lost the faint staining for nonspecific esterase seen in the undifferentiated cells. Flow cytometric analysis after treatment with human IgE and FITC-labeled anti-human IgE demonstrated that the proportion of fluorescent cells increased from 7% in control KU812 cells to 26% in cells induced to differentiate with actinomycin D for 5 days. Basophilic granules could not be detected by the Wright stain or by o-toluidine blue metachromasia. The other listed compounds had no apparent differentiation-inducing activity. We conclude that KU812 cells can be specifically induced to undergo terminal maturation with actinomycin D or butyric acid. However, their ability to fully express basophil characteristics appears to diminish with continued passage in tissue culture, and may possibly be a capability of only a subpopulation of the cells.

D 315 GROWTH FACTOR MEDIATED PROLIFERATION IN HUMAN B CELL LYMPHOMA.
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U.T. M. D. Anderson Hospital, Houston, Texas 77030
Human B cell lymphomas are a heterogeneous group of lymphoid neoplasms, roughly corresponding to the various recognized stages in B cell differentiation. We have established long term cell lines from patients representing the spectrum of mature B cell lymphomas using purified human B cell growth factor (BCGF). The cell lines were found to be monoclonal by Ig-light chain typing and Ig gene rearrangements, and to have non-random cytogenetic abnormalities associated with the corresponding histopathologic type of lymphoma. The lymphoma cells were found to not only respond to exogeneous BCGF, but also to secrete a similar growth factor activity in vitro. High MW BCGF activity was also found in the cytoplasm of the tumor cells, that was found to have activity on normal BCGF dependent human B cell lines. Both cytoplasmic and secreted lymphoma cell-derived BCGF had growth activity on authochthanous tumor cells, suggesting that an autocrine-loop mechanism was involved in lymphoma cell growth in vitro. Homogeneously purified cytoplasmic BCGF isolated by immunoaffinity chromatography was found to be biochemically similar to that found in normal T cells. These studies suggest that a neoplastically transformed B cells express the gene(s) for the lineage homologous growth factor that is not expressed in normal B cells which may be utilized for autonomous cell proliferation.

D 316 PHORBOL MYRISTATE ACETATE INDUCES INTERLEUKIN 2-DEPENDENT IN VITRO COLONY FORMATION FROM A PRE-B LEUKEMIC CELL LINE THROUGH EXPRESSION OF INTERLEUKIN 2-RECEPTORS.
Vassilis Georgoulias, Andreas Tsapis, Michèle Allouche, Maria Perraki, Dimitis Thanos, Corinne Clémenceau, Vassilis Choulakis. INSERM U 268, Hôp. P. Brousse, 94800 Villejuif, France; INSERM U 108, Hôp. St Louis, 75010 Paris, France; School of Medicine, Univ. of Crete and Molecular Biology Institute of Crete, 71409 Iraklion, Greece.
The colony growth capacity of leukemic cells from a pre-B cell line (Reh-6) in methycellulose was studied. Reh-6 cells could not generate colonies in the absence of added growth factors. Low concentrations of Phorbol Myristate Acetate (PMA; 1-10 ng/ml) induced colony formation from these cells and recombinant IL2 (rIL2) was capable to enhance the plating efficiency in a dose-dependent manner. This colony-enhancing effect of rIL2 could be inhibited by a monoclonal antibody against the IL2 receptor (IL2-R). However, cell incubation with PMA resulted in a decreased cell proliferation (as assessed by the ^{3}H-Thymidine uptake assay) which could not be enhanced by increasing concentrations of rIL2.
Although Reh-6 cells did not constitutively express IL2-R, PMA induced their expression in a dose-and time-dependent manner. IL2-R expression on Reh-6 cells requires RNA and protein synthesis. Binding experiments with radiolabelled rIL2 showed that these IL2-R displayed a low affinity for IL2. However, IL2-R-bearing Reh-6 cells absorbed specifically rIL2 in a dose-dependent manner, allowing a time-dependent "down"-regulation of IL2-R. These findings indicate that colony formation from pre-B-leukemic cells in methycellulose in the presence of PMA and rIL2 is a specific phenomenon revealing a small number of clonogenic cells bearing low affinity IL2-R.

D 317 CORRELATION OF EXPRESSION OF A NOVEL GROWTH FACTOR AND TUMORIGENICITY OF LYMPHOMA CELLS IN AKR MICE, Esther F. Hays and Shinichi Kitada, University of California Los Angeles, CA 90024.
Studies indicate that cloned tumor cell populations from T cell lymphomas of AKR mice have remarkably different tumorigenic properties. All of the T lymphoma cells produce an autocrine growth factor called lymphoma-derived growth factor (LDGF). This factor is not Interleukin 2 or any known hemopoietic growth factor. It cross-reacts with a similar factor produced by human T leukemia cells. This factor is assayed by its ability to stimulate growth of subthreshold numbers of lymphoma cells in vitro. It is secreted by the lymphoma cells into the surrounding medium and is found intracellularly in both murine and human cells. Studies of four cloned AKR tumor cell lines showed one of high tumorigenicity (SL 12.3), one of intermediate T tumorigenicity (BW 5147.3) and, one of low tumorigenicity (SL 12.4). SL 12.4 was of interest because in continuous culture, revertants of high and low tumorigenicity could be found. There was a positive correlation between local tumor growth, as measured by intradermal inoculation; metastatic tumor growth, as measured by intravenous inoculation and intracellular LDGF activity, as measured by growth stimulation of low doses of AKR T lymphoma cells in serum-free medium. Also when the low tumorigenic line, SL 12.4, reverted to high tumorigenicity, intracellular LDGF activity increased and the threshold for exponential growth decreased from 10^4 cells to 10^3 cells. Thus, we have demonstrated a correlation of intracellular growth factor activity with the ability of the T lymphoma cells to form tumors in syngeneic hosts and to grow in vitro.

D 318 CELL CYCLE ANALYSIS OF AN ENRICHED HEMATOPOIETIC STEM CELL POPULATION, Shelly Heimfeld and Irving L. Weissman, Stanford University, Stanford, CA 94305.
Included among the cells found in the bone marrow of adult mice are the hematopoietic stem cells responsible for the generation of a variety of differentiated cell types, including the cells of the myeloid and lymphoid lineages. Direct analysis of these stem cells has been hampered by their low frequency within the bone marrow. Using a panel of monoclonal antibodies that recognize mature cell subsets in conjuction with fluorescent-activated cell sorting, a population of cells has been defined which contains virtually all CFU-S, pre-B cell, and pre-T cell activity (Muller-Sieberg et al., 1986). Combining this isolation protocol with vital staining using the DNA specific dye, Hoechst 33342 the cell cycle distribution of this stem cell population in normal animals and after a variety of different in vivo and in vitro treatments has been determined.

D 319 DIFFERENTIAL EXPRESSION OF PROTEIN KINASE C ACTIVITY IN THE KG-1 AND KG-1a MYELOBLASTIC LEUKEMIA CELL LINES, W. Craig Hooper, Robert T. Abraham, Curtis L. Ashendel*, and Gayle E. Woloschak, Department of Immunology, Mayo Clinic, Rochester MN 55905 and *Department of Medicinal Chemistry and Pharmacognosy, Purdue University, West Lafayette IN.

The human KG-1 myelogenous leukemia cell line will differentiate into macrophages in response to 12-O-tetradecanoyl-phorbol-13-acetate (TPA), whereas its variant, KG-1a, is nonresponsive. Nonetheless, the KG-1a cell line has been reported to have the same numbe of [3H]-PDBu binding sites as the KG-1 line (1). We have demonstrated significant reproducible differences in total cellular protein kinase C (PKC) activity between the KG-1 ar KG-1a cell lines. These differences were maintained after ion exchange chromatography. After a 15 min. exposure to 50 ng/ml TPA, translocation of the PKC activity to the membrane was observed in both cell lines. Preliminary studies have indicated that whole cell binding of [3H]-TPA to both cell lines was nearly identical. These results togethe with ongoing molecular studies examining modulation of gene expression by TPA in these cells will be presented.

(1) Lehrer, R.I. et al. Cancer Res. 43, 3563 (1983).

D 320 SYNERGISM BETWEEN RECOMBINANT GROWTH FACTORS, GM-CSF AND G-CSF, ACTING ON THE BLAST CELLS OF ACUTE MYELOBLASTIC LEUKEMIA, Colm Kelleher, Jun Miyauchi, Gordon Wong, Steven Clark, Mark D. Minden and E.A. McCulloch, The Ontario Cancer Institute, Toronto, Canada and The Genetics Institute, Cambridge, Mass.

The genes for the hemopoietic growth factors, GM-CSF and G-CSF have been cloned and recombinant material is available for both. We have tested these recombinant factors for their effects on the blast cells of Acute Myeloblastic Leukemia (AML). Culture methods are available that support both colony-formation by AML blasts and the growth of blast stem cells in suspension. Recombinant GM-CSF has been shown to be active in both culture systems, although to a varying degree. We found that recombinant G-CSF was also effective; however the two recombinant factors showed striking synergism for the stimulation of blast growth of cells from 5 of 8 AML patients. In these cases the combination was equivalent to the stimulating activity of supernatants from the continuous cell line HTB9 (HTB9-CM). This conditioned medium is considered the standard for blast growth. Blasts from one of the patients grew without added factor. In another instance, recombinant GM-CSF alone was almost as effective as HTB9-CM. In the third case both recombinant factors were active, but synergism was not seen and their combined effect was not equivalent to that of HTB9-CM. Both GM-CSF and G-CSF were active on normal bone marrow granulopoietic progenitors, but synergism was not observed. We conclude that the marked hetergeneity observed when AML blasts are examined by other criteria is also seen when their response to growth factors is evaluated.

D 321 IDENTIFICATION OF A NOVEL PROTEIN IN HUMAN LEUKEMIA CELL LINES BY SYNTHETIC PEPTIDE ANTISERUM TO THE IMMUNE SUPPRESSIVE DOMAIN OF FELINE RETROVIRAL p15(E). W. Kloetzer, P. MacIsaac, K. Kabat, M. Miller, R. La Polla and J. Warner. Johnson & Johnson Biotechnology Center, La Jolla, CA 92038.

Fresh leukocytes and established cell lines derived from human myeloid leukemia patients secrete several factors which inhibit in vitro growth of normal bone marrow (Leukemia Res 4:437 1980 and 8:387 1984) and mitogen induction of peripheral blood lymphocytes (PNAS 83:3432 1986). Expression of these suppressive factors diminishes in HL60 and K562 cells upon induction of differentiation. Feline leukemia virus (felv) is an RNA tumor virus which can induce aplastic anemia and immune suppression in persistently viremic cats. The virus encodes a transmembrane protein, called p15(E), which may be the principle cause of immune suppression, in that partially-purified p15(E) blocks in vitro mitogen stimulation of spleen cell cultures (J Exp Med 158:885 1983). A 17 amino acid synthetic peptide of murine p15(E) is biologically-active in suppressing mitogen-induced lymphocyte proliferation (Science 230:453 1985). Sequence alignment of p15(E)s from murine, feline and human T cell leukemia viruses shows conservation in the hydrophobic transmembrane and immune suppressive domains.

We have generated a polyclonal rabbit antiserum to a synthetic peptide from the the felv p15(E) region homologous to the murine p15(E) suppressive domain. Electroblot analysis shows that this serum specifically recognizes p15(E) and the envelope precursor polyprotein in felv-infected cells. The antiserum, in a similar analysis, detects a 30,000 to 35,000 mol. wt. protein in cell extracts from the K562, EM2, CEM and Raji human leukemia cell lines. Immunocytochemistry with peptide affinity purified antibody strongly detects antigen in HL60, EM2 and K562 cells, but not control murine NIH/3T3 cells. Phorbol 12-myrisate 13-acetate (PMA) alters expression of the bcr-abl oncogene product (see L. Smith et al abstract) by increasing and decreasing the levels of $P210^{bcr-abl}$ in EM2 and K562 cells, respectively. Electroblot comparison of cell extracts from untreated and PMA-induced cells shows a direct correlation between detection of the 35K protein and $P210^{bcr-abl}$ expression.

D 322 EXPRESSION OF THE MACROPHAGE SPECIFIC COLONY-STIMULATING FACTOR (CSF-1, M-CSF) DURING HUMAN MONOCYTIC DIFFERENTIATION, Donald Kufe and Junko Horiguchi, Dana-Farber Cancer Institute, Boston, MA 02115, M. Kim Warren and Peter Ralph, Cetus Corporation, Emeryville, CA 94608.

We and others have previously demonstrated expression of the c-fms proto-oncogene during human monocytic differentiation. The c-fms gene has since been shown by Sherr et al. to encode for the CSF-1 receptor. Our recent findings demonstrate that both CSF-1 and c-fms transcripts are induced during monocytic differentiation of human HL-60 leukemia cells. In contrast, the c-fms gene is constitutively expressed in normal human monocytes and phorbol ester treatment of these cells is associated with induction of CSF-1 RNA. CSF-1 transcripts are also induced following treatment of human monocytes with GM-CSF. Moreover, the detection of CSF-1 transcripts in HL-60 cells and in monocytes is associated with the production of the CSF-1 gene product. These findings suggest that monocytes are capable of regulating their own survival, growth and differentiation by CSF-1 production.

D 323 B-LYMPHOPOIETIC FACTORS FROM STROMAL CELL LINES ISOLATED FROM MYELOID-LONG TERM BONE MARROW CULTURES. Kenneth S. Landreth and Kenneth Dorshkind, Dept. Microbiology, West Virginia University Medical Center, Morgantown, West Virginia, and Division of Biomedical Sciences, University of California, Riverside, California 92521.

Progenitor cells for B lymphocytes develop in bone marrow (BM) from the same hemopoietic stem cell population which gives rise to myeloid and erythroid cells. There is now considerable information about cell interactions and molecules which regulate hemopoiesis, but we still know little about regulation of B lymphopoiesis. We addressed this problem using long term BM cultures (LBMC). LBMC were initiated under myeloid conditions (M-LBMC, MEM, 20% horse serum, 10^{-6}M hydrocortisone). M-LBMC were depleted of hemopoietic cells at 2 weeks by mycophenolic acid (MPA) treatment. Cell lines derived from MPA treated cultures were capable of supporting M-LBMC. However, when stromal cell cultures were switched to lymphoid permissive conditions (L-LBMC, RPMI-1640, 5% fetal calf serum, 10^{-5}M 2ME), parent cultures and line S17 supported L-LBMC, but S10 did not. Conditioned media (CM) from stromal cell cultures were added to cultures of BM cells depleted of B cells and pre-B cells on antibody coated polystyrene dishes (anti-Ig and 14.8 respectively). Pre-B cells were generated in cultures potentiated with S17 CM or mixed stroma CM, but not when S10 CM was added. Chromatographic separation of CM by HPLC suggests that (1) pre-B cell generating activity resides in at least two distinct peaks by gel permeation separation, (2) both mixed stroma cultures and S17 appear to make these activities, and (3) the failure of S10 CM to potentiate pre-B cell generation does not result from inhibitory factors. (Our work is supported, in part, by NIH grants AI-23950, AI-21256, and HL-36951).

D 324 EXPRESSION AND CHARACTERIZATION OF GENES ENCODING HUMAN MYELOID MEMBRANE ANTIGENS AFTER DNA-MEDIATED GENE TRANSFER. A. Thomas Look, Stephen C. Peiper, Richard A. Ashmun and Charles J. Sherr. St. Jude Children's Research Hospital, Memphis, TN. 38105.

We have isolated the genes encoding five cell surface molecules expressed by human myeloid cells after DNA-mediated gene transfer of human cellular DNA into NIH 3T3 mouse fibroblasts. Recipient cells that incorporated and expressed amplified levels of the human genes were isolated by fluorescence-activated cell sorting with use of monoclonal antibodies directed to the gene products of interest. The human myeloid polypeptide antigens (with the antibodies used for cell sorting) that have been expressed by mouse fibroblasts are (1) gp150 (MY7) expressed by human CFU-GM, monocytes, granulocytes and myeloid leukemias; (2) p67 (MY9) expressed by CFU-GEMM, CFU-GM, BFU-E, monocytes and myeloid leukemias; (3) gp115 (MY10) expressed by CFU-GEMM and their precursors, CFU-GM, BFU-E, CFU-Meg and both lymphoid and myeloid leukemias; (4) gp55 (MY4) expressed by monocytes and monocytic leukemias; and (5) gp85 (5F1) expressed by CFU-E, monocytes, platelets and myeloid leukemias. Using human repetitive DNA sequences as a probe, we have molecularly cloned portions of the gene encoding gp150 from DNA of tertiary mouse cell transformants selected for high levels of gp150 expression. Molecular subclones were then used to isolate the complete gp150 gene (35 kb) from a human placental DNA library. The intact gp150 gene, assembled from three recombinant phages, proved to be biologically active when transfected into NIH 3T3 cells. The genes encoding gp150 and other myeloid cell surface glycoproteins are ideal candidates for studies of lineage-restricted transcriptional regulation by normal and malignant human myeloid cells.

D 325 REGULATION OF PDGF AND TGFB DURING MEGAKARYOCYTIC DIFFERENTIATION OF CML CELLS, A.R. Lopez, T. Pantazis, H. Antoniades, and A. Deisseroth, Department of Medicine, UCSF, San Francisco, CA and Blood Research Center, Harvard Medical School. Chronic Myelogenous Leukemia, (CML), which begins as an excess of circulating mature myeloid cells, evolves into an acute leukemic syndrome, called blast crisis, which may exhibit features of myeloid, lymphoid, erythroid, or megakaryocytoid differentiation. In order to further characterize the regulation of lineage specific genes in blast crisis CML, we studied the expression of two platelet proteins: platelet derived growth factor (PDGF) and tumor derived growth factor beta (TGF-Beta), before and after exposure to the phorbol ester TPA. (TPA induces differentiation of K562 cells into megakaryocyte like cells). Poly A+ mRNA was isolated from the K562 CML blast crisis cells before and after the cells were exposed to TPA for 18 hours. Cells were also exposed 18 hours to TPA following which the cells were exposed to cycloheximide, which has been shown to stabilize mRNA. Northern transfer and RNA slot blot analysis showed that the levels of PDGF and TGF-B mRNA were increased 40-fold during induction of megakaryocytic differentiation by TPA. Analysis of ^{35}S-cysteine labeled proteins from TPA treated chronic phase and blast crisis cells showed that TPA stimulated the secretion of a protein, identical to PDGF with respect to molecular weight, PDGF receptor binding, immunoprecipitation and mitogenic activity. These data suggest that the proteins PDGF and TGF-B are normally regulated during induction of megakaryocytic differentiation in the chronic phase and blast crisis CML cells.

D 326 REGULATORY ROLE OF IL-3 ON HEMATOPOIETIC PRECURSOR CELLS. J. P. McKearn and J. A. McCubrey. E. I. du Pont de Nemours & Co., Glenolden, PA 19036. Multipotent precursors of all myeloid/erythroid lineages as well as multipotent B lymphocyte precursors can be selectively enriched with monoclonal antibody (mab) AA4, a mab which recognizes committed B cell precursors but not mature B cells. When liquid cultures are supplemented with IL-3, B lymphocyte precursor clones can be grown long-term. These cells remain factor-dependent and they possess properties of multipotent B stem cells. We have begun to characterize requirements which dissociate growth- and differentiation-promoting signals for IL-3 responsive cells. Our rationale in these studies involve: 1) mechanism of action of IL-3 and other bioactive ligands, 2) accessory cell requirements for growth and/or differentiation, 3) selective abrogation of factor-dependency via retroviral (v-onc) gene infection and the consequence of such events and 4) biochemcial characterization of novel membrane glycoproteins expressed by precursor clones. Our results indicate that IL-3 acts as a competence and progression factor with a well-defined activation sequence which can include rapid changes in membrane potential, increases in oxidative phosphorylation enzymes and less rapid increases in total cell volume. A distinct set of proto-onc genes are activated by IL-3 as cells transit from a G_0 resting state. Stable integration of the v-abl gene, but not v-mos or v-src, abrogate the requirement for exogenous IL-3 without endogenous production. Biochemical analysis of membrane glycoproteins expressed by multipotent IL-3 dependent clones and committed B cell precursors will also be discussed.

D 327 FACTORS INFLUENCING THE GROWTH OF CLONOGENIC LYMPHOMA CELLS IN CULTURE. Hans A. Messner, Mary Tweeddale, Mark D. Minden, Nazir Jamal, Anh Nguyen. Ontario Cancer Institute, Toronto, Ontario, Canada, M4X 1K9
A culture system has been described that promotes growth of clonogenic lymphoma cells from bone marrow, peripheral blood, and lymph node samples of some patients with intermediate and high grade malignant lymphoma. These colonies can be propagated as EBV negative cell lines that show the same immunoglobulin rearrangement pattern as the primary tumor sample. Colony formation in some patients is dependent upon exogenous growth factors; some patients form colonies without their addition. In our system growth factors were added in form of PHA-LCM. Growth factor dependent lymphoma cell lines were used as target cells to identify and characterize growth promoting activities. They are produced by normal adherent cells and mitogen stimulated T-cells. Partially purified BCGF (12Kd) replaced the requirement for PHA-LCM of some of the clonogenic cells; recombinant G- and GM-CSF were inactive in the system. Factor independent lymphoma cell lines, when used in increasing concentrations as irradiated feeder cells, promoted colony formation of factor dependent target cells in a dose dependent fashion. Factor dependent cell lines were inactive. Factor independent lymphoma cell lines released activities into their supernatant that completely replaced the requirement for PHA-LCM of factor dependent target cells; supernatants of factor dependent cell lines did not contain any stimulatory activities. Single cells of factor independent cell lines were plated individually without addition of further growth factors or feeder cells. Colonies derived from individual cells were observed indicating an autocrine release of growth promoting activities by lymphoma cells.

D 328 AN ANALYSIS OF SOLUBLE INTERLEUKIN-2 RECEPTORS IN PATIENTS WITH LYMPHOPROLIFERATIVE DISORDERS. David L. Nelson, Metabolism Branch, NCI, NIH, Bethesda, MD 20892.
Using two monoclonal antibodies (anti-Tac and 7G7/B6) directed against non-overlapping epitopes on the human interleukin-2 receptor (IL-2R), a "sandwich" Enzyme-Linked Immunoassay (ELISA) has been developed for the quantitative measurement of soluble IL-2R in sera. Normal individuals (n=33) were found to have a low, but measurable, serum level of IL-2R (mean=186, range 83-335). Serum from patients in the United States with the HTLV-1 associated adult T-cell Leukemia (ATL) had high levels of serum IL-2R (mean=35,000, range 9,500-69,500) and similar results were obtained with sera from 21 Japanese ATL patients. Significantly elevated levels (>20 times normal) were also found in the sera of patients with hairy cell leukemia (HLC). Favorable responses to therapy were associated with reductions in the level of soluble IL-2R in patients with ATL and HCL. Elevated levels of serum IL-2R were also observed in certain patients with Hodgkins disease, the Sezary syndrome, and chronic lymphocytic leukemia. Among patients with undifferentiated (n=58) and lymphoblastic (n=18) lymphoma, serum levels of IL-2R <1,000 U/ml had a predicted long term survival of 84% compared to 36% for those with levels >1,000. Measurement of soluble IL-2R in sera may be useful in monitoring disease activity and also of prognostic significance in patients with leukemias and lymphomas.

D 329 IDENTIFICATION OF REGULATORY SEQUENCES IN THE 5' FLANKING REGION OF GM-CSF GENE, S.D. Nimer, J. Chan, D.W. Golde, J.C. Gasson, UCLA School of Medicine, Los Angeles, CA 90024
The regulation of lymphokine gene expression in activated T lymphocytes has been under intense investigation. Human granulocyte-macrophage colony stimulating factor (GM-CSF), like interleukin-2 and γ-interferon, is produced by activated but not resting T cells. Though GM-CSF is also produced by cytokine-stimulated fibroblasts and endothelial cells, constitutive production of GM-CSF by normal human cells has not been observed. To identify the regulatory sequences responsible for the increased expression of GM-CSF in activated T lymphocytes, we prepared recombinant constructions linking 660bp of 5' flanking sequences of the GM-CSF gene to the marker gene CAT. We have previously demonstrated that this 660 bp fragment could direct increased expression of CAT in stimulated T-lymphoblast cells, suggesting the presence of T cell-specific regulatory sequences. We have prepared various deletion mutants and using our transient transfection system, have determined that deletion of 460 bp from the 5' end of this 660 bp region does not abolish the increased CAT activity seen with stimulation by PHA and PMA. Thus a region of 150 nucleotides upstream from the CAP site of the human GM-CSF gene contains sequences involved in enhanced GM-CSF expression in activated T cells. Additional deletion mutants are currently being characterized.

D 330 **MULTIVARIATE FLOW CYTOMETRIC ANALYSES FOR IDENTIFICATION OF PROBES TO DISCRIMINATE LOW FREQUENCY HEMOPOIETIC SUBPOPULATIONS,** **Maria G. Pallavicini, Per N. Matsson, Leslie J. Summers, Larry H. Stanker, and Joe W. Gray, Biomedical Sciences Division, Lawrence Livermore National Laboratory, Livermore, CA 94550.**
We are using multivariate flow cytometry and sorting to identify antibodies which allow discrimination of low frequency murine clonogenic hemopoietic subpopulations. A cocktail of monoclonal antibodies conjugated with fluorescein (FITC), is being developed to positively mark non-clonogenic cells. Antibodies are selected in two steps. First, antibodies that detect unique subpopulations are identified during bivariate flow cytometric analyses of antibody cocktail vs. hybridoma supernatant (antibodies indirectly labeled with phycoerythrin (PE)). Second, the reactivity of antibodies selected in step #1 with clonogenic cells is determined. Antibodies that do not react with clonogenic cells are added to the cocktail. Those that do are evaluated further by indexed cell sorting. Indexed cell sorting allows precise mapping of the antibody binding profile of individual clonogenic progenitors (CFU-GM, BFU-E, etc.). Ultimately cocktail antibodies binding to non-clonogenic cells will be labeled with FITC and those positive for clonogenic cells will be labeled with a different fluorochrome to allow discrimination of clonogenic subpopulations. Our cocktail now consists of 5 antibodies that label 95% of nucleated cells, and <5% of the clonogenic precursors. Flow cytometric analyses and sorting of bone marrow cells with low antibody-cocktail-FITC fluorescence, high wheat germ agglutinin-PE fluorescence and high forward angle light scatter allows us to obtain 100-fold enrichment in CFU-GM, with greater than 70% of all CFU-GM. Discrimination of clonogenic cells will improve as new antibodies are added to the cocktail. **This work was performed under the auspices of the U.S. Department of Energy by the Lawrence Livermore National Laboratory under contract number W-7405-ENG-48 and NIH grants CA 25782 and GM 33016.**

D 331 CHARACTERIZATION OF THE HIGH AFFINITY CELL SURFACE RECEPTOR FOR MURINE B CELL STIMULATING FACTOR-1. Linda S. Park, Kenneth Grabstein, and David L. Urdal, Immunex Corp., Seattle, Wa., 98101.

^{125}I-labeled recombinant murine BSF-1 was used to characterize receptors for this lymphokine on the surface of primary B cells, primary T cells, and *in vitro* cell lines representing the B-cell, T-cell, mast cell, macrophage and myelomonocytic lineages. On all cell types examined, BSF-1 bound to a single class of high affinity receptor (< 2000 receptors/cell) with a K_a of 10^{10} - 10^{11} M^{-1}. Receptor expression on resting primary B and T cells was found to be exceedingly low (< 100 receptors/cell), while activation of B cells with LPS and T cells with Con A produced a 5-10 fold increase in receptor number. Extensive characterization of the BSF-1 receptor on IL-3 dependent FDC-P2 and IL-2 dependent CTLL cells showed that binding was rapid and saturable at both 37°C and 4°C with a slow dissociation rate. Among a panel of lymphokines and growth hormones, including IL-2 and IL-3, only labeled BSF-1 was able to compete for binding of ^{125}I-BSF-1. In, addition, an anti LFA-1 monoclonal antibody (M17/5.2) directed against the α-chain of LFA-1 did not inhibit ^{125}I-BSF-1 binding. Affinity crosslinking experiments with the homobifunctional crosslinker Bis(sulfosuccinimidyl)suberate resulted in the identification of a receptor protein with an average M_r of 75,000 on FDC-P2, CTLL, 32D, P815 and BCL-1 cells. These studies show that the BSF-1 receptor exhibits very similar properties on cells of B-cell, T-cell, and hematopoietic origin, demonstrating that the ability of BSF-1 to mediate a diverse array of biological events is not due to overt differences in the receptor for this lymphokine on different cell types.

D 332 INTERLEUKINE 2 PRODUCTION IN LYMPHOMAS. - B. PEGOURIE, M.C. JACOB, T. BONNEFOIX, M.P. PICCINNI, J.J. SOTTO. Laboratoire de Recherche sur les lymphomes - CHRUG - BP 217 X - 38043 GRENOBLE Cédex - FRANCE.

Interleukine 2 (Il_2) production by lymph node cells was investigated in 9 patients with B lymphomas. Cell suspensions were prepared by teasing lymph node in tissue culture medium. It was then filtered through sephadex G 10 to remove adherent cells. Cells were rosetted using sheep red blood cells (SRBC) and then separated into SRBC rosette positive (E+) or negative (E-) populations on ficoll-hypaque gradient. Initial cell suspensions, E+ cells and E- cells were cultured 2 days (10^6 cells/ml) in the presence of phytohemagglutinin (PHA) (5γ/ml) and in the absence of PHA. Il_2 was assayed using the murine Il_2-sensitive cell line $CTLL_2$.

When cells were cultured with PHA, mean Il_2 activity produced by 7 healthy patients' total and E+ peripheral blood mononuclear cells (PBMC), used as controls, were respectively 31 ± 6 and 33 ± 21 u/ml. Il_2 activity produced by cells from 3 hyperplasic lymph nodes was similar to controls. Il_2 activity produced by E+ cells from lymphomas was heterogeneous : 5 times, it was very high (more than 55 u/ml), twice, it was similar to Il_2 activity produced by E+ PBMC, and twice, no activity was observed which was never noticed among controls.

When cells were cultured without PHA, no activity was observed in any case.

D 333 DIFFERENTIAL EXPRESSION OF CLASS II MHC ANTIGENS AND RESPONSE TO GROWTH REGULATORS IN SUBPOPULATIONS OF HUMAN HEMATOPOIETIC PROGENITOR CELLS. Louis M. Pelus, Oliver G.Ottmann, Yee-Pang Yung. Sloan Kettering Institute, New York, N.Y. 10021.

Expression of Class II antigens HLA-DR,-DP and -DQ on CFU-GM, BFU-E, CFU-GEMM, CFU-Eos and basophil/mast cells was examined. Marrow progenitor cells were enriched 10-12 fold by depletion of mature myeloid, monocytic, lymphoid and erythroid cells by immunoadherence and labelled with anti-HLA-DR,-DP or -DQ. In a sorting window of low perpendicular and intermediate forward light scatter encompassing all precursors (blast window), 80-90% of cells were DR+,30-40% DP+ and 0-2% DQ+. This population was then sorted into DR-, total DR+, intermediate intensity DR++ and high intensity DR+++ fractions, as well as DP+ and DP- fractions. Sorting and C' cytotoxicity data indicate that ~95% of progenitor cells are DR+, with differing intensity, whereas DQ was undetectable. The plating efficiencies for d7 and d14 CFU-GM were similar for DR+++,DR+,DP+ and DP- fractions. Using r-human G-CSF, d7 CFU-GM segregated with DR++ and d14 CFU-GM with the DR+++ fractions. Sensitivity of sorted CFU-GM to inhibition by prostaglandin E (PGE) increased with DR intensity (DR- 0%I; DR++ 15%I; DR+++ 67%I). Erythropoietin responsive BPA dependent and independent BFU-E were present in the DR+ DP- fraction. Enhancement of BFU-E by PGE was associated with higher DR intensity. CFU-GEMM could not be further separated based upon DP. Precursors for basophil/mast cells were enriched 14 fold in the DR++ fraction and segregated predominantly to the DP- fraction while CFU-Eos were enriched in the DR+ and DR++ fractions but segregated to the DP+ fraction. In conclusion, differential expression of Class II antigens define subpopulations of progenitor cells and their responses to growth stimulators and inhibitors.

D 334 NEOPLASTIC TRANSFORMATION OF AN INTERLEUKIN-3-DEPENDENT HEMATOPOIETIC CELL LINE CORRELATES WITH THE ABROGATION OF FACTOR DEPENDENCE, J. E. Pierce*, J. Falco*, S. A. Aaronson*, and J. E. Greenbergert, *National Cancer Institute, Bethesda, MD 20892; and †University of Massachusetts Medical Center, Worcester, MA 01605.
The long-term murine basophilic cell line, 32D, is interleukin-3 (IL-3) dependent for growth and nontumorigenic. IL-3-independent variants were isolated by two approaches to assess the mechanisms by which growth-factor dependence can be subverted and to determine whether the acquisition of factor-independence correlates with conversion to a leukemogenic phenotype. One series of autonomous clones was obtained by infection of 32D cells with the Abelson-murine leukemia virus (Abelson-MuLV) which reproducibly converted 32D cells to factor independence at a high frequency. A second set was obtained by cultivating 32D cells at a high density in medium lacking IL-3. Spontaneous acquisition of IL-3 independence occurred at a low frequency and only three lines were obtained. All of the IL-3-independent clones were shown to be tumorigenic in nude mice and grew in soft agar. By contrast, the parental IL-3-dependent 32D line remained nontumorigenic and did not grow in soft agar. None of the Abelson-MuLV and only one of the three spontaneous variants autogenously produced IL-3, as determined by Northern analysis. These results suggest that progression of a hematopoietic cell regulated by a particular growth factor network to a leukemogenic state may be invoked by an autostimulatory mechanism but does not necessarily depend on this process for induction of the neoplastic state. Additional studies are now in progress to determine if release of a second growth factor, IL-4, might be responsible for the acquisition of factor independence by the other variants.

D 335 CLONING AND TISSUE-SPECIFIC EXPRESSION OF MOUSE MACROPHAGE COLONY STIMULATING FACTOR mRNA, Tripathi B. Rajavashisth, Ronald Eng, Richard K. Shadduck, Abdul Waheed, Chad M. Ben-Avram, John E. Shively and Aldons J. Lusis, Departments of Medicine and Microbiology and Molecular Biology Institute, UCLA, Los Angeles, CA 90024.
Macrophage colony stimulating factor (CSF-1) stimulates the production of macrophages from bone marrow progenitor cells. We have identified a cDNA clone for murine CSF-1 by antibody screening of a mouse L-cell cDNA library in the expression vector λgt11. A screen of about 150,000 recombinant plaques yielded six clones which reacted well with an antibody raised against denatured and reduced mouse L-cell CSF-1. These clones were further screened with synthetic oligonucleotides based on the amino terminal amino acid sequence of CSF-1. One clone which hybridized to the oligonucleotides was sequenced and found to contain a single open reading frame. This encompassed 68 amino acids of the mature protein, including the entire amino terminal sequence we previously reported. This is preceeded by what appears to be a 31 amino acid signal peptide. Northern blotting analysis showed that this cDNA hybridizes to a major mRNA species of about 4.5 kb as well as several small mRNA species (3.8,2.3 and 1.4 kb) present in mouse L-cells. Striking differences in the qualitative and quantitative expression of mRNA species for CSF-1 were observed in various mouse tissues. Liver expressed primarily a 1.4 kb species, heart and lung expressed primarily a 4.5 kb species, brain expressed high levels of both the 4.5 kb and 1.4 kb species, and intestine lacked detectable CSF-1 transcripts. Southern blotting analysis suggests that the CSF-1 gene is present as a single copy in the mouse haploid genome and that it is not rearranged or amplified in L-cells. Thus, the multiple transcripts arise as a result of alternative processing.

D 336 GRANULOCYTE-MACROPHAGE COLONY STIMULATING FACTOR (GM-CSF) COUNTERACTS ANTIPROLIFERATIVE AND DIFFERENTIATION-INDUCING EFFECTS OF CHEMICAL AGENTS ON K562 HUMAN LEUKEMIA CELLS. P.T.Rowley, J.M.Leary*, B.F.Farley*, S.LaBella*, and R.Giuliano*, Depts. Medicine and Pathology, Univ. of Rochester, Rochester, NY 14642
We have analyzed the effects of GM-CSF on a human leukemic cell line in conjunction with known chemical inducers. K562 human leukemic cells were chosen because they can be induced to display features of erythroid, granulocytic, monocytic and megakaryocytic differentiation and their erythroid differentiation can be conveniently monitored by benzidine positivity. K562 cells (passage 191) were cultured at 5 x 10^4 cells/ml in RPMI 1640-10% fetal bovine serum. Without other additives, cells grew exponentially to reach 80 x 10^4 cells/ml with 1% benzidine positive cells in 3 days. With thymidine-hypoxanthine (each 10^{-4} M), growth arrested at about 20 x 10^4 cells/ml and 64% of the cells were benzidine positive. However, if GM-CSF (10-20 u/ml) was also present, growth continued to 40 x 10^4/ml with only 25% benzidine positive cells. A shorter (4h), higher (32 x 10^{-4}M each) thymidine-hypoxanthine exposure also arrested growth at about 40 x 10^4 cells/ml with 35% benzidine positive cells in 4 days. But if GM-GSF was also present throughout, growth reached 70 x 10^4 cells/ml and benzidine positivity was only 12%. Daunorubicin (1.9 x 10^{-6}M, 1 h exposure) completely prevented growth and induced 77% benzidine positive cells in 2.5 days. But if GM-CSF (2 u/ml) was present during exposure, growth reached 70% of normal and benzidine positivity was only 18%. Analysis of the absolute numbers of benzidine positive and negative cells revealed that GM-CSF had an effect solely on the number of benzidine negative cells. GM-CSF also caused a decrease in the percentage of cells recognized by granulocyte-specific antibodies RIB-19, B4.3, UJ308, and AHN-1. These studies demonstrate that GM-CSF antagonizes the effects of several chemical inducers on K562 cells. It antagonizes both the restriction of growth and the appearance of differentiated characteristics, viz. hemoglobin and granulocyte antigens.

D 337 EVIDENCE FOR TWO EXTRACELLULAR DOMAINS IN THE HUMAN INTERLEUKIN-2 RECEPTOR: LOCALIZATION OF IL-2 BINDING.

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The human interleukin-2 receptor from HUT102B2 cells was quantitatively cleaved into two large disulfide-bonded fragments by either trypsin or endoproteinase lys-C. The smaller fragment contains both N-linked oligosaccharides found in the intact receptor and is derived from the amino-terminus of the molecule. The larger proteolytic fragment was metabolically labeled with $^{32}PO_4$ and represents the carboxy terminus. The predicted cleavage sites of both lie in the region of the molecule encoded by exon 3. This pattern of limited proteolysis provides biochemical evidence that the extracellular region of the receptor is organized into two domains. This supports a structural model of the receptor in which the regions of internal homology encoded by exons 2 and 4 form separate domains connected by a hydrophilic segment. To determine the role of these domains in IL-2 binding, [^{125}I] IL-2 was chemically crosslinked to the proteolytically cleaved receptor on the cell surface. The ^{125}I-labelled complex obtained displayed N-linked oligosaccharides and had an apparent M_r consistent with one molecule of IL-2 crosslinked to the smaller proteolytic fragment of the receptor. Thus, the amino-terminal domain of the IL-2 receptor appears to form an integral part of the IL-2 binding site.

D 338 HUMAN B CELL GROWTH FACTOR DEPENDENT REGULATION OF THE C-MYC AND IMMUNOGLOBULIN GENE EXPRESSION IN HUMAN MALIGNANT B CELLS.

Surendra Sharma[1], S. Mehta[1], J. Jackson[1], S. Jannath[2], and R. Ford[1], Department of Molecular Pathology[1], and Bone Marrow Transplantation[2], U.T. M.D. Anderson Hospital, Houston, Texas 77030

It is now clear that a 12-14 kd T cell-derived human B cell growth factor (BCGF) promotes cell-cycle progression and S-phase entry of antigen activated B cells. In case of lymphoid malignancies of B cell origin, it is invariably found that cells have either lost dependence on T cell-derived growth factors or they do not require antigen activation to respond to these growth factors. Moreover, malignant B cells have recently been shown to produce a high molecular weight (60 kd) BCGF which may support the autocrine growth. Therefore, an uncontrolled growth in malignant cells implies that dependence on T cell-derived B cell growth factors may be a prerequisite for normal growth. To study the molecular events which may contribute to the transition from a controlled to an uncontrolled cell growth, we have studied the effects of the human BCGF on the expression of genes whose gene products have been associated with cell proliferation (c-myc and β-interferon) and differentiation (immunoglobulin). B cells from patients with diffuse large cell lymphoma are used for these studies. Our preliminary results indicate that T cell-derived BCGF may play a different role than the autocrine high molecular weight BCGF.

D 339 *C-KINASE ACTIVATORS ALTER P210$^{bcr-abl}$ EXPRESSION IN CELL LINES DERIVED FROM CHRONIC MYELOGENOUS LEUKEMIA PATIENTS.*

L. Smith, K. Kabat, R. Arlinghaus and W. Kloetzer. Johnson & Johnson Biotechnology Center, La Jolla, CA 92038.

Synthetic peptides from the predicted amino acid sequence of the bcr-abl gene product (Nature 315, 550 1985) were used to generate antisera that recognize the bcr and abl domains of P210$^{bcr-abl}$. Rabbit polyclonal antisera and murine monoclonal antibodies to peptide:KLH conjugates detected P210$^{bcr-abl}$ in two cell lines established from leukocytes of chronic myelogenous leukemia patients: K562 (from an erythroleukemic blast crisis patient) and EM2 (from a myeloid blast crisis patient). The antibodies recognized bcr-abl gene products, as detected by in vitro autophosphorylation, metabolic incorporation of [^{3}H]leucine and immunocytochemistry. Changes in expression of enzymatically-active bcr-abl gene products were detected upon exposure to the tumor promotors phorbol 12-myristate 13 acetate (PMA) and mezerein. Continuous incubation of K562 cells in growth medium containing 10nM PMA inhibited cell growth and turned off the expression of P210$^{bcr-abl}$ within 48 to 72 hours. Similar treatment of EM2 cells stimulated cell growth and increased the level of P210$^{bcr-abl}$ beginning 12 hours after exposure. Very brief exposure of EM2 cells to PMA (as little as 5 minutes) was sufficient to initiate the chain of events leading to altered P210$^{bcr-abl}$ expression. This activation step was essentially irreversible, at least within cells directly exposed to PMA, since EM2 cells briefly exposed to PMA did not revert to untreated levels of P210$^{bcr-abl}$ kinase activity for several weeks. Mezerein, another activator of C-kinase with weak tumor promoting activity, induced a similar change in P210$^{bcr-abl}$ expression. We conclude that very brief exposure to PMA is sufficient to induce irreversible alterations in the steady state levels of P210$^{bcr-abl}$ in CML cell lines. We suggest that the changes in P210$^{bcr-abl}$ expression upon exposure to C-kinase activators are strongly influenced by the specifically induced hematopoietic cell lineage.

D 340 RF/J MICE ARE DEFICIENT IN MULTI-CSF (IL-3) RESPONSIVE BONE MARROW COLONY-FORMING CELLS. Ted Albert Torrey and Thomas B. Tomasi. University of New Mexico, Albuquerque New Mexico, and Roswell Park Memorial Institute, Buffalo New York.
Normal mouse bone marrow contains committed progenitor cells capable of forming colonies in soft agar in response to various colony-stimulating factors (CSFs). Multi-CSF (a.k.a. IL-3; interleukin-3) induces the formation of colonies consisting of pure clones or mixtures of macrophages, granulocytes, erythroid bursts or undifferentiated blast cells from normal bone marrow. Bone marrow from the myeloid leukemia-prone RF/J mouse strain was found to produce no colonies in response to cell conditioned medium containing multi-CSF, but did produce colonies in response to conditioned media containing GM-CSF (granulocyte - macrophage CSF) or M-CSF (macrophage CSF; CSF-1). Purified multi-CSF likewise failed to stimulate RF/J bone marrow colony formation, while purified GM-CSF readily promoted colony formation. Therefore the RF/J strain bone marrow cells are deficient in multi-CSF responsive colony formation, and not simply sensitive to various colony inhibiting factors potentially present in the conditioned medium. The possible relationship between this observation and the RF/J mouse predisposition to myeloid leukemia will be discussed.

D 341 REVERSAL OF DEXAMETHASONE HEMOPOIETIC SUPPRESSIVE EFFECTS BY INTERLEUKIN-1. Robert Tushinski, Eric Spoor and Diane Mochizuki, Immunex Corp., Seattle, Wa. 98101.
Glucocorticosteriods have been shown to have suppressive effects on immune responses and hemopoietic cells. We have confirmed that dexamethasone is capable of suppressing bone marrow colony forming ability in response to granulocyte/macrophage (GM) and macrophage (CSF-1) stimulating factors. Recent studies have revealed that recombinant interleukin-1 (IL-1) abrogates the suppressive effects of pharmacological concentrations (10^{-6} M - 10^{-7} M) of dexamethasone on murine bone marrow colony forming ability in response to both CSF-1 and GM-CSF. IL-1 alone does not stimulate bone marrow colony formation. IL-1 is not acting through T-cell or accessory cell stimulation of CSF production, since the experiments were carried out in the presence of excess CSF-1 or GM-CSF. Experiments using marrow from 5-Fluorouracil treated mice strongly suggests that IL-1 is acting at the level of immature cells (CFC or earlier). A model of IL-1 regulatory effects on hematopoiesis will be presented.

D 342 PROMOTER ANALYSIS IN HL-60 CELLS, Mark L. Tykocinski, Robert R. Getty, and Christopher A. Hauer, Case Western Reserve University, Cleveland, OH 44106.
The human leukemia (M-3) cell line HL-60 can be chemically induced to differentiate along neutrophilic, monocytic or eosinophilic pathways. As a prelude to sense and anti-sense RNA transfection analyses of the HL-60 differentiation program, we have optimized transfection procedures and evaluated promoter activities in these cells. Protoplast fusion was optimally performed by co-pelleting suspended protoplasts and HL-60 cells (5000:1 protoplast:target cell ratio) in the presence of 50% PEG 1540. The function of a panel of six eukaryotic promoter/enhancer elements was assessed in HL-60 cells by transient expression assays. Cells were transfected with promoter-chloramphenicol acetyltransferase (CAT) plasmids and ^{14}C-chloramphenicol acetylating activity was assessed at 48h by chloroform:methanol (95:5) thin layer chromatography and autoradiography. The Rous sarcoma virus (RSV) and rat p3C5 gene calcium ionophore-inducible promoters demonstrated the highest activities. In contrast, the early SV40, human metallothionein II_A, lymphotropic papovavirus and thymidine kinase gene promoters were significantly weaker. To determine if the strong RSV promoter would function in a high copy number episomally-replicating vector, we subcloned RSVCAT into the unique BamHI site of the Epstein-Barr virus (EBV)-based vector p220.2 (obtained from Dr. B. Sugden). Transient CAT expression assays established that the RSV promoter maintained its high activity in this plasmid construct. Stable hyg^R HL-60 transformants were successfully derived by transfecting with p220.2 which carries the hygromycin resistance gene. We conclude that EBV-based vectors using the RSV and perhaps other retroviral promoters offer a promising approach for high-level expression of transfected genes in HL-60 cells.

D 343 EXPRESSION OF HIGH MOLECULAR WEIGHT B-CELL GROWTH FACTOR (HMW-BCGF) RECEPTORS IN B-CELL PRECURSOR ALL. Fatih M. Uckun, Anthony S. Fauci, Kevin G. Waddick, and Julian L. Ambrus. University of Minnesota Health Sciences Center and National Institute of Allergy/Infectious Diseases.

We investigated the specific binding of ^{125}I-HMW-BCGF to freshly obtained marrow blasts from 15 B-cell precursor ALL patients. The specific binding inhibitable by excess cold HMW-BCGF varied between 5% and 44% of the total cell associated radioactivity. The percentage of blasts expressing specific receptors for HMW-BCGF was determined by indirect immunofluorescence and flow cytometry using BA-5, a monoclonal antibody that recognizes the HMW-BCGF receptor. The percentage of BA-5$^+$ cells varied from 7% to 59%. HMW-BCGF induced stimulation of ^{3}H-thymidine incorporation was determined to evaluate the functional integrity of the detected receptors. Blasts from 8 of 15 cases responded to HMW-BCGF. Seven cases failed to respond although 6 expressed specific receptors. We also examined the effects of HMW-BCGF at the level of leukemic lymphoid progenitor cells using B43$^+$/CALLA$^+$ FACS sorted marrow blasts and a novel colony assay system. Of 16 cases studied, 9 cases did not respond whereas in the remaining 7 cases, HMW-BCGF resulted in a marked stimulation of leukemia progenitor cells. To our knowledge, these findings represent the first demonstration that functional HMW-BCGF receptors are expressed on B-cell precursor ALL blasts and their progenitors in a number of B-cell precursor ALL patients.

D 344 LEUKEMIA DERIVED GROWTH FACTOR PROMOTES THE GROWTH OF NORMAL IMMATURE T CELLS, Christel Uittenbogaart, Yoshiki Ueno and Esther Hays., UCLA School of Medicine, Los Angeles, CA 90024.

In previous work we have shown that the immature human T leukemia cell lines MOLT-4f, CCRF-CEM and CCRF-HSB-2 constitutively produce an autostimulatory growth factor, Leukemia Derived Growth Factor (LDGF). LDGF was isolated from the supernatants and the cytoplasm of these cell lines. This factor is T cell specific; in addition to its autostimulatory activity, it promotes the growth of malignant T lymphoid cells of a similar stage of differentiation, but not of mature malignant T cells or of B or myeloid cells. In order to determine if LDGF promotes the growth of normal immature T lymphocytes, thymocytes were tested for their response to LDGF. Thymocytes were isolated from normal thymus tissue using Ficoll Hypaque and nylonwool adherence and cultured in serum-free medium with and without activation with antigen. These cells were more than 96% reactive with the Leu-5 monoclonal antibody and more than 98% reactive with the OKT10 monoclonal antibody. The growth of both unstimulated and stimulated thymocytes was promoted by LDGF. However, normal peripheral blood T lymphocytes, tested in the same way as thymocytes, did not respond to LDGF. These findings indicate that LDGF may be a normal growth factor, which is disregulated in malignancy.

D 345 AUTOCRINE STIMULATED GROWTH OF A LYMPHOBLASTOID LINE IMMORTALIZED BY INFECTION WITH A PARASITE
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We have been studying the reversible lymphoblastoid transformation of bovine lymphocytes induced by infection of the intracellular parasite *Theileria parva* T.p. is a protozoan parasite related to malaria that causes a fatal lymphoproliferative disease in cattle. Infection by this organism commits the infected T cell to rapid uncontrolled growth. In the early stages of infection one observes extensive proliferation and spread of infected cells in the lymphnodes. The infected lymphocytes can invade other tissues, be cloned in soft agar and cause tumors in nude mice. Infected cells very easily establish themselves in tissue culture where they remain immortalized. Experimental drugs (Wellcome Labs) have been developed that specifically eliminate the parasite. The removal of the parasite returns the dividing cell to a resting state. This reversible transformation provides a novel system for the analysis of the molecular events responsible for uncontrolled growth. An initial screening with oncogenes revealed that both the *myc* and *raf* oncogenes are concomitantly expressed in the parasitized cells. The bovine raf cDNA has been cloned from infected cells. In addition to an oncogene analysis, we will present data to show that the infected cells secrete a growth factor and that the cells need this factor for proliferation. Recombinant human IL-2 can substitute for the endogenous factor. We show that the IL-2 receptor is present and that following treatment with the experimental drug the receptor drops to levels of resting cells. TPA treatment of drug cured cells in the presence of added IL-2 stimulates the cells to divide for a further several months after which they die out. The parasite appears to create an immortalized cell that is driven in an autocrine fashion with a growth factor probably bovine IL-2.

D 346 PHORBOL ESTERS RESTORE THE DEFECTIVE MEMBRANE L-SYSTEM AMINO ACID TRANSPORT IN B-LYMPHOCYTES OF CHRONIC LYMPHOCYTIC LEUKEMIA (CLL). T.J. Woodlock, G.B. Segel, and M.A. Lichtman, University of Rochester School of Medicine, Rochester, N. Y. 14642.
L-system (leucine favoring) transport is uniquely and selectively diminished in CLL B-lymphocytes with a maximal velocity less than 10% of normal B-lymphocytes. We have produced marked enhancement of L-system transport by CLL B-lymphocytes in each of 11 patients following stimulation with the maturational agent, tetradecanoyl phorbol acetate (TPA), at a concentration of 0.16 uM. The L-system-specific synthetic amino acid, 2-amino-2-carboxy-bicycloheptane (BCH) was used to characterize L-system transport. The mean initial velocity of uptake (V_i) of untreated CLL cells measured at 2, 16 and 40 hours was 78, 149, and 141 umole/liter cell water per min respectively and increased to 92, 1172, and 1994 umole/liter cell water per min in TPA treated cells. At 40 hours, this represents a 14-fold increase in L-system transport. Early features of plasmacytoid maturation of the TPA treated CLL cells were observed at 40 hours. Cycloheximide, 100 ug/ml, which inhibited $>$ 90% of protein synthesis in TPA treated CLL cells, blocked $>$ 90% of the enhanced membrane transport of BCH. A fifty-fold excess of non-radioactive BCH inhibited $>$ 90% of ^{14}C-BCH uptake in both untreated and TPA treated CLL cells indicating that uptake is a saturable process and L-system specific. TPA treatment also produced enhanced uptake of the naturally occurring L-system amino acid, leucine (0.1 mM), with a four-fold increase in the Vi observed at 12 hours. TPA corrects the selective L-system transport defect in CLL B-lymphocytes by increased synthesis and expression of the L-system transport protein as part of the in vitro maturational process induced by this agent.

D 347 EVIDENCE THAT INTRACELLULAR GUANINE RIBONUCLEOTIDE(G-NTD) POOLS REGULATE THE GROWTH AND MATURATION OF HUMAN MYELOID PROGENITORS BY AFFECTING A PHOSPHOLIPASE C(PLC) MEDIATED SIGNAL TRANSDUCTION PATHWAY FOR GROWTH FACTORS. Daniel G. Wright and Marti Jett. WRAIR, Washington, DC. 20307
In previous studies of the human myeloid cell line, HL-60 (JCI, 72:1889, 1983), we found that induced maturation of these cells is associated with a down-regulation of G-NTD synthesis from inosine monophosphate (IMP) and with depletion of GTP and GDP pools. We also found that inhibitors of IMP-dehydrogenase [e.g. tiazofurin(RTC)], which block G-NTD synthesis from IMP, are potent inducers of HL-60 maturation, while guanosine(GUO), which is salvaged for G-NTD synthesis by-passing the pathway from IMP, supports high G-NTD levels, prevents induced maturation and promotes cell growth. In order to examine the possibility that G-NTD depletion and repletion might affect a GTP-regulated signal transduction pathway for myelopoietic growth factors, we studied functional and biochemical responses of HL-60 cells to high specific activity human recombinant GM-CSF (Genetics Inst, Cambridge, MA). GM-CSF(50-100 u/ml) stimulated the proliferation of HL-60 cells in serum-free suspension culture and in 0.8% methylcellulose. This effect was further enhanced by GUO(10^{-5}M) but blocked by RTC (10^{-6}M). RTC and GUO also affected PLC activity in HL-60 cells. In serum-free medium, these cells actively incorporated ^3H-inositol into phosphatidylinositol-4,5- bis-phosphate(PIP_2). Endogenous PLC mediated hydrolysis of PIP_2 was then readily detected in $LiCl_2$(10mM) treated cells by the methods of Berridge et al. This PLC activity was enhanced by GUO(10^{-5}M) and diminished by RTC(10^{-6}M). Moreover, stimulation of PLC by GM-CSF(100 u/ml) was reflected by a significant rise in IP_3 within 15 sec following exposure of cells to the CSF and by an increased accumulation of inositol phosphates thereafter. This GM-CSF effect was completely blocked by RTC(10^{-6}M). These studies suggest that intracellular G-NTD supplies influence the growth and maturation of primitive myeloid cells via a GTP-regulated, PLC-mediated signal transduction pathway for myelopoietic growth factors.

Immunology

D 400 CHARACTERIZATION OF ACUTE LEUKEMIAS WITH THE HLA-DR+/TdT+/CD10− PHENOTYPE.

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The immunological phenotype HLA-DR+/TdT+/CD10− is found in immature leukemias of both the lymphoid and the myeloid lineage. We characterized 15 of such leukemias morphologically and by use of immunological marker analysis and cytogenetic analysis. In addition the configuration of immunoglobulin (Ig) genes and T cell receptor (TcR) genes was determined and several leukemias were analyzed for the presence of CD3 transcripts. Five cases were classified as null acute lymphoblastic leukemia (ALL), based on positivity for the B cell markers CD24 (BA-1), CD9 (BA-2) or CD19 (B4) and based on rearrangement of both Ig heavy chain (IgH) gene alleles. Four out of these five cases carried the t(4;11). Two of the 15 leukemias were classified as prothymocytic ALL, based on the expression of the T cell markers CD7 (3A1) and CD2 (OKT11), the presence of CD3 transcripts but the absence of T cell receptor β chain (TcR-β) gene rearrangement. It is remarkable that in both cases a part of the TdT positive cells was also positive for the myeloid marker CD13 (My7). The other eight leukemias were classified as acute undifferentiated leukemias (AUL) or acute myeloid leukemias (AML). In these eight leukemias TdT positive cells appeared to be positive for the myeloid markers CD13 (My7) and CD33 (My9) and in four of them the TdT positive cells were also partly positive for the T cell marker CD7 (3A1). The tested AUL and AML had both germline IgH genes and germline TcR-β genes. Two AML cases carried the t(6;9). The expression of the myeloid marker CD13 (My7) by early T cells and the expression of the T cell marker CD7 (3A1) by early myeloid cells suggests the existence of a common progenitor for these cells. This is supported by the observation that the immunological phenotypes of one of the prothymocytic ALL and of one of the AML were rather similar at relapse, probably due to a phenotypic shift to a more immature differentiation stage.
We propose a differentiation scheme with a common progenitor cell for the T cell and myeloid lineages.
Supported by the Sophia Foundation for Medical Research.

D 401 SPECIFICITY AND FUNCTIONAL ROLE OF A MONOCLONAL ANTIBODY 4B8 REACTIVE WITH PROLYMPHOCYTIC LEUKAEMIA B CELLS,

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We have produced a mouse monoclonal antibody (Mab) 4B8, from a fusion following immunization of BalB/c mice with prolymphocytic leukaemia (PLL) B cells. 4B8 is an IgG1 subclass antibody and reacts with the majority of B cells from all PLL patients tested and a small proportion of both centroblasts from the blood of non-Hodgkins lymphoma (NHL) patients and normal tonsil B cells. 4B8 is unreactive with normal peripheral blood (PB), chronic lymphocytic leukaemia (CLL) and hairy cell leukaemia (HCL) B cells as well as EBV-transformed B-cell lines. Resting and activated T cells, monocytes and granulocytes also fail to stain with 4B8. On tonsil sections 4B8 reactivity is almost exclusively confined to areas in the germinal centres. We show here that although 4B8 alone has little effect on B-cell function it can exert a strong proliferative effect on PLL and normal tonsil B cells in conjunction with staphylococcus aureus Cowan I (SAC) or mixed leucocyte reaction (MLR) supernatant and can augment IgM secretion in a pokeweed mitogen (PWM) driven Ig synthesising system. The functional role of 4B8 will be discussed in relation to other known B-cell stimulatory agents.

D 402 PROGNOSTIC VALUE OF THE CD19+/CD10- PHENOTYPE IN ACUTE LYMPHOBLASTIC LEUKEMIAS,

Marie C. Béné, Gilbert C. Faure, Jean P. Vannier and the GEIL (Groupe d'étude immunologique des leucémies). Lab. Immunol. Fac. Médecine 54500 Vandoeuvre les Nancy. FRANCE.

A collaborative immuno-haemato-clinical study of hematopoietic proliferations, including 18 French hospitals has been working since early 1984. Acute lymphoblastic leukemias steadily represent 80-85% of the recruitement. An extensive panel of monoclonal antibodies was designed and its applicability tested in the participating centers, assessing the validity of phenotyping techniques. In August 1985, a first analysis of 334 ALL patients defined a subgroup of pejorative prognosis: 21 patients of CD19+/CD10- phenotype were either dead of in second remission. Only two children who had received an allogeneic bone marrow transplant immediately after remission was obtained still were in good health. This subgroup kept increasing in the following months, representing about 7% of the ALL group. No significant positive or negative effect was observed as related to other membrane markers. Different therapeutic protocols were applied, limiting the possible therapy biais. When available, cytogenetic studies showed an elevated frequency of t(4,11) in this subgroup. Updated proportions (450 were registered in September 1986), patients' characteristics and follow-up will be presented.

D 403 A NOVEL CLONAL T LYMPHOCYTE IN THE PERIPHERAL BLOOD OF A PATIENT WITH TYPICAL HELPER PHENOTYE CUTANEOUS T CELL LYMPHOMA (CTCL), James R. Berenson, Janis Giorgi, Ian Okazaki, David Hudnall, and Edward Tobinick, UCLA, Los Angeles,CA 90024

Prior investigations have established that the malignant cell infiltrating skin and in the circulation of patients with CTCL are helper T lymphocytes that demonstrate clonal T cell receptor (TCR) gene rearrangements. We describe a case of CTCL with peripheral blood lymphocytes (PBLs) containing clonal T cells which lacked the helper phenotype despite skin lesions demonstrating helper T lymphocytes. The patient is a 75 year old white male with a ten month history of generalized pruritus. Chief findings were generalized erythroderma, poikiloderma and generalized lymphadenopathy. Peripheral blood revealed a normal lymphocyte count without obvious circulating Sezary cells. Skin biopsy revealed Pautrier's microabscesses consistent with the diagnosis of CTCL. Peripheral blood lymphocytes and granulocytes were obtained by density gradient centrifugation. Flow cytometry utilizing monoclonal antibodies revealed that the majority of circulating lymphocytes were a distinct phenotype- dim Leu4+, Leu2-, Leu3-, Leu1+ and Leu5+. However, indirect immunofluorescence of the patient's skin lesions revealed the vast majority of cells to be Leu4+, Leu2-, Leu3+. In addition, functional assays showed inability of the PBLs to respond to PHA or tetanus antigen. Highly purified populations of this dim Leu4+ PBL were also unable to respond in these assays. Moreover, Southern blot analysis demonstrated clonal TCR gene rearrangements in these lymphocytes without clonally rearranged antibody genes. Thus, these data reveal a novel clonal T lymphocyte in the circulation of a patient with helper phenotype CTCL.

D 404 PRE-B CELL ORIGIN OF A NODULAR B CELL LYMPHOMA, Gary Borzillo, Luigi F. Bertoli, Hiromi Kubagawa, Peter D. Burrows and Max D. Cooper, University of Alabama at Birmingham, Birmingham, AL 35294.

These studies were designed to test the hypothesis that B cell malignancies are the consequence of cumulative transformation events, the earliest of which occur in the bone marrow in mammals. To analyze clonal involvement at a pre-B cell level in a patient with nodular, poorly-differentiated lymphoma, we produced a novel monoclonal antibody with specificity for a V_H idiotope (Id) on the free μ-heavy chains of the lymphoma IgMκ molecules. This anti-V_H probe identified a subset (3-26%) of an initially expanded population of bone marrow pre-B cells as the clonal ancestors of the nodular B lymphoma cells present in the lymph nodes. Examination of pre-B enriched marrow cell DNA by Southern blotting with immunoglobulin gene probes revealed the expansion of multiple clones of early B-lineage cells, including members of the neoplastic clone. A conventional anti-Id monoclonal antibody specific for the intact lymphoma IgMκ molecules recognized 10% of marrow B cells, 10% of circulating B cells, and >95% of B cells in the lymphomatous lymph nodes. The idiotypic and Ig gene markers were retained following tumor conversion to a diffuse large cell lymphoma. We conclude that this patient's lymphoma arose through a progression of transformational events beginning in the bone marrow, creating a pool of pre-neoplastic pre-B cells, followed by homing of low-grade neoplastic B cells to the lymph node follicles, and finally progressing to a high grade B cell lymphoma.

D 405 A MURINE MODEL FOR PURGING MARROW OF MALIGNANT T CELLS. E. Copelan, S. Johnson, M. Grever and P. Tutschka, Ohio State University, Columbus, Ohio 43210.

T cells are uniquely sensitive to the congenital absence or pharmacologic inhibition of the purine nucleoside pathway enzyme adenosine deaminase (ADA) due to their high level of deoxyadenosine kinase activity which leads to rapid accumulation of dATP. We have developed a murine model for in vitro purging of malignant T cells from marrow using the ADA inhibitor deoxycoformycin in combination with deoxyadenosine. The sensitivity of murine T cell malignancies to in vitro incubation with deoxycoformycin and deoxyadenosine can be predicted from their levels of ADA, deoxyadenosine kinase and 5' nucleotidase. Selection of sensitive tumors by profile of these enzymes, purging of marrows contaminated with 5% malignant T cells and long-term (greater than 9 mos.) leukemia-free survival of these transplanted mice has been successfully carried out.

D 406 EXPRESSION OF CD25 (IL2 RECEPTOR) ON NON HODGKIN LYMPHOMAS. Gilbert C. Faure, Marie C. Béné. Laboratoire d'Immunologie. Fac. Médecine. 54500 Vandoeuvre les Nancy. FRANCE.

The presence of IL2 receptors was initially described on activated lymphocytes of T-lineage. In vitro stimulation and IL2 fixation tests however showed the presence of several types of IL2 receptors of different affinity, both on T- and B-cells. In a previous study, we used a monoclonal antibody (gift of T.Waldmann) on a series of human haematopoietic proliferations, and showed the frequent expression of CD25 on chronic lymphocytic leukemia cells, together with B-cell markers. We report a similar analysis performed on cell suspensions obtained from the blood or bone marrow of 23 patients with non Hodgkin lymphoma. All samples contained more than 50 % of atypic cells. Indirect immunofluorescence was used on cell suspensions using a classical technique to assess the numbers of CD25+ cells. Twelve patients had 0 to 20 % of positive cells, while 20 to 85% of the cells brightly expressed CD25 in the 11 others. The concomitent use of an extensive phenotyping panel showed that most of these positive lymphomas were of B-lineage. These data support previous observations describing the spontaneous expression of IL2 receptors (althgouh of unknown affinity) on human proliferations.

D 407 T-CELL RECEPTOR ANALYSIS IN ACUTE AND CHRONIC T-CELL LEUKEMIAS, Robert Foa, M.Cristina Giubellino, Giulia Casorati, M. Teresa Fierro, Francesco Lauria, Gianpietro Semenzato, Nicola Migone, Clinica Medica A and Istituto di Genetica Medica, Torino; Istituto di Ematologia, Bologna; Istituto di Medicina Clinica, Padova, Italy.

Analyses of the configuration of the T-cell receptor (TCR) β chain gene were carried out in a wide spectrum of human acute and chronic T-cell leukemias. A clonal rearrangement of the TCR β chain gene was found in 28 of the 34 T-cell acute lymphoblastic leukemias (T-ALL) studied, while 6 were in a germ-line position. One of them was rearranged at the Tγ region. Correlation with the immunotyping showed that the 5 germ-line cases had an immature, pre-T-ALL phenotype (TdT+, Leu9(CD7)+, Leu1(CD5)±, but negative for all other T-cell markers). These findings demonstrate that the rearrangement of the TCRβ chain gene follows the expression of the p40 antigen (Leu9/CD7), and thus does not recognize pre-T-ALL cases. In chronic T-cell disorders, a clonal rearrangement was documented in all T4(CD4) expansions (prolymphocytic leukemia and Sèzary syndrome). Studies at the DNA level were of particular relevance in establishing the origin of cases with a picture of granular lymphocyte proliferation. Within the most frequent group displaying a T3(CD3)+, T4(CD4)-, T8(CD8)+ phenotype, a monoclonal or a polyclonal configuration of the TCR β chain gene could be demonstrated, thus providing a valuable tool to dissect between the neoplastic or reactive nature of the process. The rare granular expansions with a T3(CD3)-, T4(CD4)-, T8(CD8)-, Leu11(CD16)+, NK picture were germ-line, pointing therefore to the non-T cell origin of these cases. (Work supported by CNR, PF Oncologia, Rome).

D 408 IMMUNOLOGICAL STUDIES IN A TRIAL EVALUATING ^{131}I-T101 FOR RADIOIMMUNOIMAGING AND RADIOIMMUNODETECTION OF CUTANEOUS T CELL LYMPHOMA, Robin E. Goldman-Leikin, Robert Marder, Ed H. Kaplan, A. Michael Zimmer, Joann M. Kazikiewicz, and Steven T. Rosen, Northwestern University, Chicago, IL 60611.

Seven cutaneous T-cell lymphoma patients (CTCL) have been imaged with ^{131}I-labeled T101 murine monoclonal antibody. Six of these patients received a therapeutic dose of 100-200 mCi ^{131}I on 10 mg T101 antibody. Responses were of two weeks to 3 months duration, with 2 patients experiencing a partial response and 4 patients undergoing minor responses. Major toxicities included dypsnea in one patient, generalized urticaria and angioedema in one patient and marrow suppression in the 3 patients treated with 150-200 mCi of ^{131}I-T101. Three patients were retreated at the time of disease progression. This presentation will focus on the pharmokinetics of T101 antibody in serum samples and the human anti-mouse antibody responses (HAMA) seen in all patients. HAMA responses were of both μ and γ isotypes and were detectable 14 days following antiody infusion. HAMA were predominantly anti-idiotypic. We have also investigated the effects of plasmapheresis on circulating HAMA antibody levels in patients prior to retreatment and antigenic modulation of the T101 specific antigen from T lymphocytes following T101 infusions. Our results demonstrate the importance of immunological analyses in optimizing the design of monoclonal antibody trials.

D 409 IMMUNOLOGICAL PHENOTYPING OF ACUTE LYMPHOBLASTIC LEUKEMIA IN CHILDREN WITH CNS-RELAPSES. Jesper Heldrup, Department of Pediatric, University Hospital, S 221 85 Lund, Sweden.

Since 1981 we have immunologically phenotyped and chromosomally kariotyped all children in southern Sweden with lymphoblastic leukemia and lymphoma before any chemotherapy was given. During the last year the same techniques were applied to the cerebrospinal fluid in patients with relapses in the central nervous system. It enables

1) the right diagnosis in dubious cases of CNS-engagement and

2) comparison between the immunological phenotype and chromosomal kariotype of the leukemic cells in the CNS with those from the bone marrow and blood at diagnosis.

D 410 THE ACTIVATION ASSOCIATED ANTIGEN 4F2 PREDICTS PATIENT SURVIVAL IN LOW GRADE B-CELL LYMPHOMAS, Harald Holte[1], Catharina Davies[2], Stein Kvaløy[3], Erlend Smeland[1], Per Marton[4], Olav Kaalhus[2], Arne Foss-Abrahamsen[4], and Tore Godal[1].
[1]Lab. for Immunology, [2]Dept. of Biophysics, [3]Dept. for Clinical Oncology and Radiotherapy, [4]Dept. of Pathology; The Norwegian Radium Hospital, Montebello N-0310 OSLO 3, Norway

Expression of the activation-associated antigens 4F2, transferrin receptor (TrR) and interleukin-2 receptor (IL-2R) of cell suspensions from 75 biopsied low grade B-cell lymphomas was correlated to patient outcome, DNA synthesis and clinical prognostic parameters. 4F2 expression was independently correlated to DNA synthesis as estimated from spontaneous ^{3}H-thymidine incorporation ($p = 0.0037$), TrR expression ($p = 0.058$) and patient outcome ($p = 0.004$). TrR expression was associated with DNA synthesis and treatment response (complete response = CR vs. non-CR), but not to patient survival. On the other hand, IL-2 did not behave as an activation associated antigen, but rather related to differentiation/maturation. Our results show that monoclonal antibodies against activation-associated antigens may play an important role in the subclassification of B-cell lymphomas.

D 411 LYMPHOKINE-ACTIVATED KILLER (LAK) CELL THERAPY OF LEUKEMIA IN MICE, Candace S. Johnson and Philip Furmanski, AMC Cancer Research Center, Denver, CO, 80214.
Adoptive immunotherapy with LAK cells and recombinant interleukin-2 (rIL-2) has been shown to be an effective approach to the treatment of tumors in mice. To determine the effectiveness of LAK therapy in causing regression of leukemia, we examined the effect of LAK cells plus rIL-2 on progressive Friend virus (FV) induced leukemia in mice. For these studies, we utilized a model system developed in our laboratory for the study of immunologically mediated regression of FV erythroleukemia. LAK cells were generated by incubating normal spleen cells for 72 hrs in the presence of rIL-2 (1000U/ml). At the time of injection, the LAK cells were cytotoxic in vitro against FV-infected fibroblasts and not against normal fibroblast control lines. To determine in vivo activity, FVA progressively leukemic mice were injected IP at 14 and 17 days post-virus inoculation with either PBS or LAK cells (10^8) and rIL-2 (10,000 units 3X, every 8 hrs). By day 98 post virus, 45% of the progressively leukemic mice had experienced permanent leukemia regressions following LAK cell/rIL-2 therapy. When leukemic animals were given either LAK cells/rIL-2, LAK cells only or IL-2 only, both LAK cells/rIL-2 and rIL-2 only animals experienced leukemia regressions, whereas LAK cells alone had no effect. Based on productive virus infection of erythroid (CFU-E) and macrophage (CFU-C) progenitor cells, only leukemic animals given LAK cells/rIL-2 experienced permanent regressions; IL-2 only regressed animals had productively infected CFU-C and CFU-E, an accurate indicator for the subsequent recurrence of disease. These results demonstrate that LAK cells and rIL-2 can efficiently induce permanent regressions in progressively leukemic mice. Supported by grant CA33939 and a gift to AMC from Irving Ash.

D 412 STUDIES ON B LYMPHOID TUMORS TREATED WITH MONOCLONAL ANTI-IDIOTYPE ANTIBODIES. CORRELATION WITH CLINICAL RESPONSES. J.N. Lowder, T.C. Meeker, M. Campbell, C.F. Garcia, J. Gralow, R.A. Miller, R. Warnke, and R. Levy.

Monoclonal anti-idiotype antibodies can be made which are exquisitely specific for B lymphocytic malignancies. We have conducted a clinical trial in which some patients' tumors regressed after infusion of such antibodies. Here, we evaluated characteristics of the antibodies, the tumors, and the patients to determine which features best correlated with the clinical response. Neither the isotype of the murine antibodies, nor their avidity were predictive of clinical outcome. The specific epitope to which the antibodies bound was characterized by immunochemical techniques. Reactivity with a heavy-light chain combinatorial determinant correlated somewhat with clinical effect. Variations in the characteristics of the individual tumors such as antigen sites per cell and ability to modulate the surface immunoglobulin (Ig) were not predictive of response. In one patient with prolymphocytic leukemia the anti-idiotype antibody had a direct antiproliferative effect on tumor cells in vitro. This patient's tumor response was explainable by such a direct mechanism. In the other patients, who had lymphomas, therapeutic outcome correlated with the number of host non-tumor cells infiltrating the tumor. The vast majority of these non-tumor cells were mature T lymphocytes of the Leu 4, Leu 3 (T3, T4) phenotype. Thus, a preexistent host-tumor interaction seems to be important in the in vivo effect of anti-idiotype antibodies in B cell tumors.

D 413 LYMPHOID IRRADIATION INCREASES NATURAL KILLER CELL POPULATIONS, Roger Macklis, Peter Mauch, Nancy Tarbell, Steven Burakoff and Brian Smith, Harvard Medical School.

The long-term effects of radiation on the human immune system are poorly understood. Previous studies have suggested that lymphoid irradiation (LI) results in a functional and phenotypic T cell cytopenia with a reversal of the Leu3+ (CD4+) "helper" to Leu2+ (CD8+) "suppressor" T cell ratios. We have used dual immunofluorescence flow cytometry and functional cytolytic assays to investigate the effects of LI either alone or in combination with chemotherapy on 41 patients aged 10-72 treated for Hodgkin's Disease. (HD). In contrast to previous reports, we find that the "true" T cell phenotypic ratios (i.e., Leu4+Leu3+ : Leu4+Leu2+ or CD3+CD4+ : CD3+CD8+) for patients treated with LI alone are generally within normal limits. An apparent reversal of the T cell subset ratio is seen in many patients due to a large increase in the population of Leu 11+ natural killer (NK) cells, a significant proportion of which simultaneously label with Leu2. These cells show variable expression of the NK marker Leu7, and are not labeled by Leu4 or Leu3. Blood from patients with increased numbers of these NK cells shows proportionately increased in vitro cytotoxicity against a panel of tumor targets. These changes are generally not seen in patients treated with combined chemoradiotherapy. Serial studies show that these changes are fairly stable over time and persist for many years after treatment. Prospective evaluation of LI-treated patients who were studied while in complete remission but who eventually relapsed with HD show that high levels of circulating NK cells do not correlate with the durability of remission.

D 414 IMMUNOREGULATORY PROPERTIES OF CSF-1, Veronica E. Miller, Cheryl L. Willman, Latif Kazim, and Thomas B. Tomasi, University of New Mexico, Albuquerque, New Mexico, 87131.

Murine amniotic fluid is known to have immunosuppressive properties, including the inhibition of Ia antigen expression and of mitogen induced proliferation, which has been attributed to alphafetoprotein. However, when neonatal (up to 21 days post-partum) spleen cells are cultured in the presence of 10% amniotic fluid there is marked proliferation. The factor responsible for the proliferation appears to be CSF-1 based on the morphology, esterase staining and cell surface characteristics of proliferating cells in bulk cultures and soft agar colonies. The CSF-1 activity was still present in preparations of partially purified alphafetoprotein, and could be depleted by passage over anti-CSF-1 antiserum columns. We therefore tested the possibility that CSF-1 may be responsible for some of the immunoregulatory properties of amniotic fluid and found that: 1) Lcell conditioned media (containing CSF-1 and less than 4 units/ml of interferon), and stage 4 purified CSF-1 down-regulate the expression of Ia genes in bone marrow cultures, and 2) Lcell conditioned media inhibits the induction of Ia surface expression induced by IFN-gamma on bone marrow derived macrophages. These results suggest that CSF-1 may have immunoregulatory properties during fetal development.

This work was supported by NIH grants HD17013 and CA22105.

D 415 IMMORTALIZATION OF T-LYMPHOCYTES DERIVED FROM PATIENTS WITH HAIRY CELL LEUKEMIA OF B-CELL TYPE. F. Naeim, V. Kermani-Arab, D. Hoon, D. Gatti, J. Rosenblatt, and P. Concannon, UCLA School of Medicine (LA) and California Institute of Technology (Pasadena) CA

The vast majority of hairy cell leukemias (HCL) are of B-cell type. There is a controversy whether the scattered reported cases of T-cell HCLs are true HCLs or represent a T-cell variant of CLL/lymphoma, or in some instances, merely represent proliferation of non-neoplastic T-cells (or cell lines) derived from patients with HCL. We have established two T-cell lines, independent from exogenous interleukin-2 (IL-2), from splenectomy specimens of two patients with B-cell HCL.

The first T-cell line (66% Leu 4^+, Ig^-) was derived from a splenectomy specimen which contained 42% T-cells but demonstrated a monoclonal pattern for IgGλ $^+$ cells (IgGκ32%, M 3%, λ31%, κ2%). The second T-cell line grew out from an EBV-induced B-cell line derived from another HCL patient. The B-cell line (IgG $^+$, Ia^+, TdT^-, Leu 4^-) which was phenotypically similar to the fresh HCL cells and demonstrated Ig gene rearrangement (JH), after 8 weeks in culture was completely replaced by clonal expansion of a T-cell line (Ig^-, Ia^-, Leu 4^+, Leu 3^+) which was HLA identical to the original B-cell line, showed T-cell receptor gene rearrangement (TCRβ) and was negative for HTLV-II. The mechanism of this unusual event of establishment and growth of a T-cell line over an EBV-transformed B-cell line is not clear. However, establishment of human T-lymphocytes after transfection of EBV-DNA has been recently demonstrated. We conclude that a) T-cells from HCL patients could be easily transformed to established cell lines and, b) clonal growth of T-cell lines derived from HCL patients does not necessarily establish diagnosis of T-cell HCL.

D 416 Ability of Lymphokine Activated Killer Cells to Destroy Epstein Barr Virus Transformed B-Cell Lines and Lymphoma Cells. Allen Norin, Christine Lackner and Rimma Mushnitsky. Montefiore Med Ctr, Albert Einstein College of Medicine, N.Y. 10467.

Lymphokine Activated Killer (LAK) cells have been shown to destroy Natural Killer (NK) cell resistant solid tumors. Remission following repeated injection of autologous LAK cells into tumor bearing animals and cancer patients have been described. LAK cells also have the capability of destroying Epstein Barr Virus (EBV) associated tumor cells (Daudi, Raji) in vitro, suggesting the use of such therapy for patients with lymphoma and possibly leukemia. These B-cell lines have a 50-fold lower cloning efficiency at limiting dilution compared to the lymphoma cell lines. We examined the susceptability of EBV transformed B-cell lines and lymphomas to LAK cell cytotoxicity. LAK activity was generated by culture of freshly isolated Ficoll-Hypaque and nylon column purified peripheral blood lymphocytes in a serum free medium (supplemented with Il-2) for 6 days. LAK activity was assessed on a NK resistant lung carcinoma cell line and on EBV transformed B-cells in a standard 3 hour ^{51}Cr release assay. LAK cytotoxicity was 70% on the carcinoma targets, 46% on Daudi, 37% on Raji, but only a mean of 8% on three newly transformed B-cell lines. The data suggest that EBV induced transformation to immortal B-cell lines is not sufficient in itself for expression of a LAK sensitive cell surface phenotype. In this regard further studies will determine whether low cytotoxicity is due to inability of LAK cells to bind to EBV-B cells or whether "programming for lysis" does not occur after binding. This experimental system may thus be useful in identifying cell membrane determinant(s) which are the molecular target(s) for LAK cell lysis. Supported in part by NIH grant HL17417.

D 417 GROWTH OF SPONTANEOUS RCS OF SJL/J MICE IS REGULATED BY HOST $L_3T_4^+$ AND LYT-2^+ T CELLS, Kazunori Ohnishi and Benjamin Bonavida, Dept. of Microbiology & Immunology, UCLA School of Medicine, Los Angeles, CA 90024.

Previous studies have shown that RCS (IA^+) tumor cells are dependent on host cells for maintenance and growth. The mechanism of this dependence was investigated. Spontaneously arising RCS of SJL/J (IA^+ IE^-) express on their surface aberrant IE-like specificities recognized by snygeneic Lyt1^+2^-, $L_3T_4^+$ T-cells and are dependent on host T cells for maintenance and growth. The nature of these tumor associated IA specificities was investigated by the generation of tumor specific T cell hybridomas. Two hybridomas were specific for the RCS, and two responded to RCS and allogeneic cells expressing $IE^{k,d}$ specificities. The response of the hybridomas to RCS was only blocked by anti-IA^s mAb and the response of the hybridomas to IE specificity was blocked by anti IA/$E^{k,d}$ mAb. The T-cell hybridomas formed conjugates with RCS and allogeneic B cell blasts with the same specificity as obtained in the IL-2 response. These studies demonstrate that RCS tumor cells express aberrant IE-like specificities recognized by cloned T cells. Passive administration of anti-L_3T_4 mAb showed that RCS tumor growth is dependent on the L_3T_4 T cells and both prevention and cure can be achieved. The studies also suggest that other regulatory cells such as Lyt2^+ T-cells play a regulatory role in suppression of tumor growth. In conclusion, RCS growth in vivo depends on stimulation of tumor specific $L_3T_4^+$ T-cells and the presence of regulatory Lyt2^+ T-cells.

D 418 MOUSE MONOCLONAL ANTIBODIES DEFINING HUMAN HEMOPOIETIC CELLS. M. Tanimoto*, D.A. Scheinberg, C. Cordon-Cardo, B.D. Clarkson, and L.J. Old. *Nagoya University, Nagoya, Japan and Memorial Sloan-Kettering Cancer Center, New York, New York 10021.
Mouse monoclonal antibodies were generated against HL-60, MOLT-4, K562 cells, and fresh AML cells. The specificity of the antibodies were determined by immune RBC rosetting on 90 hemopoietic and tumor cell lines, 50 samples of fresh leukemia cells, and on peripheral blood elements. Tissue specificity was determined by immunoperoxidase on frozen sections of normal human tissues. These antibody panels may be useful for diagnostic and therapeutic purposes and in studies of hemopoietic differentiation and function. Several are described:

		Specificity:			
mAb	Immunogen	Hemopoietic lines	Fresh Hemopoietic Malignancies	Sold Tumor lines	Normal Tissues
M195 (IgG2a)	Fresh AML Cells	Myeloid, null cells	60% of AML, 25% of ALL	none	none
M31 (IgM)	K562	Myeloid, T cells	CML, Mult. Myeloma, 25% of AML, 10% of ALL	Some breast, colon, lung	many, including myeloid series
T37 (IgM)	MOLT-4	T cells	T-ALL	none	Prostate, uterus esoph, others

D 419 IDENTIFICATION OF HUMAN cDNA CLONES FOR THE MO1 ALPHA CHAIN GENE AND ITS EXPRESSION IN MYELOID CELLS, Daniel G. Tenen, Linda Clayton, Rachel Neve, Mark L. Pierce, and M. Amin Arnaout, Harvard Medical School, Boston, MA 02115
Mo1 is a surface membrane glycoprotein present on human granulocytes, monocytes and large granular lymphocytes. It is a heterodimer with an alpha subunit of 155kD non-covalently associated with a beta subunit of 94kD. Mo1 is identical with complement receptor type 3 (CR3) and also promotes adhesion of granulocytes to each other and to certain substrates. Rabbit polyclonal antibodies were developed by immunizing rabbits with purified human Mo1 alpha protein and used to screen a lambda gt11 human peripheral blood leukocyte expression library. One clone which reacted with the anti-alpha antiserum (alpha 155L4) contained a 1.5 kb insert which produced a fusion protein of 180 kD. This fusion protein reacted on Western blots with anti-Mo1 alpha polyclonal antibody that had been further affinity purified. Northern blots revealed that alpha 155L4 hybridizes to a messenger RNA of about 8 kb in human monocytes and induced HL60 cells. This cDNA clone was used to isolate a cosmid genomic clone for the Mo1 alpha chain which cross-reacts with an oligonucleotide made against the amino acid sequence from a tryptic peptide derived from purified Mo1 protein. This clone should be helpful in understanding the factors regulating transcription, translation, and surface expression of this leukocyte differentiation antigen.

D 420 EVIDENCE FOR IMMUNOREGULATORY CONTROL OF MuLV-INDUCED LYMPHOMAGENESIS BY CLASS II GENES: THE I-A REGION REGULATES BOTH SUSCEPTIBILITY TO LYMPHOMAGENESIS AND PHENOTYPE OF VIRUS-INDUCED LYMPHOMAS.
Wies L.E. Vasmel, Maarten Zijlstra, Thaddaus Radaszkiewicz*, Cornelis J.M. Melief, Division of Immunology, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands, *Institute of Pathology, Spitalgasse 4, Vienna, Austria.

Neonatal infection of C57BL/10 mice with a cloned dualtropic mink cell focus-inducing murine leukemia virus (MuLV), MCF 1233, induces a wide spectrum of lymphomas of T, B and non-T/non-B cell type. In a mapping study using intra H-2 recombinant C57BL/10 mice, evidence was obtained that a) resistance to early development of T-cell lymphomas is controlled by the H-2 I-A locus, b) susceptibility to early T-cell lymphomagenesis is associated with an I-A regulated low anti-MCF 1233 envelope antibody response and persistent viral infection of the thymus, c) H-2 I-A^b mice, although resistant to early T-cell tumors, develop more B-cell lymphomas late in life. On some B10.A (H-2^a) B-cell lymphomas imbalanced class II MHC expression was observed. The immunoregulatory mechanisms underlying the pleiotropic influence of the I-A locus on the outcome of MuLV-induced lymphomagenesis will be discussed.

D 421 B-CELL LEUKEMIA/LYMPHOMA RECAPITULATES THE NORMAL IMMUNE RESPONSE, D.D. Weisenburger, D.S. Harrington, J.O. Armitage, Univ. of Nebr. Med. Ctr., Omaha, NE 68105.
Surface marker studies of 240 cases of B-cell leukemia/lymphoma indicate that the various types of B-cell neoplasia represent successive stages at which neoplastic differentiation is arrested. Pre-B acute lymphoblastic leukemia arises from the rapidly-dividing pool of normal bone marrow lymphoblasts (cyto. μ, CALLA +). Chronic lymphocytic leukemia/small lymphocytic lymphoma usually arises from an immature, bone marrow-derived virgin B-cell that mediates the primary immune response (sIgM+D, Leu 1 +). Intermediate lymphocytic (mantle-zone) lymphoma arises from a slightly-more differentiated cell that resides in primary follicles and the mantle zones of secondary follicles (sIgM,D +); thus, the intermediate lymphocyte appears to be the precursor cell of the normal germinal center. The follicular center cell lymphomas are derived from reactive germinal centers, where clonal expansion and Ig-class switching (IgM,D → IgG/A) occur and from which memory B-cells arise. The sequence of differentiation within the germinal center is thought to be: small non-cleaved → large non-cleaved → large cleaved → small cleaved cell (IgG/A, CALLA +). Lymphoplasmacytoid lymphoma may be derived from either mature IgM-producing B-cells of the primary response or IgG/A-producing memory B-cells of the secondary immune response (cyto. Ig +). Immunoblastic lymphomas may be derived from large non-cleaved cells of the prolonged germinal center reaction, from mature small lymphocytes of the primary response, or from memory B-cells (cyto. Ig +). Partial blocks in differentiation result in the development of "mixed-cell" lymphomas. Thus, relationships of the various types of B-cell leukemia/lymphoma may be understood in the context of the normal immune response.

D 422 RELATIONSHIPS BETWEEN B-LINEAGE LYMPHOCYTES AND STROMAL CELLS IN LONG TERM BONE MARROW CULTURES. PL Witte, PW Kincade, M Robinson, D Stiers, and M Low. UTHSC at Dallas and Oklahoma Med Res Found, Oklahoma City 73104.
Hemopoietic microenvironmental cells may provide stimuli necessary for the differentiation of early B-lineage lymphocyte precursors. In long term cultures of bone marrow, lymphocytes are supported by a pleomorphic adherent stroma. We examined the identity and characteristics of the adherent cells and the specificity of the lymphocyte-stromal cell contact. Two types of adherent cells were distinguished by immunoperoxidase staining with an extensive panel of monoclonal antibodies against lymphoid and hemopoietic cells. A majority were macrophages that lacked Ia. These were localized around very large, highly spread stromal cells that expressed no hemopoietic antigens. Large stromal cells did not endocytose acetylated-LDL and could be separated from macrophages on this basis. Sorted acyl-LDL negative stromal cells supported the growth of lymphocytes after reculture. In primary cultures, lymphocyte clusters were physically associated with large stromal cells only, and suspended lymphocytes rebound specifically to a subset of large stromal cells. A specific adhesion mechanism is suggested by the ability to release bound lymphocytes with EDTA or with a phospholipase C specific for phosphatidyl inositol. The findings suggest that cultured B-cell precursors are supported by nonhemopoietic-derived stromal cells and that at least two subsets of these can be distinguished by their association with lymphocytes.

Hematopoiesis; Therapy

D 500 PROPERTIES OF DENDRITIC CELL PRECURSORS IN RAT BONE MARROW, William E. Bowers and Mary R. Berkowitz, Bassett Research Inst. for Medical Research, Copperstown, NY 13326
Dendritic cells provide accessory activity for responses of T lymphocytes initiated by mitogens or antigens and act as potent stimulators of a mixed leukocyte reaction. Although not detectable in fresh preparations of bone marrow cells, both dendritic cells and accessory activity developed when bone marrow cells were cultured for five days in serum-free medium. Virtually all dendritic cell precursors in bone marrow were recovered in a low-density fraction containing only 5% of the fractionated bone marrow cells. Removal of the 2-5% Ia^+ low-density bone marrow cells by panning prior to culture did not decrease the production of dendritic cells. The dendritic cells produced in cultures of bone marrow cells were strongly Ia^+. Irradiation of low-density bone marrow cells prior to culture prevented the development of dendritic cells. When irradiation was delayed by daily intervals, progressive increases in the number of dendritic cells resulted up to the 5th day. ^{3}H-thymidine in the culture medium was incorporated into dendritic cells, as detected by autoradiography. We conclude that the bone marrow-derived dendritic cell arises from the division of a low-density precursor that does not express, or minimally expresses, Ia. Supported by USPHS grant AI-17887 and The Stephen C. Clark Fund of the Mary Imogene Bassett Hospital.

D 501 ABVD + LIMITED-FIELD LOW-DOSE RADIOTHERAPY (RT) FOR CHILDHOOD HODGKIN'S DISEASE (HD).
Franca Fossati-Bellani, Marco Gasparini, Rado Kenda, Fabrizio Lombardi, and Paolo Pizzetti, Istituto Nazionale Tumori, Milan 20133, Italy.
Splenectomy, high-dose extended-field RT and MOPP produce important acute and late complications in children with HD. In 1979 the treatment approach for children with stage I-III HD was changed: laparotomy was no longer required and substituted by laparoscopy with liver and spleen biopsies, MOPP was replaced by ABVD, and doses and fields of RT were reduced. The initial treatment in each case consisted of 3 monthly cycles of ABVD, followed by RT (30 and 35 Gy to involved area(s) to complete and partial responders, respectively, and 25 Gy to the adjacent area(s)). Children with stage III or B symptoms received 3 additional courses of ABVD. 44 consecutive children (age range 3-15 yrs) were treated. Stages were: IA 12, IIA 10, IIB 10, IIIA+B 12. As to histologic subtypes, 24 were NS, 15 MC, 4 LP, 1 LD. Following the initial 3 ABVD courses, of the 22 stage I and IIA 18 (82%) achieved CR and 4 PR. Of the 22 stage IIB and III, CR was reached in 8 (36%) and PR in 14 (64%). No patient progressed or relapsed during initial ABVD. At the end of RT all 44 pts. attained CR. The actuarial 5-yr RFS is 95%, with a median follow-up of 47 mos. (range 18-74 mos). 2 children have relapsed: one stage IIB with a single lung metastasis, one stage IIIB with disseminated disease. Except for nausea and vomiting ABVD produced no severe acute side effects. Long-term sequelae are under evaluation.

D 502 DEXAMETHASONE, HIGH DOSE ARA-C AND CISPLATINUM (DHAP) FOR RELAPSING HODGKIN'S DISEASE,
Fredrick Hagemeister, William Velasquez, Sundar Jagannath, and Fernando Cabanillas. Univ. Texas, M.D. Anderson Hospital, Houston, TX 77030.
Twenty-two patients (pts) with progressive Hodgkin's disease were treated with DHAP. All had received prior MOPP and adriamycin-containing combinations, and 18 had prior radiation. Eight had no response to prior chemotherapy and 14 had recurrence in irradiated areas. The median age was 27 (19-52) and 13 had B symptoms. Seventeen had extranodal (EN) disease, including pleura or lung (14 pts), bone marrow or bone (5), liver (2), and kidney (1). Sixteen had mediastinal and eight had abdominal nodal disease. DHAP consisted of dexamethasone 40 mg IV days 1-4 and platinum 100 mg/M2 continuous infusion over 24 hours on day 1, followed by Ara-C 2 gm/M2 over 3 hours q12h x 2 on day 2. Among the 22 pts, 16 received 2-4 cycles of DHAP and subsequent high dose cyclophosphamide, BCNU, etoposide, and autologous bone marrow transplantation (ABMT). Twelve (75%) responded to DHAP with 5 CR and 7 PR. Eight responders have undergone ABMT for two additional CRs. Only one of the 12 had disease progression with a median follow-up of 4 months. All but 2 of the 16 are alive. Six other pts were not considered for ABMT; 3 had prior ABMT, one was elderly, and 2 had bone marrow disease. These 6 received 2-6 cycles of DHAP, and 3 responded. Response to DHAP was related to tumor extent. For the 8 having 2 sites of EN or nodal disease (ND), there were 2 PR and one CR. By contrast, among the 14 pts having only one EN site or ND, there were 5 CR and 7 PR. Myelosuppression was common; however, only one pt died with neutropenic infection. No pt had permanent renal damage. DHAP has significant activity in this heavily pretreated population with a 68% response rate.

D 503 HANDICAPPED RETROVIRAL VECTORS EFFICIENTLY TRANSDUCE FOREIGN GENES INTO HEMATOPOIETIC STEM CELLS,
Robert G. Hawley, Luis Covarrubias, Teresa Hawley, and Beatrice Mintz, Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, PA 19111
A novel retroviral vector has been designed for introducing nonselectable genes, governed by their own (or experimentally chosen) regulatory sequences, into cells of intact animals. The prototype vector contains a deletion of enhancer and promoter sequences in the 3' long terminal repeat (LTR), from which a deletion in the 5' LTR would ensue. Recombinant helper-free viruses with the c-*myc* proto-oncogene under the control of immunoglobulin (Ig) gene regulatory elements have been generated. Infected lymphoma cell lines of the pre-B- and B-cell types expressed the retroviral c-*myc* sequences. *In vivo* experiments were then undertaken with retrovirally infected hematopoietic stem cells. Recipient mice were unirradiated and had a stem cell (W/W^v) or lymphoid (*scid*) deficiency. They were successfully repopulated with donor stem cells that gave rise to lineages with the appropriate genetic markers in erythrocytes (hemoglobin electrophoretic variant) and lymphocytes (restriction fragment length polymorphism in the Ig μ heavy chain gene). Intact recombinant viral sequences were detected in recipient spleen DNA. Engraftment with multiple stem cells was suggested by the complexity of the viral restriction enzyme patterns. Studies of expression and effects of the introduced c-*myc* gene are in progress.

D 504 EBVP (EPIRUBICINE, BLEOMYCINE, VINBLASTINE, PREDNISONE) CHEMOTHERAPY BEFORE RADIOTHERAPY IN LOCALIZED HODGKIN'S DISEASE. B. Hoerni for the Pierre-et-Marie-Group. Fondation Bergonié, 180, rue de Saint-Genès, 33076 Bordeaux Cedex France.

In usual and good presentation, Hodgkin's disease is highly curable. Thus the therapeutic search is mainly aimed to decrease toxicity of treatment. With this objective, a new regimen of chemotherapy, was tried in a cooperative phase II trial, derived from ABVD : doxorubicine is replaced by epirubicine and dacarbazine by prednisone. Thirty eight consecutive patients with stage I to IIIA, previously untreated, received 3 courses (= 3 months) of this regimen before radical radiotherapy. Gastro intestinal and alopecia were far less marked than with ABVD used in the same cooperative group. Immediate efficacy, after 3 courses, was similar with 80% of complete remission (one third observed at day 28) and only 2 failures (5%). All patients with only partial remission were put in complete remission by following radiotherapy. This regimen appears as a clear improvement in the treatment of patients with Hodgkin's disease and deserves larger comparison with other previously used chemotherapy in a more extensive controlled trial.

D 505 BIOLOGY OF THE NORMAL AND LEUKEMIC CD-7+ STEM CELL. J. Kurtzberg, S. Denning, P. Le, K. Singer, M. Hershfield and B. Haynes, Duke Medical Center, Durham NC 27710.

We previously described the complete lymphoid to myeloid conversion of leukemic blasts in a patient with CD-7+ acute lymphoblastic leukemia following therapy with 2'deoxycoformycin (PNAS USA 81:253, 1984). We subsequently established a permanent CD-7+ cell line (DU.528) from leukemic cells from this patient that were capable of *in vitro* multilineage differentiation (J Exp Med 162:1561, 1985). We hypothesized that the malignant cell in this patient was not irreversibly committed to the T lineage, but rather was a lymphohematopoietic stem cell (LHSC) bearing the CD-7 antigen. In this report we characterize the clinical and pathophysiologic features of 9 patients with CD-7+ stem cell leukemia and report on studies to identify the normal counterpart of this malignant pleuripotent CD-7+ cell. Immunophenotypic analyses of pretreatment leukemic blasts revealed that 9/9 patients were CD-7+, 2/9 CD-3+, 4/9 CD-2+. All were negative for other mature T-lymphoid, B-cell, myeloid, and erythroid cell surface antigens. Pretreatment leukemic blasts from 8 patients were evaluated in *in vitro* clonal assays for lymphoid and non-lymphoid (myeloid, erythroid, megakaryocytoid) differentiation. Blasts from 7/8 were able to give rise to the full complement of lymphohematopoietic progeny. Normal CD-7+ LHSC, found in the CD-7+, CD-4-, CD-8- subset of normal thymocytes and bone marrow cells, were capable of self renewal as well as lymphoid and nonlymphoid differentiation. Factors that regulate the commitment of this cell to lymphoid or non-lymphoid differentiation pathways will be discussed.

D 506 DOWN REGULATION OF CFU-GM DERIVED FROM PATIENTS WITH UNTREATED CML, I.Z. Leiderman and M.L.Greenberg, Mt. Sinai School of Medicine (CUNY), New York, NY 10029

Patients with untreated CML have an increased number of CFU-GM. We have reported that bone marrow (BM) cells from patients within the AIDS spectrum inhibit normal CFU-GM. Conditioned media (CM) prepared by the liquid culture of these BM cells contain the inhibitory activity that has been isolated in a 8kD glycoprotein (gp84) fraction. We investigated the possibility that leukemic CFU-GM could also be inhibited by AIDS BM cells and CM. Nucleated cells isolated from three Ph^1 positive CML BM were cultured over feederlayers (FL) containing 0.5, 1.0, 2.0, and 4.0 X 10^5 nucleated BM cells from patients with AIDS. CSF was not added to the FL. The CML derived CFU-GM were inhibited significantly in a dose response fashion (23.5, 36.4, 44.9, and 61.5% respectively) when compared to CML BM cultured over FL without BM cells (P <0.001). AIDS CM equivalent to 4 X10^5 BM cells inhibited the CFU-GM to a statistically significant greater degree than the AIDS BM cells themselves (74%, P <0.01). CM from the BM cells of two healthy HIV negative, gay men, two healthy controls, two patients with myelodysplastic syndrome, and one with Ph^1 positive CML had no inhibitory effect. Western blots of CML CM were probed with an antibody raised against gp84. No band was found to be cross reactive in lanes containing CM from the CML patients though five out of nine healthy normal controls had faint bands in the 84kD region. These data suggest that leukemic granulopoiesis can be inhibited in a manner similar to normal granulopoiesis and, thus far, there is no evidence that this newly described inhibitor is produced by the leukemic cells.

D 507 ETOPOSIDE FOR REMISSION INDUCTION OF ADULT ACUTE NON-LYMPHOCYTIC LEUKAEMIA (ANLL),
Raymond M. Lowenthal, James F. Bishop, Douglas E. Joshua and Jane P. Matthews, on behalf of the Australian Leukaemia Study Group, University of Tasmania, Australia, 7005.
In January 1984 the Australian Leukaemia Study Group initiated a multi-centre randomised controlled study of protocol "73" (cytosine arabinoside 100 mg/sq m/day by continuous infusion for 7 days plus daunorubicin 50 mg/sq m/day for 3 days) versus protocol "737" (cytosine and daunorubicin as above, plus etoposide 75 mg/sq m/day for 7 days). This trial is designed to define the place of etoposide in the induction treatment of patients with adult acute non-lymphocytic leukaemia. The trial is open to previously untreated patients between the ages of 15 and 70. By September 1986, 243 patients had been entered. Preliminary analyses of toxicities showed patients receiving "737" had more WHO grade 3 or 4 stomatitis ($p < 0.01$) and diarrhoea than patients receiving "73", but without more infective sequelae; otherwise toxicities were similar. In preliminary comparisons between the two protocols, overall complete remission (CR) rates were similar (54% for "73", 61% for "737") as were durations of CR. However, sub-set analysis showed that for patients aged < 55, there were significant benefits of "737" in terms of CR rate (70% versus 52%, $p < 0.03$) and median survival (23 months versus 11 months, $p < 0.02$). The addition of etoposide to standard induction therapy for adult ANLL may be of benefit to patients aged < 55; follow-up of patients in this study is continuing.

D 508 RECOMBINANT HUMAN GM-CSF INDUCES LEUKOCYTOSIS AND ACTIVATES PERIPHERAL BLOOD POLYMORPHONUCLEAR NEUTROPHILS (PMNs) IN NON-HUMAN PRIMATES,
Peter Mayer, Charles Lam, Hubert Obenaus, Ekke Liehl and Jürgen Besemer, Sandoz Forschungsinstitut, Brunner Straße 59, 1235 Vienna, Austria.

The *in vivo* efficacy of glycosylated and non-glycosylated recombinant human granulocyte macrophage colony stimulating factor (rh GM-CSF) was studied in rhesus monkeys. The monkeys responded to the rh GM-CSF with a prompt rise in circulating white blood cells within 24 hrs. Thereafter, the total cell counts increased steadily in a dose-dependent manner with repeated dosing to numbers six times over the pretreatment levels. Within one week after the end of treatment, the leukocytosis had disappeared. In addition to inducing leukocytosis, parenterally administered rh GM-CSF primed mature circulating granulocytes for enhanced oxidative metabolism and killing of an *E.coli* strain. These results show that exogenously administered glycosylated or non-glycosylated rh GM-CSF is both an effective stimulator of leukocytosis and a potent activator of the phagocytic function of mature granulocytes in monkeys.

D 509 Predictors of Response To Low Dose Ara-C.
K.B. Miller, H. Grunwald, R. Larson, A. Raza, J. Goldberg, R. Vogler, J. Bennett, G. Browman, H. D. Preisler. A Leukemia Intergroup Study. Roswell Park Memorial Institute, Buffalo, NY
Thirty patients with either A.N.L.L. or a myelodysplastic syndrome were treated with Ara-C 10 mg/m2 subcutaneously q/12hrs for 21 days. The overall response rate was 30% (19% CR: 11% PR). Responses ranged from 8-75 weeks, mean 20 weeks. The predictors for response to low dose Ara-C included the pretherapy WBC and BUdr labeling index. The median WBC of the responder was lower than for the non-responder 1,400 vs 20,100 respectively ($P < .05$). There was no correlation between the pretherapy bone marrow biopsy or aspirate cellularity, percent abnormal cells and the response to Ara-C.

A similar finding was observed in the labeling index (BUdr). Responders had a lower labeling index (4.8%) than non-responders (8.8%). No patient with a labeling index greater than 7% responded to Ara-C. There was no apparent correlation between the pretherapy *in vitro* Clonogenic assay, or Ara-c inhibition of DNA synthesis and the outcome of therapy. Bone marrow biopsies were performed on days 7, 14, 21, and 28 of therapy. All responders demonstrated cytoreduction on their day 14 bone marrow biopsy. However, there was no correlation with the degree of cytoreduction and the outcome of therapy.

In summary, the predictors of response to low dose Ara-C include the pretherapy WBC and *in vitro* labeling index. These studies may define which patients might benefit from a prolonged course of low dose Ara-C.

D 510 CLINICAL RELEVANCE OF PROTO-ONCOGENE EXPRESSION, Harvey D. Preisler, Azra Raza, Roswell Park Memorial Institute, Buffalo NY 14263.

The expression of 5 proto-oncogenes (c-myc, c-myb, c-fos, c-fms, c-fes) and of histone H3 were assessed in 30 patients with newly diagnosed leukemia. The level of c-myc and c-myb expression were significantly correlated ($r = .5836$, $p = .001$). No other correlations were noted. The pattern of gene expression and the level of expression were unrelated to FAB type suggesting that the level of expression of differentiation associated genes is not necessarily reflected in the morphologic appearance of the cells. All patients received remission induction therapy. While there were patients with high and low levels of c-myc expression in the group that entered CR, high c-myc expression appeared to characterize patients whose leukemia failed to respond to treatment (RD or resistant disease failures). In addition, two different sized c-myc transcripts were detected in each patient. If the smaller sized transcript predominated the patient was unlikely to respond to therapy ($p=.007$). The effect of chemotherapy in vivo on c-myc and on histone H3 expression were measured and related to response. The histone H3 mRNA level fell in all 10 patients who entered remission while the same was true for only 1 of 9 patients with RD ($p=<.001$). With respect to c-myc, the level of expression fell in 7 of 14 patients who entered CR but in only 2 of 13 RD patients ($p=.103$). Remission duration appears to be longer for those CR patients in whom c-myc expression was reduced by chemotherapy but longer follow-up is necessary. These data demonstrate that assessment of the pattern of proto-oncogene expression in leukemic cells at diagnosis and the effects of chemotherapy on expression provides important clinical information regarding the likely response to chemotherapy.

D 511 ROLE OF THE CHICK EMBRYO MICROENVIRONMENT IN CONTROLLING AMV-TRANSFORMED NONPRODUCER CELLS: A MODEL SYSTEM FOR STUDYING HOMING AND CELL DIFFERENTIATION, M. Siegel, S. Vidan, M.G. Moscovici, and C. Moscovici, University of Florida, Gainesville, FL 32610 and Tumor Virology Laboratory, Veterans Administration Medical Center, Gainesville, FL 32620.

Avian myeloblastosis virus (AMV) is a replication-defective retrovirus which induces an acute monocytic leukemia in birds and is capable of transforming myeloid hematopoietic cells in vitro. Nonproducer, AMV-transformed cell lines have been obtained by infecting both outbred (SPAFAS) and inbred (HYLINE) yolk sac or bone marrow cells at a low multiplicity of infection. The cell lines proliferate in vitro as non-adherent mononuclear monoblasts but when injected intravenously into 13-day old embryos produce no overt leukemia after hatching. Phenotypically identical cells can, however, be recovered from animals sacrificed at different intervals from several days post injection to two weeks after hatching. Prior attempts to recover nonproducer cells from older birds were unsuccessful. We have used recombinant DNA probes to (i) confirm the identity of recovered cells by proviral integration analysis and (ii) screen the bone marrow and other tissues of older birds in order to study the homing and differentiation of the nonproducer cells in vivo.

D 512 THE EFFECT OF ALTERSOLANOL A AND MACROSPORIN ON NEOPLASTIC AND TRANSFORMED LYMPHOBLASTOID CELL LINES, Leo F. Skinnider and Albert Stoessl, University of Saskatchewan, Saskatoon, Sask., S7N OWO, Canada and Agriculture Canada, London, Ont.

Altersolanol A is a naturally occurring tetrahydroanthraquinone, a metabolite of the fungi _Alternaria solani_ and _Dactylaria lutea_. The anthraquinone macrosporin is biosynthesised in _A. Solani_ from altersolanol A. The compounds were tested against the mouse leukemic L1210 cell line and the human lymphoblastoid cell line Raji. Altersolanol A was cytotoxic to both cell lines at a concentration of 2 ug/ml. Inhibition of [^{3}H] thymidine and [^{3}H] leucine uptake in both L1210 and Raji cells occurred at 1 ug/ml. Macrosporin was cytotoxic at 10 ug/ml but at 5 ug/ml growth and viability of both cell lines were normal. With normal human lymphocytes cytotoxicity of Altersolanol A was found at a concentration of 5 ug/ml.

D 513 HIGH RISK LYMPHOID MALIGNANCIES: FRACTIONATED TOTAL BODY IRRADIATION AND HIGH DOSE CYCLOPHOSPHAMIDE FOLLOWED BY BONE MARROW TRANSPLANTATION DURING FIRST COMPLETE REMISSION, David S. Snyder, Auayporn P. Nademanee, Philip J. Bierman, Gerhard M. Schmidt, Margaret R. O'Donnell, Stephen J. Forman and Karl G. Blume, City of Hope National Medical Center, Duarte, CA 91010.

Between April 1983 and June 1986, 28 adult patients (pts) [age range 16-38 years, median: 23 years] with high risk lymphoid malignancies were treated during their first complete remission (CR) with a uniform preparatory regimen [fractionated total body irradiation with 11 x 120 cGy and cyclophosphamide 100 mg/kg] followed by allogeneic bone marrow transplantation (BMT) from histocompatible sibling donors. Six pts had malignant lymphoma with extranodal involvement [CNS, bone marrow or skin] at the time of diagnosis and four of them are alive and in continued CR 4, 10, 17 and 42 months after BMT; one pt died with chronic graft-versus-host disease and one relapsed after BMT. Twenty-two pts had acute lymphoblastic leukemia (ALL) and 17 of them had one or more of the following risk factors at presentation: WBC >25,000/μl, chromosomal translocations, extramedullary leukemia, age >30 or requirement for more than six weeks of chemotherapy to reach CR. Currently, 17 of the 22 ALL pts are alive and in continued CR for 7-40 months [median: 22 months] after BMT; the actuarial disease-free survival rate is 74%. Three ALL pts relapsed after BMT [actuarial relapse rate: 18%] and 2 pts developed acute graft-versus-host disease followed by fatal interstitial pneumonia. Our data indicate that bone marrow ablation with high dose radiochemotherapy followed by allogeneic bone marrow transplantation leads to prolonged disease-free survival and probably cure in approximately two-thirds of patients with high risk lymphoid malignancies.

D 514 HIGH-DOSE CYTOSINE ARABINOSIDE (HDARA-C) AND DAUNORUBICIN AS BRIEF CONSOLIDATION OF ACUTE NON-LYMPHOCYTIC LEUKEMIA (ANLL) IN FIRST REMISSION: AN UPDATE, SN Wolff, GL Phillips, RH Herzig, HM Lazarus, RS Stein and GP Herzig, Vanderbilt University, Nashville TN 37232, Cleveland Clinic Foundation, Cleveland OH 44106, Case Western Reserve University, Cleveland OH 44106 and Washington University, St. Louis MO 63110. Beginning in 1979, 52 adult patients with ANLL induced in first remission with standard therapy received HDARA-C and Daunorubicin as intensive consolidation. HDARA-C at a dose of 3 gm/m^2 was infused over 1 hour every 12 hours for 6 days (12 doses) followed by Daunorubicin at a dose of 30 mg/m^2 daily for 3 days. The median age of all patients was 38 years (range 14-65). The median interval from obtaining complete remission to beginning HDARA-C was 1 month (range 1-14) with however only 2 patients having an interval greater than 2.5 months. 31 patients received 1 cycle of consolidation, 20 patients 2 cycles and 1 patient 3 cycles. Severe infection was the most common reason for not administering a second cycle although only 3 patients (6%) died during consolidation. After consolidation, no further therapy was administered. As of August 1986, the overall actuarial probability of continued complete remission (ccr) is 52%. 13 patients have been in remission for more than 3 years from diagnosis and the survival curve appears to plateau. The probability of ccr for the 22 patients less than 35 years old is 76%. As expected with this intensive but non-marrow ablative treatment, myelosuppression was formidable. As shown by this study, brief intensive consolidation with HDARA-C has resulted in a large proportion of patients remaining in ccr. These results are comparable to bone marrow transplantation in first remission and suggest that comparative studies be performed.

D 515 INFLUENCE OF LOW MOLECULAR WEIGHT B CELL GROWTH FACTOR (BCGF) AND IL-2 ON HUMAN B CELL PRECURSOR ALL

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We have studied the influence of the 12 kD BCGF on proliferation and differentiation of B cell precursor ALL (non-B/non-T ALL). Partially purified BCGF was obtained from Cytokine Tech., Buffalo, N.Y., and contained no detectable IL-1, IL-2 or -IF. Bone marrow aspirates (>90% leukemic blasts) from 36 patients with B cell precursor ALL were incubated with various concentrations of BCGF for 96 h and pulsed with ^{3}H-TdR for the last 8 or 16 h. In 27/36 patients (75%) BCGF induced a significant increase of ^{3}H-TdR incorporation, with stimulation indices ranging from 2-129. The leukemic cell specificity of the BCGF response was confirmed on CALLA$^+$ cells isolated by cell sorting. After incubation of bm from 11 patients for 24 h, 6-54% of the cells expressed IL-2 receptors as determined by anti-Tac binding. Recombinant IL-2 in concentrations from 10-1000 U/ml, however, did not induce proliferation of the cells. A synergistic effect of IL-2 with BCGF was observed in 1/10 patients. The B cell antigen CD20/p35 is acquired during B cell maturation. Cells from 7 patients with CALLA$^+$, CD20$^-$ ALL were incubated with either BCGF, IL-2 at 250 U/ml, BCGF + IL-2 or medium alone for 48 h. BCGF induced CD20 expression in 3/7 patients. IL-2 did not induce CD20 but enhanced its expression in the 3 patients who responded to BCGF. We conclude that BCGF can induce proliferation and differentiation of leukemic B cell precursors.