LEUKEMIA: ADVANCES IN BIOLOGY & THERAPY--PROGRESS & CONTROVERSIES

Organizers: Robert Peter Gale, Melvyn Greaves, Donald Metcalf and David Baltimore

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Poster Sessions

Leukemia: Advances in Biology and Therapy — Progress and Controversies

Etiology: Epidemiology, Risk Factors, Radiation, Immune Deficiency

S 001 **FANCONI ANEMIA AND LEUKEMIA - TRACKING THE GENE.** Arleen D. Auerbach[1], William R. Mann[1], V.S. Venkatraj[1], R.G. Allen[1], Qian Liu[1], Drew A. Olsen[1], Barbara Adler-Brecher[1], Stephanie L. Sherman[2]. [1]The Rockefeller University, New York, NY 10021 and [2]Emory University School of Medicine, Atlanta GA 30322.

We report the results of a genetic linkage study involving 34 families enrolled in the International Fanconi Anemia (FA) Registry. FA is an autosomal recessive disorder characterized clinically by a progressive pancytopenia, diverse congenital abnormalities and increased predisposition to malignancy. The incidence of acute myelogenous leukemia (AML) in FA patients may be more than 15,000 times that observed in children in the general population, and is associated with an exceedingly poor prognosis with a mean age of death of 15 years. We suggest that all FA patients may be considered preleukemic, and that this disorder presents a model for the study of the etiology of AML.

Of the 34 families studied for linkage, 12 were consanguineous with one or more affected siblings, 20 non-consanguineous families were multiplex, and two had irregular structures with affected children appearing in collateral or more distant sibships. DNA was obtained from a total of 199 individuals, all of whom were diagnosed as affected/unaffected on the basis of the diepoxybutane (DEB) test. After excluding approximately 40% of the genome (73 RFLP probes) for linkage to FA, a significant lod score was obtained between D20S20, an anonymous DNA segment from distal 20q, and FA ($Z_{max} = 3.04$, $\Theta_{max} = 0.12$). However, six other chromosome 20q markers, including D20S19 which is highly polymorphic and tightly linked to D20S20, revealed only weak or no evidence for linkage in these families by two point linkage analysis, and the intervals between all chromosome 20q markers examined were excluded for linkage to FA by multipoint analysis. The admixture test for linkage heterogeneity revealed significant evidence for linkage heterogeneity in the region defined by markers D20S19 and D20S20. It is noteworthy that lymphoblastoid cell lines established from two affected individuals in one of the larger kindreds have previously been shown to belong to FA complementation group A. Lod scores suggestive of linkage between Fanconi anemia and D20S19 were obtained with two of the largest kindreds studied (lods = 2.6 and 2.1, respectively, at $\Theta = 0.001$). Thus, our data support the provisional assignment of a Fanconi anemia gene to chromosome 20q.

Among genes localized to distal chromosome 20q that have pertinence to FA are topoisomerase I (TOPI), proliferating cell nuclear antigen (PCNA, cyclin), hemopoietic cell kinase (HCK), c-*src*1(human), protein phosphotyrosyl phosphatase 1B (PTP1B) and phospholipase C-148 (PLC1); deletions of this chromosomal region are associated with myelodysplasia and AML. The regional placement of an FA-determining gene is a first step towards the identification of an FA gene, which should elucidate the basis for the leukemia predisposition in these patients.

S 002 **ATAXIA-TELANGIECTASIA (A-T): A DISEASE PROVIDING CLUES TO THE REGULATION OF THE IMMUNOGLOBULIN GENE SUPERFAMILY?**, Raymond D.A. Peterson, Jane D. Funkhouser and Richard A. Gatti, Depts. Pathology and Biochemistry, University of South Alabama, Mobile, AL. 36688 and Dept. of Pathology, University California Los Angeles, CA 90024.

A-T is one of several genetically determined syndromes that has cancer as one of its prominent characteristics. Approximately 35% of patients with this disorder die of leukemia/lymphoma or other malignancies if they don't succumb earlier as a consequence of infection due to their immunodeficiency or inanition due to their progressive neurological disability. Heterozygotes for the A-T gene also have a significantly increased risk of cancer, i.e. 2.3-fold for men and 3.1-fold for women, relative to non-carriers. The relative risk of breast cancer for the female A-T heterozygote is 6.8-fold. It is estimated the 9 to 18% of all breast cancer cases may be A-T heterozygotes.

A prominent molecular feature of this syndrome is the occurrence of non-random chromosome breaks and rearrangements in lymphocytes. They occur at sites where somatic recombinatorial DNA rearrangements occur prior to gene expression: 7p14, 7q35, 14q11.2, 14q32, 2p12 and 22q11, the sites of TCRγ, TCRβ, TCRα, IGH, IGκ, and IGλ respectively. Leukemias and lymphomas in these patients generally exhibit one or more of these rearrangements. Longitudinal studies suggest that a particular rearrangement may be exhibited by a gradually enlarging clone of lymphocytes for years before an overt malignancy becomes evident. The rearrangements present in lymphocytes are not commonly observed in fibroblasts, despite the increased instability of chromosomes in these cells.

The gene responsible for A-T has been localized to a region of less than 8 cM on chromosome 11q23. Several members of the Ig gene superfamily also reside in this locale. These include N-CAM, Thy-1, and CD3. 11q23 may be a chromosomal segment where extensive early duplication of the Ig superfamily genes occurred.

The protean manifestations of A-T all may be the consequence of a mutation in the gene coding for a protein that regulates the genes of the immunoglobulin superfamily. Huebner et al (Cancer Res.49,4071-4074, 1989) have developed the notion that characteristic rearrangements/deletions occur in lineage-specific regions of the genome as part of the normal differentiation program of cell of many lineages. Identification of the A-T gene may provide insight into the molecular mechanisms regulating such processes.

Viruses

S 003 PROPHYLAXIS AGAINST HTLV-I IN A RABBIT MODEL, Isao Miyoshi, Department of Medicine, Kochi Medical School, Kochi 783, Japan

We have investigated the effect of HTLV-I immune globulin (HTLVIG) against transmission of HTLV-I in rabbits. HTLVIG containing 77 mg/ml of IgG was prepared from pooled plasma from seropositive healthy persons. This preparation had an immunofluorescence antibody titer of 1:5,120 and a vesicular stomatitis virus (HTLV-I) pseudotype neutralizing titer of 1:6,250. In the first experiment, 4 groups (A, B, C, and D) of 3 rabbits weighing about 3 kg were transfused with 5 ml of blood from an HTLV-I-infected rabbit. Groups A, B, and C were infused 24 hours later with 10, 5, and 2 ml of HTLVIG, respectively, while group D was infused with 10 ml of HTLVIG 48 hours later. Seroconversion for HTLV-I occurred in none of group A, 1 of group B, and 3 of groups C and D after 2-5 weeks. In the second experiment, 4 litters (E, F, G, and H) born to another virus-infected rabbit and consisting of 7, 5, 7, and 7 newborns, respectively, were used. Litters E and H were allowed to grow normally, while litters F and G were infused intraperitoneally with 3 µl/g of HTLVIG weekly 4 times until weaning. Although 3 of litters E and H seroconverted for HTLV-I after 5-8 weeks, none of litter F and 1 of litter G became antibody-positive after 10 weeks. Presence or absence of HTLV-I infection in all these animals was confirmed by transfusion assay or gene amplification. These results indicate that passive immunization protects rabbits against not only blood-borne but also milk-borne transmission of HTLV-I, when given within 24 hours of transfusion or during breast feeding. In view of the proven safety of human immunoglobulin, a clinical trial of HTLVIG would be warranted.

Kataoka, R., Takehara, N., Iwahara, Y., Sawada, T., Ohtsuki, Y., Dawei, H., Hoshino, H., and Miyoshi, I.: Transmission of HTLV-I by blood transfusion and its prevention by passive immunization in rabbits. Blood 76: 1657-1661, 1990.

S 004 VIRUSES AND HODGKIN'S DISEASE: Ruth Jarrett and David Onions. LRF Virus Centre, University of Glasgow G61 1QH, UK.

Epidemiological evidence suggests that a transmissable agent is involved in the pathogenesis of Hodgkin's disease (HD) and there is accumulating evidence that EBV is involved. EBV genomes are detectable in tumour biopsies from approximately one third of non-selected cases of HD and analysis of the terminal fragments of these genomes indicates that the EBV-infected cells are clonal. EBV has been localised to Reed-Sternberg cells and the EBV latent gene product LMP is expressed. Over 70% of samples from HD patients over 49 years of age are EBV positive and >50% of paediatric cases are positive. In contrast <15% of young adult cases are positive; these differences are highly significant statistically. A slight excess of EBV genome positivity is found in patients with mixed cellularity disease as opposed to nodular sclerosing HD but this does not attain stastical significance. The above findings provide good evidence that EBV is involved in the pathogenesis of HD in the majority of old and paediatric cases. However, the lack of EBV genome positivity in young adult cases suggests that another as yet unidentified virus may be involved in this group.

Hematopoiesis

S 005 **DETECTION OF MINIMAL RESIDUAL DISEASE IN ACUTE LEUKEMIA**

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Sensitive techniques for the detection of low numbers of leukemic cells may be useful for adaptation or stratification of treatment in acute leukemias and may result in individualization of treatment protocols. During the last decade several methods for the detection of minimal residual disease (MRD) have been evaluated, such as cyto-morphology, cytogenetics, cell culture systems, immunologic marker analysis and molecular-biological techniques. In most studies the detection limits of these techniques appeared not to be lower than 1-5%. However, depending on the immunophenotype or the genotype of the acute leukemias, immunologic marker analysis and the polymerase chain reaction (PCR) technique are able to detect lower frequencies of leukemic cells, down to 0.001-0.01% (1-10 leukemic cells between 100,000 normal cells).

Although leukemic cells generally have immunophenotypes comparable to their normal counterparts, it is possible to use immunologic marker analysis for the detection of MRD based on the assumption that the presence of positive cells outside their normal "homing areas" is indicative of malignancy. For example, cells positive for terminal deoxynucleodityl transferase (TdT) do normally not occur in cerebrospinal fluid (CSF), implying that the TdT immunofluorescence (IF) staining technique can be used for early detection of central nervous system (CNS) involvement in acute lymphoblastic leukemia (ALL) and other TdT^+ malignancies. Another example is the use of double IF stainings for the detection of TdT^+ cells which also express a T cell marker (such as CD1, CD5 or cytoplasmic CD3) in patients with a T-ALL or another TdT^+ T cell malignancy. This is based on the finding that such double positive cells normally only occur in the thymus and not on extrathymic locations such as bone marrow (BM) and peripheral blood (PB). A comparable approach is possible for the detection of MRD in TdT^+ acute myeloid leukemias (AML) by use of double IF stainings for a myeloid marker (such as CD13, CD33 or CDw65) and TdT, because myeloid $marker^+/TdT^+$ cells are extremely rare in BM (<0.03%, if they occur at all) and are not detectable in PB. The value of immunologic marker analysis for detection of MRD is limited by the possible occurrence of phenotypic shifts. Especially loss of TdT expression will lead to false negative results of the above described methods, but this has not been seen frequently in acute leukemias.

The second sensitive technique for detection of MRD is the PCR-mediated amplification of leukemia specific sequences. In the initial MRD-PCR studies well-defined chromosome translocations were used as tumor-specific markers (e.g. t(14;18) and t(9;22)). For this purpose oligonucleotide primers were designed to recognise sequences at opposite sides of the breakpoint recombination area so that the PCR product represented the tumor-specific hybrid DNA segment. However, only ~10% of ALL and ~25% of AML have a specific cytogenetically-detectable chromosome translocation. Recently, it has been suggested to detect MRD by use of PCR-mediated amplification of the junctional regions of rearranged immunoglobulin (Ig) and T cell receptor (TcR) genes, using V and J gene specific oligonucleotides as primers. This junctional region is different in each lymphocyte or clone of lymphocytes. Therefore it is assumed that junctional regions of rearranged Ig and TcR genes in leukemias can be regarded as "tumor-specific" markers. The main pitfall of this application is the occurrence of multiple rearrangements at diagnosis (oligoclonality) and changes in rearrangement patterns at relapse (clonal evolution), which will lead to false negative results of this MRD-PCR technique. Such heterogeneity occurs at high frequency (>50%) at the IgH gene level in precursor-B-ALL, but seems to occur less frequently in TcR genes in T-ALL and "cross-lineage" rearranged TcR genes in precursor-B-ALL.

In conclusion, the technique of choice for the detection of MRD is dependent on the immunophenotype of the leukemia, the presence of a well-defined chromosome translocation, the presence of a rearranged Ig and/or TcR gene and the chance of phenotypic or genotypic shifts. The origin of the cell sample, its volume and its cellularity will influence this choice as well.

Hematopoietic Growth Factors and Interleukins

S 006 GENETIC LESIONS OF THE INTERFERON SYSTEM IN LEUKEMIA. Manuel O. Díaz, Bruce Porterfield, Olufunmilayo Olopade, Stefan Bohlander, Helen Pomykala, Leon Plantanias and Oscar Colamonici. Department of Medicine, University of Chicago, Pritzker School of Medicine, Chicago, IL 60637.

The interferon system is involved in the regulation of cell proliferation, apparently through negative feedback loops triggered by various growth factors. It is possible that genetic lesions in different components of this system are involved in cell transformation, or neoplastic progression. Deletions of the interferon genes located on the short arm of human chromosome 9 have been observed in acute lymphoblastic leukemia (ALL) primary cells and cell lines. These deletions are frequently submicroscopic, and sometimes homozygous. Several of these deletions have breakpoints within the interferon gene cluster on 9p. The minimum region of overlap of these deletions seems to map proximal to the interferon gene cluster. Our interpretation of these findings is that a tumor suppressor gene closely linked to the interferon genes is the main relevant gene lost in the deletions. Nevertheless, it is possible that the deletion of the interferon genes contributes additional features to the transformed cell phenotype, through interruption of autocrine feedback regulation. We are testing this hypothesis by using microcell hybridization to introduce normal or deleted chromosomes into cells with complete deletions of the interferon genes.

Since interferons can produce their effects also through paracrine mechanisms, a lesion at the level of its receptor, or intracellular signalling pathways, would be a more efficient way of interrupting negative feedback loops controlling cell growth. Leukemia cells and cell lines are frequently resistant to the antiproliferative effects of interferon. We are searching for abnormalities of the interferon receptor in leukemia cells as a possible cause for this resistance. Abnormalities in the receptor can be detected in many primary leukemia cells and some derived cell lines.

S 007 MOLECULAR CONTROL OF B LYMPHOPOIESIS, Paul W. Kincade, Kensuke Miyake, Katsuhiko Ishihara, Qi He, Kay Medina, Eugene Butcher, Jayne Lesley, Robert Hyman, Anthony E. Namen, Norman Boiani, William Wood, and Randy Wall, Oklahoma Medical Research Foundation, Oklahoma City, OK 73104, Stanford University, Stanford, The Salk Institute, San Diego, Immunex Corporation, Seattle and Molecular Biology Institute, U.C.L.A, Los Angeles.

De novo production of B lymphocytes within bone marrow depends on transient interactions between hemopoietic cells and cells of the microenvironment. Stromal cell and lymphocyte clones derived by many laboratories from murine bone marrow have been important tools for understanding the molecular basis for this process. At least some of the 12 cytokines now known to be made by stromal cells must regulate proliferation and differentiation of committed precursors in the B lineage. The relative importance of one of these, IL-7, is being assessed with a new neutralizing monoclonal antibody (M25). Proliferation of the stromal cell dependent 2E8 lymphocyte clone was totally inhibited by M25 addition. Proximity of responding cells to such cytokines, retention of immature cells in the bone marrow, and reception of other signals may all depend on cell adhesion molecules. A number of adhesion molecules have now been defined on immature lymphocytes and/or stromal cells and their functional significance tested by addition of Mab to long term bone marrow cultures. Such experiments indicate that CD44 may be critical to the formation of myeloid, as well as lymphoid, cells. This cell adhesion molecule has multiple ligands, including hyaluronate, whose recognition is easily demonstrated on plasmacytomas, hybridomas and cytokine treated lymphocytes. However, most unstimulated $CD44^+$ blood cells do not bind hyaluronate and the receptor appears to be maintained in an inactive state. Transfection experiments are being done to determine how its function is controlled. Thymoma cells expressing an intact, but not a cytoplasmic tail deleted, molecule bind to hyaluronate and undergo homotypic aggregation in response to epitope specific Mab. However, CD44 does not mediate adhesion of pre-B cells to stromal cells. Rather, this interaction can be inhibited or disrupted by a new Mab to VLA-4 (expressed on pre-B cells) and by two new Mab to a possible ligand for VLA-4 on stromal cells. These antibodies preferentially inhibit lymphocyte growth in long term cultures. The stromal cell antigen has similarities to the VCAM-1/INCAM-110 adhesion molecule described on inflamed human endothelial cells. Indeed, these studies suggest that cellular recognition within bone marrow may be mediated by molecules which have other functions in peripheral tissues. Information is also being obtained about the timing and sequence of acquisition of recognition molecules by maturing B cells. Transcription of the B lineage specific B29 gene is initiated as a very early event and continues in mature B cells. We have now prepared an antibody to the product of this gene, and determined that it appears on the B cell surface in concert with IgM, to which it is intimately, but non-covalently, associated. We are just beginning to appreciate the molecular complexity involved in B lymphocyte formation and export from the bone marrow.

S 008 PROLIFERATIVE RESPONSES OF GRANULOCYTE-MACROPHAGE PRECURSORS TO COMBINATIONS OR EXCESS LEVELS OF GROWTH FACTORS, Donald Metcalf, The Walter and Eliza Hall Institute of Medical Research, P.O. Royal Melbourne Hospital, Victoria 3050, Australia.

There are now six defined growth factors with apparently direct proliferative actions on granulocyte-macrophage precursors - the four colony stimulating factors, interleukin-6 and the stem cell factor (SCF). In each case, regulator action is mediated by unique membrane receptors that, for some combinations, exhibit receptor-receptor interactions possibly based in at least one case on shared adaptor molecules.

Experiments using the FDC-Pl cell line, where at least seven naturally expressed or inserted growth factor receptors can initiate mitotic signals, have led to the conclusion that the final stages of mitotic signalling are likely to be shared in common. This arrangement suggests that the superadditive responses seen with G-CSF plus M-CSF or G-CSF plus SCF must involve unique elements in the signalling pathways, which for some regulators like G-CSF tend to abort proliferative responses.

Sustained stimulation by excess levels of these regulators does not result in down modulation of receptors but, at least in GM-CSF transgenic mice, does not achieve sustained proliferative responses. The peritoneal macrophage population in such mice initially exhibits excess proliferative activity, but with time, as in normal animals, the cells exhibit reduced proliferative responses although still remaining functionally responsive to GM-CSF stimulation.

This acquired proliferative unresponsiveness to sustained overstimulation may contribute to the failure of simple overstimulation to result in leukemic transformation in any of the model systems analyzed.

Leukemia: Advances in Biology and Therapy — Progress and Controversies

Molecular Biology - I

S 009 CHROMOSOME TRANSLOCATIONS AND CLONAL EVOLUTION OF HUMAN LEUKAEMIAS, T.H. Rabbitts, T. Boehm, L. Foroni, R. Gonzalez-Sarmiento and P. Sherrington, Medical Research Council Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, England.

Chromosome abnormalities play a role in the aetiology of tumours by altering the characteristics of genes near to the breakpoints. In T cells such translocations and inversions are found in association with the rearranging T cell receptor genes because of errors of the VDJ recombinase. We have studied the timing of normal recombinase expression in thymus by *in situ* RNA hybridisation of the two recombinase activating genes (RAG). Both RAG-1 and 2 are predominantly expressed in the cortical (i.e. immature) regions of the developing thymus, in accord with the idea that the chromosome abnormalities arise as early events in tumorigenesis, giving rise to the pre-leukaemic cell. This role in establishment of pre-leukaemia is pertinent to the occurrence of inversions or translocations involving the chromosome 14q32.1 in patients with Ataxia Telangiectasia. We have studied such patients with T cell-associated chromosome abnormalities in both leukaemic and non-leukaemic, clonally expanding phase. The characteristics of clonal evolution in these patients show that the abnormal clone remains for many years without eliciting leukaemic symptoms, with eventual conversion to overt disease.

The role of translocations in clonal evolution has been further studied in T cell tumours, and has defined a new family of oncogenes, designated the LIM-domain oncogenes, initially from the study of a translocation t(11;14)(p15;q11). This gene, the rhombotin gene, encodes a highly conserved protein with 15 cysteine residues arranged in two LIM domains. A second LIM-domain oncogene has been found near a different site of frequent translocations in childhood T-ALL at chromosome 11p13, and a comparison to rhombotin will be made.

Molecular Biology - II

S 010 ANIMAL MODELS OF CML AND ALL. John Groffen, William Voncken, Paul Pattengale and Nora Heisterkamp, Department of Pathology, Childrens Hospital of Los Angeles, Los Angeles, CA 90027.

The Philadelphia chromosome is characteristic for chronic myeloid leukemia (CML) and one type of acute lymphoblastic leukemia (ALL). It has been well established that as a consequence of the formation of this abnormal chromosome, two genes, BCR and ABL are joined together. The resulting chimeric mRNA is translated into a fusion protein with an aberrant enzymatic activity. Two types of fusion proteins exist: a BCR/ABL P190, found in approximately 50% of patients with Ph-positive ALL and a BCR/ABL P210, found in the other 50% of Ph-positive ALL patients and virtually all of the CML patients.

Notwithstanding this progress in the understanding of the molecular events underlying this type of cancer, little is known about the normal cellular function of these genes or the cellular events resulting in this type of leukemia. In an attempt to start addressing these issues, we decided to generate BCR/ABL transgenic mice. Our original construct contained the BCR promoter and a combination of BCR/ABL cDNA and genomic DNA sequences. The choice of the construct was determined by a desire to reconstruct the chimeric BCR/ABL gene as present on the Ph-chromosome as much as possible. Surprisingly, introduction of this construct into fertilized one cell-embryos did not result in living progeny. We found that the BCR gene is expressed in the course of embryogenesis; our data indicate that the BCR/ABL gene product appears to have a pleiotropic lethal effect during development and can severely affect the process of normal embryogenesis.

To circumvent this detrimental effect on embryogenesis, we decided to exchange the normal BCR gene promoter for the promoter of the mouse metallothionein gene. This new construct encoded a P190 BCR/ABL. Resulting BCR/ABL transgenic progeny developed rapid leukemia of the lymphoid or myeloid type. Of the leukemias generated in different mice, some were polyclonal while others were oligoclonal. These findings confirm the association of the Philadelphia chromosome with leukemia in man. The availability of a mouse model for Ph-positive leukemia should improve our understanding of the disease and will allow the rapid evaluation of therapeutic protocols.

S 011 MOLECULAR CHARACTERIZATION OF THE t(6;9) TRANSLOCATION IN ANLL, Marieke von Lindern, Maarten Fornerod, Sjozef van Baal, Anne Marie Poustka* and Gerard Grosveld, Dept. of Cell Biology and Genetics, Erasmus Univeristy, Rotterdam, The Netherlands, *DKFZ, Heidelberg, Germany.

Human acute lymphocytic leukemia, characterized by t(6;9)(p23;q34) exhibits a variable phenotype, indicating that the translocation probably takes place in a primitive cell of myeloid origin. In this subtype of acute myeloid leukemia, t(6;9) is often the sole karyotypic abnormality which suggests a causative role of this translocation. Abrerrant expression of genes at or near the translocation breakpoints at 9q34 and 6p23 may be responsible for the generation of this leukemia. In an attempt to characterize these genes, a combination of chromosome jumping and walking techniques identified the position of chromsome 9q34 breakpoints in several t(6;9) ANLL patients, 360 kb telomeric from *c-abl.* Subsequent cDNA cloning showed that these breakpoints are located in an intron in the middle of a gene called Cain *(can)* encoding a 7.5 kb mRNA. Disruption of *can* by the translocation results in expression of a new 5.5 kb *can* mRNA from the 6p- chromosome. This mRNA apppears to be the product of a fusion gene that consists of 5' chromosome 6 linked to 3' *can* sequences. The chromosome 6 sequences are part of a gene on 6p23 called *dek*, encoding a 3 kb mRNA.
The *can* open reading frame (ORF) encodes a protein of 220 kD and the *dek* ORF a protein of 43 kD. Neither of the two predicted protein sequences show substantial homology with known protein sequences. The chimeric 5.5 kb mRNA encodes a fusion protein of 165 kD; $p165^{dek\text{-}can}$.
The consistent formation of the *dek-can* fusion gene in 16 out of 16 cases of t(6;9) ANLL analyzed, strongly argues for a direct role of $p165^{dek\text{-}can}$ in the generation of this leukemia.

Molecular Biology - III

S 012 BCL-2 PROTO-ONCOGENE: LIFE, DEATH AND NEOPLASIA, Stanley J. Korsmeyer, David Hockenbery, Gabriel Nunez, Timothy J. McDonnell and Robert Young, Howard Hughes Medical Institute, Washington University School of Medicine, St. Louis MO, 63110

Follicular lymphoma, the most common human lymphoma, characteristically possesses the t(14;18) interchromosomal translocation. It is typically an indolent disease, initially comprised of small resting B cells, but frequently progresses to high grade lymphoma. The t(14;18) chromosomal translocation of human follicular B cell lymphoma juxtaposes the Bcl-2 gene with the immunoglobulin (Ig) heavy chain locus. The Bcl-2-Ig fusion gene is markedly deregulated resulting in inappropriately elevated levels of Bcl-2 RNA and protein. Transgenic mice bearing a Bcl-2-Ig minigene demonstrate a polyclonal expansion of resting yet responsive IgM/IgD B cells which display prolonged cell survival but no increase in cell cycling. Moreover, deregulated Bcl-2 has been shown to extend the survival of certain hematopoietic cell lines following growth factor deprivation. Using immunolocalization studies we demonstrated that Bcl-2 is a 25 kD integral inner mitochondrial membrane protein. Overexpression of Bcl-2 blocks the apoptotic death of a pro B lymphocyte cell line. Thus, Bcl-2 is unique among proto-oncogenes being localized to mitochondria and interfering with programmed cell death independent of promoting cell division. To determine the prospective oncogenic potential of the t(14;18) we followed transgenic mice bearing a Bcl-2-Ig minigene that structurally recreated the t(14;18. Transgenic mice progressed from an indolent follicular hyperplasia to malignant diffuse large cell lymphoma. A long latency, progression from polyclonal to monoclonal disease, and histologic conversion implicated secondary changes. Half of the immunoblastic high grade lymphomas had a rearranged c-myc gene. This provides an animal model of the tumor progression seen in t(14;18) lymphoma and establishes that prolonged B cell life is tumorigenic. Bcl-2 constitutes the first member of a new category of proto-oncogenes that are regulators of programmed cell death.

S 013 THE CANDIDATE PROTO-ONCOGENE *BCL3* IN THE t(14;19) OF B-CELL NEOPLASIA.

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A gene, *BCL3*, has been identified immediately adjacent to a cluster of breakpoints in the 14;19 translocation, which occurs in a small fraction of cases of B-cell chronic lymphocytic leukemia and other B-cell malignancies. The predicted protein of 446 amino acids and 46,805 m. w. shows a remarkable structure (Ohno, Takimoto, McKeithan. Cell, 60:991-997, 1990). The N-terminus is highly enriched in proline (25%), and the C-terminus in proline (28%) and serine (23%). Almost the entire remainder of the protein (about half) is made up of seven tandem repeats of 33-37 amino acids. This repeated motif has been found in mammalian ankyrin, four invertebrate proteins, and three yeast proteins, among others. Many of these proteins are known to be involved in cell-lineage determination or cell-cycle control. The *BCL3* gene, about 11.5 kb in length, contains 9 exons with a partial correspondence to the structural features of the encoded protein. The gene is highly conserved, with 80% amino acid sequence identity between mouse and human. It is expressed in a wide variety of cell types. Expression in normal peripheral blood cells or spleen cells increases markedly following mitogenic stimulation, and leukemic cells with the translocation show much greater expression than controls. This increased expression presumably results from the juxtaposition of sequences from the immunoglobulin heavy chain locus.

BCL3 is the first candidate proto-oncogene to be characterized in this family of genes. The features of the gene are consistent with a role in some B-cell neoplasms.

S 014 CELL TRANSFORMATION BY *FMS*,

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Colony-stimulating factor 1 (CSF-1 or M-CSF) acts on cells of the mononuclear phagocyte series to support their proliferation, differentiation, and survival, and potentiates the effector functions of mature macrophages during the inflammatory response. CSF-1 exerts its stimulatory effects by binding to high affinity receptors (CSF-1R) encoded by the *FMS* proto-oncogene. CSF-1 binding triggers the intrinsic protein tyrosine kinase (PTK) activity of CSF-1R, resulting in the cross-phosphorylation of ligand-aggregated receptor subunits and triggering the tyrosine phosphorylation of intracellular effector proteins whose actions ultimately affect the transcription of CSF-1-responsive genes. One basis for CSF-1's pleiotropic actions involves the ability of CSF-1R to simultaneously activate multiple second messenger pathways which act in concert to affect the specificity of the growth factor response in mononuclear phagocytes at different stages of maturation.

Certain *FMS* mutations activate the PTK activity of CSF-1R in the absence of ligand and induce persistent proliferative signals that lead to cell transformation. Mutationally activated retroviral v-*fms* genes act as potent oncogenes both *in vitro* and *in vivo* and can transform hematopoietic cells of diverse lineages. An activating mutation previously mapped within human CSF-1R resides within its extracellular domain at codon 301. The ligand-independent activity of activated *FMS* alleles bearing codon 301 mutations is also enhanced by secondary regulatory mutations within the receptor C-terminal tail (e.g., codon 969). By altering restriction sites in the human *FMS* gene without affecting its encoded protein, we created a *FMS* retroviral vector containing a series of tandem "cassettes" that could be chemically mutagenized and recloned in a single step. This procedure generates libraries of *FMS* variants containing random mutations within defined CSF-1R target sequences. After transfection into NIH/3T3 cells, transformed clones were selected, and the target sequences were amplified by PCR and sequenced directly. This method provides an unbiased approach for pinpointing the locations of *FMS* activating mutations and indicates that they may reside within the CSF-1R extracellular domain at codons other than 301.

Therapy

S 015 IMMUNOTHERAPY OF LEUKEMIA, Malcolm K. Brenner, Department of Hematology/Oncology, St Jude Children's Research Hospital, Memphis TN 38101

Of the three available treatment approaches for leukemia, chemotherapy, autologous or allogeneic bone marrow transplantation (BMT), it is only after allogeneic BMT that clear evidence exists for an anti-leukemic effect of the (incoming) immune system - the Graft versus leukemia (GvL) effect. Thus BMT between identical twins has a higher relapse rate than BMT between MHC identical but allogeneic siblings; patients who develop graft versus host disease (GvHD) after BMT are less likely to relapse than those who do not; and all effective methods of GvHD prevention may be associated with an increased risk of disease recurrence. These data imply i) that GvL effects are simply another manifestation of GvHD, and are attributable to the same alloreactive T lymphocytes ii) that the anti-leukemic effects of the immune system are important only after allogeneic BMT. If this is true then immunotherapy of leukemia will be of limited value, since attempts to cure one disease (leukemia) by inflicting another (GvHD) are unlikely ever to be successful. In fact, a number of approaches may allow us not only to separate GvL from GvHD after allogeneic BMT, but also to induce therapeutic immunomodulation after autologous BMT and chemotherapy. This review will describe specific T cell dependent and independent anti-leukemic effector mechanisms and will discuss how they can be selected and enhanced using immune system growth factors. It will also describe how gene marking of patient marrow may allow identification of the source and characteristics of the clonogenic leukemic cell. This information should help direct the anti-leukemic immune mechanisms described toward the appropriate target cells and tissue sites.

S 016 BIOCHEMICAL BASIS FOR THE REMITTIVE ACTIONS OF ADENINE DEOXYNUCLEOSIDES IN LYMPHOPROLIFERATIVE DISEASES. Dennis A. Carson, Chihiro Terai, and Carlos J. Carrera, Department of Medicine, University of California, San Diego, La Jolla, CA 92093

2-Chlorodeoxyadenosine (2-CdA), deoxycoformycin, and 2-fluoroadenine arabinoside have demonstrated potent activity in human lymphoproliferative diseases. For example, a single treatment course with 2-CdA induces complete remission in at least 75% of patients with refractory hairy cell leukemia, and also is effective in some patients with chronic lymphocytic leukemia, low grade lymphoma, and cutaneous T cell lymphoma. It is remarkable that a single, short-term exposure to a simple adenine deoxynucleoside can cause the complete ablation of a systemic lymphoproliferative disease. We have therefore investigated intensively the mechanism by which 2-CdA and deoxyadenosine impair immune function and lymphocyte survival. Unlike other purine and pyrimidine antimetabolites used in chemotherapy, 2-CdA is directly toxic to non-dividing lymphocytes and monocytes. Drug toxicity absolutely depends upon nucleoside phosphorylation by deoxycytidine kinase, with resultant formation of 2-chloro-dATP, a potent inhibitor of DNA repair. The ratio of deoxycytidine kinase to cytoplasmic nucleotidase is a principal determinant of cellular sensitivity to 2-CdA, by regulating the amount of triphosphate formed. In addition, non-dividing cells can differ in their sensitivity to toxic adenine deoxynucleoside 5'-triphosphates. This may relate to the intrinsic tendency of interphase cells to undergo apoptosis, following the accumulation of DNA single strand breaks. Our data suggest that the effectiveness of adenine deoxynucleosides in chronic lymphoproliferative diseases may depend upon (i) selective accumulation by the target cells, (ii) inhibition of monocyte and T lymphocyte growth factor production, and (iii) the capacity of a cell to undergo programmed cell death. This approach to chemotherapy may be exploitable in the treatment of other tumors with a low growth fraction, and a prolonged cell cycle generation time.

S 017 HOW TO CURE LEUKEMIA, Robert Peter Gale, Department of Medicine, UCLA School of Medicine, Los Angeles, CA 90024-1678

Is it necessary to eradicate leukemia to cure it. Probably not—at least not in all instances. Considerable data from several sources suggest that some (most) cures of leukemia result from mechanisms other than eradicating of the disease. These data will be reviewed.

Leukemogenesis is typically multi-step: it begins with clonal preference, evolves to dominance and eventuates in abnormally regulated growth, usually with clinical consequences. Phases of leukemogenesis antecedent to the final step are termed preleukemia. Recent studies in persons with acute myelogenous leukemia (AML) in remission receiving chemotherapy indicate that cells related to the leukemia clone contribute substantial numbers of normal appearing end-cells in at least one-third of cases. A similar undetectable contribution may operate in the remaining cases. In some instances, cytogenetic abnormalities typical of the leukemia clone are detected in normal appearing cells. These and other data to be discussed suggest that remission in AML may result from re-establishing preleukemia. If the final step of leukemogenesis does not recur (or is prevented) such persons will be cured. Correlates between clonal remission hematopoiesis and cure are not yet reported. Another possible means to cure AML involves immune regulation of residual (pre)-leukemia cells. This is illustrated in bone marrow transplants where few relapses occur after allogeneic grafts versus many after syngeneic grafts. There is also a correlation with severity of graft-versus-host disease (GVHD).

Acute lymphoblastic leukemia (ALL) is less studied. Here, residual leukemia or preleukemia cells (i.e. cells related to the leukemia clone) persist in some persons seemingly cured of leukemia. In ALL, mechanisms that normally control lymphocyte development and survival may operate to control or eradicate residual leukemia or preleukemia cells. Transplant studies also indicate modest immune regulation of leukemia. Eradication of all leukemia cells by chemotherapy probably occurs more often in ALL than AML.

Chronic myelogenous leukemia is frequently cured by transplants; chemotherapy is ineffective. The mechanism seems immune-mediated. Relapses after twin or T-cell depleted transplants are frequent whereas those after allografts are rare. Equally interesting is the detection of persisting (pre)-leukemia cells for a prolonged interval posttransplant in most persons eventually cured.

These data suggest that it is not always necessary to eradicate leukemia to cure it. Mechanisms contributing to cure include eradicating leukemia cells with chemotherapy and/or radiation, re-establishing preleukemia, normal homeostatic mechanisms regulating lymphocytes, and immune mechanisms operating in the context of transplants. Whether similar immune modes operate after chemotherapy is unknown. Since most drugs used to treat leukemia have little if any selectivity for leukemia cells it is fortunate that leukemia eradication is not needed for cure.

Late Abstract

ONCOGENES AND TUMOR SUPPRESSOR GENES IN BURKITT LYMPHOMAGENESIS.

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A group of B-cell malignancies including Burkitt lymphoma (BL), L3-type acute lymphoblastic leukemia (ALL) and AIDS-associated non-Hodgkin lymphoma (AIDS-NHL) are associated with specific chromosomal translocations involving the c-myc and immunoglobulin loci. The consequences of these abnormal recombinations are: i) the juxtaposition of the c-myc gene to immunoglobulin regulatory domains; ii) structural lesions including truncations and mutations of the 5' regulatory region of translocated c-myc alleles. Both these events are thought to be necessary to cause deregulation of c-myc gene expression. Constitutively expressed c-myc alleles have been shown to transform EBV-immortalized human B-lymphoblasts in vitro and to cause B-cell tumors in transgenic mice.

Deregulated c-myc expression leads to changes in the regulation of genes that are regulated by c-myc. It has been found that the gene coding for the α_L chain of LFA-1 (α_L/β_2) integrin receptor is specifically modulated by c-myc by a posttranscriptional mechanism in B-lymphoblastoid cells transfected with c- myc oncogenes as well as in Burkitt lymphoma cells which carry activated c-myc oncogenes. Since LFA-1 is involved in B-cell adhesion to cytotoxic T-cells and NK cells, as well as to vascular endothelia, these results imply novel functions for the c-myc gene both in controlling normal B-cell development and in determining the ability of tumor cells to escape immunosurveillance and metastasize. Consistent with this notion, transformation by c-myc oncogenes has been found associated with resistance to autologous T-cell mediated cytotoxicity in vitro.

Multiple genetic alterations, in addition to c-myc oncogene activation, are likely to be required for lymphomagenesis. While EBV infection is likely to play a role in BL and AIDS-NHL, recent evidence indicates that loss/mutation/inactivation of p53 and RB "tumor suppressor" genes may also be involved. In particular, loss/inactivation of the p53 gene is associated at high frequency (50-70%) with all types of neoplasms carrying activated c-myc oncogenes including L3-type ALL, whereas it is very rarely found in other types of ALL and NHL (see Abstract by Ballerini et al.). These results suggest that specific interactions may exist between c-myc and p53 in B lymphomagenesis.

Viruses

S 100 DETECTION OF CLONAL EPSTEIN BARR VIRUS IN MALIGNANT PROLIFERATION OF PERIPHERAL BLOOD CD8+ T CELLS, Li Chong CHAN[1], Yok Lam KWONG[1], Gopesh SRIVASTAVA[1], Hing Wing LIU[1] and Hui Leung YUEN[2], Department of Pathology, University of Hong Kong[1] and Queen Elizabeth Hospital[2], HONG KONG.

Epstein Barr virus (EBV) DNA was detected in a monoclonal proliferation of T cells in a three year old girl who presented with a history of fever, hepatosplenomegaly and generalised lymphadenopathy. The disease ran a rapid, fulminant course and the patient died 11 days after presentation. Examination of the blood showed a lymphocytosis of 50 x 10^9/l with all the cells showing the morphology of large granular lymphocytes. These cells were CD2+3+8+25+ and CD4-5-7-. Cytogenetic studies showed the presence of a 6q- clone. Southern blotting and hybridisation with a constant region probe for the T cell receptor (TCR) β chain gene showed clonal rearrangement of the TCRβ gene. Hybridisation of the Southern blot to the EBV-Dhet probe revealed a clonal pattern of circularised EBV DNA. This is the first report which links clonal EBV infection to a malignant proliferation of peripheral blood CD8+ T cells.

S 101 LEUKEMIA INITIATED BY HEMOPOIETIC STEM CELLS EXPRESSING THE V-ABL ONCOGENE. S.W. Chung, P.M.C. Wong, H. Durkin, Y.S. Wu, and J. Petersen. Morse Institute of Molecular Genetics, Department of Microbiology, Immunology and Pathology, SUNY Health Science Center, Brooklyn, NY.

To determine whether introduction of the v-abl oncogene into a primary hemopoietic stem cell can lead to a profound hematological disturbance. A helper-free A-MuLV was prepared for infection of hemopoietic stem cells, which were used to reconstitute lethally irradiated mice. Two weeks later, progenies of a single primitive hemopoietic stem cell carrying the v-abl oncogene could be detected in both colony forming units in spleen (CFU-S) and factor-independent colony forming cells in the bone marrow. Beginning 3 weeks post-transplantation, the recipients developed elevated leukocyte counts, splenomegaly and increase of blast cells in the bone marrow and peripheral blood. From the same animal, DNA extracted from various affected organs and factor-independent lymphoid and myeloid immortalized cells all contained an identical integrated proviral genome. Many cell lines isolated were immature in nature, and they subsequently differentiated into macrophages, T and B cells. These were determined by the use of monoclonal antibodies and examination of genetic rearragement of the T cell receptor and the immunoglobulin genes. These data demonstrate that expression of the v-abl oncogene in a primary lymphoid-myeloid stem cell is sufficient to initiate leukemia in mice. They also suggest that the 5' upstream sequences of both v-abl and bcr-abl play a similar role in stem cell-induced leukemogenesis.

S 102 IMMUNOREACTIVE EPITOPES OF HTLV1-P19. R Jarrett[1], S Gledhill[1], S Crae[1], R S Tedder[2], J Tosswill[3], J Webber[4], D E Onions[1]. [1]LRF Virus Centre, Glasgow Veterinary School, Glasgow, G61 1QH. [2]Dept of Med Microbiology, University College and Middlesex School of Medicine, London W1P 7PN. [3]CPHLS Virus Reference Laboratory, 61 Colindale Ave., London NW9 5HT. [4]Department of Medicine, RPMS, Du Cane Rd., London W12 OHS.

We have synthesized overlapping peptides covering the p19 of HTLV-1 and are using these to define the epitopes reactive with: 1)Monoclonal antibodies (MoAb) to HTLV-1 p19. 2) HTLV-I and HTLV-II +ve sera. 3) Sera that give "indeterminate" results in Western blot assays. The data indicate that the C-terminal 30 amino acids of p19 contains at least 2 antigenic sites. A MoAb reactive with HTLV-I p19 and the equivalent HTLV-II protein reacts with an epitope in this region. The location of p19 epitopes reactive with 5 HTLV-II- positive sera could not be convincingly demonstrated. Eight indeterminate sera from Papua New Guinea showed a consistent pattern of reactivity with peptides near the C-terminus. These results should assist in the development of assays to distinguish between HTLV-I and HTLV-II infection, and may help determine the significance of indeterminate reactivities in HTLV-I negative sera.

S 103 A STRATEGY TO ISOLATE GENES INVOLVED IN HEMATOPOIETIC STEM CELL DIFFERENTIATION , Jan-I. Jönsson and Robert A. Phillips, Div. of Immunology and Cancer, Hospital for Sick Children, Toronto, Ontario, CANADA M5G 1X8

Genes expressed in hematopoietic stem cells and during differentiation play a role in both self-renewal and commitment to different specific lineages. Some of these genes may also be involved in malignant transformation such as leukemia. To identify developmentally regulated genes, we have constructed a retroviral vector carrying the gene for neomycin resistance and with a deletion of the enhancer region in the LTR. Our working hypothesis is that retroviral integration occurs only in transcriptionally active regions of the genome. Thus, the neo-gene will not be expressed in infected cells unless the virus integrated near a cellular enhancer. In preliminary studies, BALB/c bone marrow was co-cultivated with an enhancer-trap virus producer cell line and selected for 2 days with factors and high concentrations of G418 (2mg/ml). 6 weeks after transfer into sub-lethally irradiated SCID mice, pre-B cell clones from reconstituted bone marrow were established under limiting dilution with IL-7. We are now investigating the presence of neo in these clones by PCR. From clones containing neo but which fail to survive growth in G418, integration sites and thus flanking host DNA will be cloned by inverted PCR. These genomic clones are potentially candidates for stem cell-specific genes and will be screened with RNA from normal bone marrow, fetal liver, stem cell lines, different leukemic cell liens, as well as with DNA from several species.

S 104 INFECTION OF THE SCID-HU MOUSE BY HTLV-I, Miriam Lieberman*, Reiko Namikawa**, Anis S. Majumdar*, Joseph D. Rosenblatt*** and Joseph M.McCune**. *Department of Radiation Oncology, Stanford University School of Medicine, Stanford, CA 94305, **SyStemix, Inc., Palo Alto, CA 94303, and ***Department of Medicine, University of California School of Medicine, Los Angeles, CA 90024.

The development of the SCID-hu mouse model (C.B.17scid/scid mice permanently engrafted with human fetal liver hematopoietic cells and human fetal thymus) provides a setting in which infection of human lymphoid cells by retroviruses may be studied within an in vivo experimental environment. The present report concerns infection of the model by HTLV-I.

The results to date indicate that: (1) Productive infection takes place after inoculation of killed virus-producing cells, not when free virus is used; it is most effective when the inoculum is injected directly into the thymic graft. (2) Products of viral replication become detectable approximately one month after infection, and are restricted to the cells of the graft. (3) Evidence of infection is not detectable in vivo, but becomes manifest on maintenance of infected graft lymphocytes in vitro and increases with time of culture. Expression of viral messenger RNA is first seen at 5-7 days by in situ hybridization with an HTLV-I specific RNA probe. Viral core proteins become detectable by Western blot after 10-14 days of culture. (4) Neoplastic transformation of the graft cells has not been observed within one year after infection.

S 105 VIRUSES AND HODGKIN'S DISEASE: David Onions and Ruth Jarrett. LRF Virus Centre, University of Glasgow G61 1QH, UK.

Epidemiological evidence suggests that a transmissable agent is involved in the pathogenesis of Hodgkin's disease (HD) and there is accumulating evidence that EBV is involved. EBV genomes are detectable in tumour biopsies from approximately one third of non-selected cases of HD and analysis of the terminal fragments of these genomes indicates that the EBV-infected cells are clonal. EBV has been localised to Reed-Sternberg cells and the EBV latent gene product LMP is expressed. Over 70% of samples from HD patients over 49 years of age are EBV positive and >50% of paediatric cases are positive. In contrast <15% of young adult cases are positive; these differences are highly significant statistically. A slight excess of EBV genome positivity is found in patients with mixed cellularity disease as opposed to nodular sclerosing HD but this does not attain stastical significance. The above findings provide good evidence that EBV is involved in the pathogenesis of HD in the majority of old and paediatric cases. However, the lack of EBV genome positivity in young adult cases suggests that another as yet unidentified virus may be involved in this group.

S 106 GROWTH OF CLONAL PRIMARY NON-HODGKIN'S LYMPHOMA IN SCID MICE

Edmund K. Waller*, Miriam Lieberman**, Irving Weissman***.
*Division of Oncology, Department of Medicine; ** Department of Radiation Oncology; ***Howard Hughes Medical Institute. Stanford University School of Medicine. Stanford CA 94305.

Heterotransplantation of 31 cases of primary Hodgkin's disease (HD) and non-Hodgkin's lymphoma (NHL) into untreated SCID mice and SCID mice reconstituted with human fetal thymus, spleen and liver (SCID-hu) resulted in the development of lymphoid tumors in 13 (41%) cases. Two T-cell NHL grew as clonal $CD3^+$ $CD4^-$ $CD8^-$ EBV^- tumors following injection of primary lymphoma cells into the thymus xenograft in SCID-hu mice; tumors failed to grow in SCID mice. Analysis of the transplanted and primary T-cell tumors demonstrated identical patterns of T-cell surface markers by flow cytometry and immunophenotyping of fixed tissue sections, and, in one case, reactivity with a specific monoclonal antibody to V beta 5.1. One heterotransplanted tumor developed from a patient with AILD that contained a TCR gene rearrangement that was identical to one of the TCR gene rearrangements present in the primary tumor. One EBV^- B-cell tumor grew as a clonal diffuse large cell lymphoma with the same histology and pattern of immunoglulin gene rearrangements as the primary tumor following intraperitoneal injection in SCID mice. Heterotransplantation of the remaining nine cases (2 HD, 2 T-cell and 5 B-cell NHL) yielded EBV^+ lymphoblastoid B-cell tumors that did not contain lymphoma cells clonally related to the patients' original lymphomata.

S 107 IMMORTALIZED HEMOPOIETIC CELLS WITH STEM CELL PROPERTIES.

P.M.C. Wong, S.W. Chung, and X.D. Han. Morse Institute of Molecular Genetics, Department of Microbiology & Immunology, SUNY Health Science Center, Brooklyn, NY.

Following a standard gene transfer protocol which includes the infection of mouse fetal liver hemopoietic stem cells with our retrovirus vector, N2-IL3, we were able to obtain a cell line from a recipient mouse with immature blast cell phenotype. Southern blot analysis of DNA from these cells indicated that the IL-3 gene within the N2-IL3 genome was deleted, so was a portion of the neo^r gene, which was also present in the retroviral genome. Northern blot analysis on these immature cells revealed the presence of a novel mRNA of about 3kb, using a LTR-neo probe. When injected into lethally irradiated mice, these cells reconstituted the recipients. Three months later, DNA from various organs of these recipients were similarly analyzed by southern blot analysis and a pattern identical to that of the parental cell line was observed in the thymus, spleen, bone marrow, lung, but not liver and kidney in one mouse. A similar pattern was observed in other recipients. Colony assay of bone marrow cells from these recipients revealed the presence of factor-independent colonies, composed of differentiated cells. These data suggest that the immortalized cells can differentiate in vivo and give rise to functionally differentiated hemopoietic cells of lymphoid and myeloid lineages. Immortalization of these cells may be the result of retroviral insertional activation of a gene flanking the proviral DNA.

S 108 LEUKEMIA IN DOWN SYNDROME (D.S.):

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Children with D.S. have a 20-30x greater incidence of leukemia than normal. All types of leukemia occur in D.S. however there is a striking increase in the incidence of acute megakaryoblastic leukemia in D.S. children in the first three years of life. There are reports also of an abnormally high incidence of erythroleukemia (E.L.) in D.S. at this age. Our own studies and those in the literature suggest that "AMKL" and "EL" in D.S. are the same disease. The leukemic blasts in these disorders have features of both megakaryocytic and erythroid precursors.

D.S. children also develop a syndrome, evidenced at birth in which abnormal numbers of megakaryoblasts appear in the peripheral blood. This syndrome, Transient Leukemia (TL), disappears in the first weeks of life. However, review of cases reported in the literature suggest that 25% of these cases recur or develop AMKL during the first three years of life. "TL" therefore would appear to be a pre-leukemic lesion. The incidence of TL is unknown, however our own prospective study revealed 2/14 DS newborns to have megakaryoblasts in the blood; similarly one report indicated that 2/25 D.S. fetuses had TL. TL therefore may be a frequent phenomenon in D.S.

It would appear therefore that a specific genetic disorder (trisomy 21) predisposes to both a benign and malignant proliferation of megakaryoblasts.

These observations and conclusions are based on a review of the literature, cases at The Hospital for Sick Children and in the Canadian Down Syndrome Leukemia Registry.

Oncogenes

S 200 SCRUTINY OF THE HUMAN K-ras-2 GENE FOR MUTATIONS USING DENATURING GRADIENT GEL ELECTROPHORESIS, Ezra S. Abrams and Leonard Lerman, Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139. Denaturing Gradient Gel Electrophoresis (dgge) can be used to detect single base changes in DNA fragments of up to several hundred bp in length. We have used the PCR to amplify the five coding exons of the K-ras-2 gene, so that mutations in each exon can be detected. However, maximal sensitivity is obtained only by careful choice of PCR oligonucleotides, and by addition of a GC clamp to each fragment. Computer programs which predict the melting properties of any DNA sequence were used to select PCR amplimers for each of the coding exons of the K-ras-2 gene. Up to three sets of PCR amplimers can be used simultaneously for both the PCR and subsequent analysis on a DGG. We have found 3 regions of the K-ras-2 gene which are polymorphic in the population; these DGG polymorphisms are two or three allele systems, and appear to be distinct from the known RFLPs in this gene. We are currently screening DNA from individuals with different tumors; DNA from both tumor and non-tumor tissue is analyzed to distinguish mutations from polymorphisms.

S 201 DETECTION OF MINIMAL RESIDUAL DISEASE IN ANLL PATIENTS BY USE OF DOUBLE IMMUNOLOGIC MARKER ANALYSIS FOR TdT AND A MYELOID MARKER

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Terminal deoxynucleotidyl transferase (TdT) is expressed in normal precursor B and T cells as well as their malignant counterparts i.e. acute lymphoblastic leukemias (ALL) and some lymphoblastic lymphomas. Using double immunologic marker analysis for a myeloid marker (CD13 or CD33) and TdT we could also demonstrate expression of TdT in the majority (45 out of 60) of acute non-lymphocytic leukemias (ANLL). In contrast to most ALL, in ANLL TdT is often expressed by a subpopulation of the ANLL cells, e.g. in 24 out of the 45 TdT^+ ANLL the frequency of myeloid $marker^+$,TdT^+ cells was <10%. The precursor antigen CD34 was expressed in higher frequencies on the TdT^+ leukemic cells than on the TdT^- cells, suggesting that the TdT^+ cells represent an immature subpopulation which might contain the clonogenic ANLL cells.

Control studies on a series of non-leukemic bone marrow samples (n=66) and blood samples (n=25), obtained from healthy volunteers as well as ALL patients in complete remission (CR), revealed that normal counterparts of myeloid $marker^+$,TdT^+ cells are rare in bone marrow samples (<0.03%, if they occur at all) and that such cells are not detectable in blood. Therefore, we started a follow-up study to monitor the myeloid $marker^+$,TdT^+ leukemic cellpopulation in ANLL patients, during and after chemotherapy.

Preliminary results are available from the follow-up of 17 patients with a TdT^+ ANLL who obtained CR according to clinical as well as cytomorphologic criteria. Ten relapses occurred in seven patients within 7-24 months after diagnosis. In a period of 14-38 weeks before relapse we observed a gradual increase of myeloid $marker^+$, TdT^+ cells. Interestingly, during CR in these seven patients as well as in the ten patients who are still in CR (4-30 months) low frequencies (0.01%-0.2%) of myeloid $marker^+$,TdT^+ cells were repeatedly detected in bone marrow and/or blood, suggesting that TdT^+ ANLL cells may survive despite chemotherapy. Three out of the ten patients who are still in CR received an autologous bone marrow transplantation (BMT) within 8 months after diagnosis. Follow-up of the ten patients will be continued to study the fate of these myeloid $marker^+$,TdT^+ cells.

Our results indicate that double immunologic marker analysis for TdT and a myeloid marker may represent a powerful tool for detection of minimal residual disease as well as the early detection of relapse in patients with a TdT^+ ANLL.

S 202 DIFFERENT SUBSTRATE SPECIFICITIES OF THE abl PROTEINS AS DETERMINED BY TYROSINE KINASE INHIBITORS. [1]Mordehai Anafi, [2,3]Aviv Gazit, [3]Chaim Gilon, [1]Yinon Ben-Neriah and [2]Alexander Levitzki. [1]The Lautenberg Center for General and Tumor Immunology The Hebrew University Hadassah Medical School, Jerusalem 91010 Israel. [2]Departments of Biological Chemistry and [3]Organic Chemistry, The Hebrew University, Jerusalem 91904 Israel.

The abl oncogene has been implicated in human and mouse malignancies. The protooncogene is expressed in all cell types tested so far, but its function remains elusive. Since different cellular phenotypes have been associated with different abl protooncogene variants and proteins, we wondered whether the different abl proteins may have different substrate specificities. To approach this question we have tested 16 tyrosine kinase inhibitors (tyrphostins) synthesized in our laboratory and checked their ability to inhibit the tyrosine kinase activity of 5 abl proteins: human $p140^{c\text{-}abl}$, $p210^{bcr\text{-}abl}$, $p185^{bcr\text{-}abl}$, mouse $p140^{c\text{-}abl}$ and $p160^{gag\text{-}abl}$. Most of the tyrphostins examined exhibited selectivity towards the abl kinases. Interestingly, $p210^{bcr\text{-}abl}$ tyrosine kinase was the most susceptible to inhibition by many of the tyrphostins tested. We believe that differences in sensitivity to tyrphostins may reflect differences in the catalytic domain or in allosteric sites which affect the substrate - protein tyrosine kinase interaction. Tyrphostins which selectivly inhibit oncogenic forms of abl have potential as chemotherapeutic agents for abl-related diseases, such as chronic myelogenous leukemia (CML).

S 203 FOLLICULAR LYMPHOMA ORIGINATES IN MATURE B CELLS AFTER SOMATIC MUTATION OF IMMUNOGLOBULIN GENES. *David W Bahler, and Ronald Levy. Deptartment of Medicine, Stanford University Medical School, Stanford CA 94305*

Multiple genetic events are likely to be required before a follicular lymphoma can develop. One such event appears to be the translocation involving chromosomes 14 and 18 which deregulates the BCL-2 gene. Since molecular analysis suggests that the t(14;18) translocation occurs as an error in recombination of immunoglobulin (Ig) heavy chain diversity and joining gene segments, it has been proposed that this event occurs in a pre-B cell when these gene segments normally recombine. To more precisely identify the stage in B cell development when transformation occurs, we have analyzed somatic mutations in multiple Ig heavy chain variable gene (VH) clones from 3 separate cases of follicular lymphoma. For each lymphoma, the VH clones were found to contain multiple common nucleotide differences compared to germline in addition to nucleotide differences restricted to particular clones. Moreover, the common nucleotide differences from germline present in all clones from a lymphoma were mostly preserved in multiple tumor specimens acquired from different sites over a period of 4 to 5 years. It appears , therefore, that nucleotide differences from germline shared among all VH clones of a lymphoma identify somatic mutations that occurred prior to transformation. This in turn would place the origin of follicular lymphoma after the stage in B cell development when somatic mutation of Ig genes occurs.

S 204 p53 MUTATIONS IN LYMPHOID MALIGNANCIES: ASSOCIATION WITH BURKITT'S LYMPHOMA AND CHRONIC LYMPHOCYTIC LEUKEMIA. Paola Ballerini, Gianluca Gaidano, Jerry Z. Gong, Giorgio Inghirami, Elizabeth W. Newcomb, Ian T. Magrath, Daniel M. Knowles and Riccardo Dalla-Favera, Department of Pathology and Cancer Center, Columbia University, New York, NY 10032; Department of Pathology and Cancer Center, New York University, New York, NY 10032; Pediatric Branch, National Cancer Institute, Bethesda, MD 20892

Mutations affecting the p53 "tumor suppressor" gene have been found associated with several types of human tumors. Since no comprehensive data are available for lymphoid neoplasia, we have investigated the frequency of p53 mutations in B- and T- cell human lymphoid malignancies, including acute lymphoblastic leukemia, the major subtypes of non-Hodgkin lymphoma, and chronic lymphoproliferative leukemia. p53 Exons 5 through 9 were studied using genomic DNA from 202 primary tumours and 27 cell lines by the single strand conformation polymorphism (SSCP) analysis; positive samples were further analyzed by direct sequencing of the PCR-amplified fragment. Mutations were found associated with: i) Burkitt lymphoma (9/27 biopsies; 17/27 cell lines) and its leukemic counterpart L_3-type B-acute lymphoblastic leukemia (5/9), which both carry also activated c-myc oncogenes; ii) B-chronic lymphocytic leukemia (6/40) and, in particular, its stage of progression known as Richter's transformation (3/7). Mutations were not found at any significant frequency in other types of non-Hodgkin lymphoma or acute lymphoblastic leukemia. Most mutations were represented by single nucleotide changes, occurring within highly conserved domains of p53 coding sequence. In many cases only the mutated allele was detectable, thus implying the loss of the normal allele. These results suggest that: i) significant differences in the frequency of p53 nutations are present among subtypes of neoplasms derived from the same tissue; ii) p53 may play a role in tumor progression in B-chronic lymphocytic leukemia; iii) the presence of both p53 loss/inactivation and c-myc oncogene activation may be important for the pathogenesis of Burkitt's lymphoma and its leukemic form L_3-type B-acute lymphoblastic leukemia.

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S 205 REGIONS OF HUMAN C-MYC INVOLVED IN INHIBITION OF MURINE ERYTHROLEUKEMIA DIFFERENTIATION. Matia Bar-Ner, Lora Messing and Shoshana Segal, NCI-Navy Medical Oncology Branch, National Cancer Institute, Bethesda, MD 20814.

Members of the myc family of oncogenes share several highly conserved areas of homology in the protein coding domain. All three cellular oncogenes can cooperate with an activated ras in transforming primary rat embryo fibroblasts and can block HMBA and DMSO induced differentiation of murine erythroleukemia (MEL) cells when constitutively expressed. These observations suggest that the conserved homology regions of the myc genes might be important for their common activities. To determine the relative significance of various structural domains of c-myc to its activity in blocking differentiation, we transfected the C19 TK$^-$ MEL cell line with several deletion mutants spanning the normal c-myc coding region. Results obtained from detailed analysis of independent transfectants indicate that sequences at the 5' and 3' ends of the coding region are required for inhibition of differentiation. Similar results were obtained with these mutants when assayed for transforming activity (Stone et al. Mol. Cell. Biol. 7:1697-1709, 1987).

We thank Dr. William Lee for providing the deletion mutants for this study.

S 206 GROWTH OF HUMAN HEMATOPOIETIC PROGENITORS IN SCID MICE. Ross S. Basch, Yamil H. Kouri and John A. Hirst. Departments of Medicine and Pathology, N.Y.U. Medical Center, N.Y., N.Y. 10016.

Bone marrow ablated SCID mice have been injected with human marrow cells. Human cells were detectable in all animals receiving 3 x 10^6 cells by one week after the transfer. Antibodies to CD45 and CD34 were used to detect the human cells in the peripheral blood, spleen and bone marrow of the recipients. These antibodies do not cross react with mouse cells On day 7, 1.8% of the nucleated cells in the peripheral blood were CD45 + ; none of these stained with CD34. 2.6% of the bone marrow cells were of human origin (CD45 +) and more than half (53%) of these also stained with CD34. 8.7% of the spleen cells were of human origin but only 16% of them stained with CD34. By 3 weeks 13.6% of the cells in the peripheral blood were CD45 + : none of these expressed CD34. At this time 38.4% of the marrow and 65.2% of the spleen cells were of human origin and approximately 60% of the marrow cells and 11 % of the spleen cells expressed CD34. The two color histogram of the staining of the bone marrow cells is shown in Fig 1. When cells isolated from the mouse marrow were subjected to a three color analysis, gating on HLA(biotin/Streptavidin Red 613) positive cells to identify human cells and anti CD33(PE) and anti glycophorin(Fl) to identify developing human myeloid and erythroid cells, the pattern shown in Fig 2 was obtained. Large numbers of developing erythroid cells were detected but virtually no staining with CD33 was apparent. Despite this lack of staining human CFU-GM could be recovered from these marrows. By 3 weeks the SCID mice had 5,300 GM-CFC/femur. These results indicate that SCID mice can serve as hosts for human hematopoietic progenitor cells and that some of these progenitors can differentiate in the absence of exogenously provided growth factors.

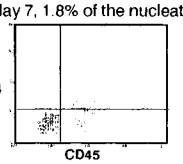

Fig.1

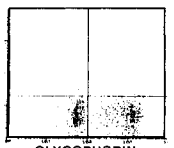

Fig. 2.

S 207 **HETEROGENEITY OF IgH GENE REARRANGEMENT PATTERNS IN PRECURSOR-B-ALL AT DIAGNOSIS AND RELAPSE: IMPLICATIONS FOR THE DETECTION OF MINIMAL RESIDUAL DISEASE BY PCR**

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Rearrangement of the variable, (diversity) and joining gene segments of the immunoglobulin (Ig) and T cell receptor (TcR) genes generates unique DNA sequences at the junctional region of these gene segments. This junctional region is different in each lymphocyte (clone) and is known as complementarity determining region III in case of Ig genes. Since a malignancy represents a clonal expansion of a single malignantly-transformed cell, junctional regions of rearranged Ig and TcR genes in leukemias can be regarded as "tumor-specific" markers.

We examined 60 consecutive childhood precursor-B-ALL for their IgH gene rearrangement patterns by use of the Southern blot technique. In 43% of cases more than two differently-rearranged IgH gene bands were found, which were not due to hyperdiploidy of chromosome 14 or other chromosome-14 aberrations. In 30 cases we also compared the IgH gene rearrangement patterns of bone marrow (BM) and peripheral blood (PB) at diagnosis. Strikingly, in five cases (17%) differences in rearrangement patterns were found. These data indicate that subclone formation leading to oligoclonality occurs in high frequency in precursor-B-ALL. Furthermore, comparative studies on IgH gene rearrangement patterns at diagnosis and subsequent relapse(s) in 18 precursor-B-ALL revealed that clonal evolution at relapse is a frequent event (50%).

We would like to emphasize that heterogeneity of IgH gene rearrangement patterns at diagnosis (oligoclonality and/or differences between BM and PB) and at relapse (clonal evolution) will severely hamper the detection of minimal residual disease by use of PCR-mediated amplification of "leukemia-specific" IgH gene junctional regions, since this heterogeneity will lead to false-negative results in a large part (~50%) of precursor-B-ALL patients. Our preliminary results indicate that the "cross-lineage" TcR-γ and TcR-δ gene rearrangements in precursor-B-ALL are more stable leukemia-specific markers than the IgH gene rearrangements. Therefore we suggest that the TcR-junctional-region-PCR method may be a more reliable technique for the detection of minimal disease in precursor-B-ALL, although it should be noted that TcR-γ and/or TcR-δ gene rearrangements occur in 60% of precursor-B-ALL cases only.

This work was supported by The Netherlands Cancer Foundation (Koningin Wilhelmina Fonds), grant IKR 89-09.

S 208 **THE IDENTIFICATION OF GENES EXPRESSED IN MOUSE PLASMACYTOMAS, BUT NOT B LYMPHOMAS,** P. Leif Bergsagel, Cynthia Timblin, and W.Michael Kuehl, NCI-Navy Medical Oncology Branch, National Cancer Institute, Bethesda, MD, 20889

Using a novel strategy incorporating PCR technology, we have constructed a murine plasmacytoma minus B lymphoma subtractive cDNA library. Analysis of 130 random clones has identified 7 different genes, each of which is expressed in most plasmacytomas but in one or none of 8 B lymphomas examined. Curiously, two of these genes are expressed in all 10 pre-B lymphomas examined whereas the other five are expressed in none of the pre-B lymphomas.

We have completed the preliminary characterization of one gene (egp314) which is expressed in 16 of 17 mouse plasmacytomas and none of 18 pre-B or B lymphomas. The cDNA sequence predicts a 314 AA intrinsic membrane glycoprotein which is 82% identical to the human epithelial antigen recognized by monoclonal antibodies GA733, HEA125, KS1/4. The patterns of expression of this gene appear to be similar in normal human and mouse tissues, i.e. expression principally in epithelial tissues. Using the mouse cDNA probe and the HEA125 monoclonal antibody, we found expression of egp314 in one of two human myeloma cell lines. It has been speculated that egp314 is an adhesion molecule, suggesting that it may function to facilitate interaction of plasma cells with epithelial cells.

We have begun the preliminary characterization of a second gene (clone 326) which is expressed in 15 of 16 mouse plasmacytomas and none of 18 pre-B or B lymphomas. In human cell lines it is expressed in at least 6 of 8 Burkitt's Lymphoma and in 1 of 2 myeloma cell lines. The cDNA sequence predicts a 748 AA protein with a 38 AA region homologous to the highly conserved G-protein ß subunit repeat. A yeast gene (CDC4) with this repeat has been found to be required for progression through the G_1 phase of the cell cycle.

S 209 EXPRESSION OF IMMUNOGLOBULIN HEAVY CHAIN VARIABLE GENE (V_H) IN B-CHRONIC LYMPHOCYTIC LEUKEMIA (B-CLL) AND B-PROLYMPHOCYTIC LEUKEMIA (B-PLL) CELL LINES: "RESTRICTED" USAGE OF V_H3 FAMILY. Pablo A. Bertin and Gerald E. Marti. LCMB, DBB, CBER, FDA, NIH Bldg 29 Room 511, Bethesda, MD. 20892.

Expression analysis of V_H genes in monoclonal Epstein-Barr virus (EBV)-transformed B cell lines derived from adult and fetal normal human tissues show that IgM-secreting EBV cell lines express V_H genes from particular families in proportion to the estimated family size. Size varies from 1 member in the V_H6 family to more than 100 in V_H3 family. Expression in patients with B-CLL suggest a biased V_H repertoire with high frequency of expression of V_H4, V_H5 and V_H6 families. 2 EBV-transformed B-PLL cell lines [(JVM-2, JVM-HH(3)) Int. J. Can 38;531,1980] and 3 EBV-transformed B-CLL cell lines [(WR#1, SeD, B-CLL-LCL) PNAS 75;5706,1978; Blood 71;9,1988] were grown to 10^8 cells in culture in RPMI 1640, 10% FBS, gentamicin sulfate 0.05mg/ml + glutamine 2mM. in a 5% CO_2 incubator at 37°C. mRNA was isolated and cDNA was synthesized. Polymerase chain reaction (PCR) was used to analyse the V_H genes expressed by these cell lines. We used a set of 7 primers, 27 mer each with an Eco RI restriction site on the 5' end; the sense primer was the leader sequence of each 6 V_H families(LS1 through LS6) and the antisense primer the complementary strand to a given sequence of the first exon of Cμ (CH1). Agarose gel electrophoresis of the PCR products show that all the B-PLL and B-CLL cell lines express genes of the largest family, V_H3, and these genes differ by enzyme restriction analysis. Cloning and sequencing are under way. This data is discordant with the findings in B-CLL patients. This suggest that biased usage of V_H families in B-CLL may not be predominant and that it is related to family size as in normal derived EBV-transformed B cell lines.

S 210 HOMEOBOX GENE EXPRESSION IN HEMATOPOIETIC CELLS. J. Bollekens, F.H. Ruddle and K. Takeshita. Departments of Biology and Medicine, Yale University, New Haven, CT, USA.

We have used the polymerase chain reaction technique to detect the expression of homeobox genes in hematopoietic cell lines. The highly degenerate PCR primers correspond to the conserved sequences located near the 5′ and 3′ ends of the homeobox and were used in a PCR reaction using as the template first-strand cDNA obtained by reverse transcription of poly A+ RNA from mouse erythroleukemia and from human erytholeukemia cell lines. Sequencing of the PCR products showed the presence of the following transcripts in the cell lines examined. MEL contained Hox 3.2 and 3.3 and the murine equivalents of Hox 3E, 4C and 3I. The relative amounts of the homeobox gene transcripts were quantitated using the RNase protection analysis before and after erythroid induction with dimethyl sulfoxide. This showed that Hox 3.3 increased, Hox 4C and 3E decreased and Hox 3.2 remained unchanged with induction by DMSO. In order to examine the array of homeobox genes expressed in primary progenitor cells, we have developed a method to create a cDNA library from 10 nanograms of total RNA. This will allow us to compare the pattern of expression of homeobox genes in leukemic cells and primary progenitor cells.

S 211 INTERLEUKIN-1 RECEPTOR ANTAGONIST INHIBITS ACUTE MYELOID LEUKEMIA CELL GROWTH IN VITRO, Federico Cozzolino, Maria Torcia, Maria Lucibello, Silvia Fabiani, Stefania Bettoni, Clara Crescioli, Charles A. Dinarello, Alessandro Rambaldi, IV Department of Internal Medicine, University of Firenze, I-50139 Italy; Tufts University, Boston, MA 02111; Istituto "Mario Negri" Bergamo, I-35100 Italy.

Interleukin-1 (IL-1) has been described as an autocrine growth factor for Acute Myeloblastic Leukemia (AML) cells. It induces production of CSFs by leukemic and stromal cells and therefore participates in autocrine and paracrine loops of growth. An IL-1 receptor antagonist (IL-1ra), a product of normal monocytes, has been recently purified and cloned. This protein, devoid of IL-1-like activity, competes with IL-1 for binding to the receptor. The effect of IL-1ra on blast proliferation was studied in 15 cases of AML; in some of these cases, production of IL-1 and GM-CSF was also assessed. The results showed that, in all of the cases studied, spontaneous AML cell proliferation was inhibited by addition of IL-1ra in a dose-dependent manner. The constitutive production of GM-CSF was strongly reduced by IL-1ra in 7 of 10 cases studied, while IL-1β synthesis was reduced in 4 of 7 cases. Northern blot analysis of 21 cases revealed that IL-1ra mRNA was expressed by the cells from 2 cases only, whereas IL-1β mRNA was detected in 19 cases. These data enforce the role of IL-1 in AML cell proliferation and cytokine production and suggest that imbalanced secretion of IL-1 and of IL-1ra may contribute to the unrestricted growth of AML.

S 212 THE INFLUENCE OF THE ABSENCE AND OVER-EXPRESSION OF *pim*-1 ON THE GROWTH OF NORMAL AND LEUKEMIC HEMATOPOIETIC CELLS, Jos Domen, Sjef Verbeek, Maarten van Lohuizen, Peter Laird, Chris Saris and Anton Berns, Division of Molecular Genetics, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands.

Mice transgenic for the Eμ-*pim*-1 or H_2K-*pim*-1 transgene have a high expression of the *pim*-1 serine-threonine specific kinases in their lymphoid compartment and are predisposed to T cell lymphomas. Eμ-*myc* transgenic mice highly overexpress c-*myc* in their B cell compartment and succumb to pre-B cell lymphomas with a mean latency of 200 days. Crossing of both transgenic strains leads to a dramatic acceleration of the development of pre-B cell lymphomas; Eμ-*pim*-1/Eμ-*myc* double transgenic mice develop lymphomas *in utero* and die at birth, H_2K-*pim*-1/Eμ-*myc* transgenic mice die shortly (approx. 1 week) after birth. Most of the Eμ-*pim*-1/Eμ-*myc* tumors can be transplanted into syngeneic hosts, while most of the H_2K-*pim*-1/Eμ-*myc* tumors cannot (with the exception of a few late tumors). Mice without *pim*-1 (after homologous recombination in ES cells) have no obvious hematopoetic abnormalities *in vivo*. *In vitro* culturing of cells from the double transgenic tumors on bone marrow feeder cells (Whitlock-Witte type cultures) showed that the tumor cells, even when derived from transplanted tumors, were strictly dependent on feeder cells for proliferation. An exception was a transplanted H_2K-*pim*-1/Eμ-*myc* tumor. These cells showed an autocrine stimulation. Conditioned medium from these cells also stimulated feeder dependent double transgenic cells. The factor produced by these cells is not identical to a number of factors tested thus far. Hematopoietic cultures with no *pim*-1 expression at all revealed an impairment in their proliferative response to growth factors. The insight these observations provide for the function of *pim*-1 will be discussed.

S 213 ISOLATION OF A YEAST ARTIFICIAL CHROMOSOME (YAC) SPANNING THE ACUTE MYELOGENOUS LEUKEMIA 8;21 TRANSLOCATION BREAKPOINT, Jizong Gao, Paul Erickson, Katheleen Gardiner, Michelle Lebeau, Manuel Diaz, David Patterson, Janet Rowley, and Harry Drabkin, Division of Medical Oncology, University of Colorado Health Sciences Center and the Eleanor Roosevelt Institute, Denver, CO 80262, Section of Hematology-Oncology, Department of Medicine, The University of Chicago, Chicago, Il. 60637

The 8;21 translocation is one of the most common specific rearrangements in acute myelogenous leukemia (AML). We have identified markers flanking the chromosome 21q22.3 breakpoint which demonstrate physical linkage in normal genomic DNA with at least three restriction endonucleases (*Not* I, *Sac* II, and *Bss* HII), and which are located not more than 250-280 kb apart. PFG analysis of somatic cell hybrid DNA containing the 8;21 translocation chromosomes demonstrates rearrangement of these markers. A 470 kb yeast artificial chromosome, YAC-Not 42, has been isolated which contains both probes. Construction and mapping of lambda subclones from YAC-Not 42 indicates that greater than 95% (25/26 probes tested) of the YAC DNA is located on the proximal side of the breakpoint. *In situ* hybridization studies using AML 8;21 translocation metaphase chromosomes confirmed these results and demonstrated translocation of the distal marker in additional direct patient samples. These findings indicate the NotI boundary clone, Not 42, which contains conserved DNA sequences, is in close proximity to the translocation breakpoint and may represent the involved gene on chromosome 21. Our data also indicate that the chromosome 21 breakpoint is likely to be quite similar among various 8;21 translocations. The 8;21 translocation occurs in the region of chromosome 21 that appears to be critical for many of the phenotypic features of Down syndrome (DS). This suggests that the same gene may be responsible for the increased risk of leukemia, or leukemia-like syndrome, in persons with DS.

S 214 THE INCIDENCE AND CLINICAL RELEVANCE OF MYELOID ANTIGEN-POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA (MY+ ALL), Hans G. Drexler, Ursula Köller and Wolf-Dieter Ludwig, German Collection of Microorganisms & Cell Cultures Braunschweig, Germany; Institute of Immunology, University of Vienna, Austria; Free University of Berlin, Klinikum Steglitz, Germany

An increasing number of reports documents cases of acute leukemia in which individual blast cells coexpress markers normally believed to be restricted to a single cell lineage. The most commonly used terms are hybrid acute leukemia and acute mixed-lineage leukemia. The incidence of phenotypically variant acute leukemia varies with the quality and quantity of parameters used and the stringency of the criteria employed for its definition. We reviewed the literature and present own new data on one particular subset, namely My+ ALL. The reported incidence ranged from 5% to 46% in 13 studies on My+ ALL totaling 3778 patients. Several studies documented a higher incidence of My+ ALL in adults (realistically in the range of 10-20%) than in children (5-10%) and in B-lineage ALL as opposed to T-lineage ALL. My+ ALL cases are more likely to display unique immunological (positivity for progenitor cell marker CD34) and cytogenetic (t(9;22), 11q23, 14q32) features. Expression of myeloid-associated antigens has no prognostic significance in childhood ALL. There is controversy whether in adults myeloid antigens identify a high-risk group of ALL patients with poorer response to standard ALL therapy.

S 215 MANIPULATION OF THE MOUSE HEMOPOIETIC SYSTEM BY CONDITIONAL ABLATION
Elaine Dzierzak, Peter Fraser, Mike Lindenbaum, Colin Miles and Bernadette Daly, Division of Gene Structure and Expression, National Institiute for Medical Research, Mill Hill, London, NW7 1AA, England

Hemopoiesis is a process which begins in the embryo and continues throughout the fetal and adult life of the animal. Stem cells responsible for hemopoiesis in the adult reside in the bone marrow, while during embryonic and fetal stages, they reside in the yolk sac and liver, respectively. It is unclear whether there is a direct lineage relationship between hemopoietic cells active in the various locations during the different developmental stages or whether they are distinct cells. We are using mice transgenic for the Thy 1 regulatory elements linked to the HSV-TK gene to yield insight into the developmental relationships of stem cells and differentiative potential of cells at various branch points of the hemopoietic system. Through drug induced ablation, we have been able to eliminate greater than 90% of the Thy 1 expressing cells of the thymus. The most efficiently ablated cells are those undergoing differentiation in the thymus of the double positive CD4 and CD8 phenotype. Interestingly, the remaining cells in the thymus consist of three highly enriched populations: 1) double negative cells, 2) mature CD4 and CD8 single positive (HSA negative) cells which would have migrated back through the thymus and 3) cortical and medullary epithelial cells. Since hemopoietic stem cells of the adult express Thy 1 to a low level, we are testing whether we can ablate these cells through in vitro or in vivo treatment. Results of fetal liver and bone marrow transplantation experiments will be shown. Furthermore, we are attempting to study the embryonic hemopoietic stem cell through transplantation of in vitro differentiated totipotent embryonic stem cells. The negative results of these experiments in lethally irradiated mice suggest that the in vivo potential of hemopoietic stem cells does not develop in this differentiation culture system or that the cells necessary for total hemopoietic reconstitution are limiting.

S 216 PRODUCTION OF LEUKEMIA INHIBITORY FACTOR (LIF) BY CULTURED MACROPHAGES AND HEPATOMA CELL LINES
Georges Baffet, Mei-Zhen Cui, Raymond G. Fletcher and Georg H. Fey, Department of Immunology, Research Institute of Scripps Clinic, La Jolla, CA 92037

Leukemia Inhibitory Factor (LIF) and Interleukin 6 (IL6) are produced by a similar spectrum of cell types and share several common functions. For example, both induce differentiation of myeloid leukemia cells and the expression of liver acute phase genes. We have recently demonstrated production of IL6 by rat hepatoma cells. Here we show that LIF was produced by primary rat macrophages, rat and murine macrophage-derived cell lines, and by certain rat hepatoma cell lines. The expression of IL6 and LIF in both macrophages and hepatoma cells was stimulated by treatment with lipopolysaccharides (LPS), and this induction was inhibited by treatment with glucocorticoids. Both IL6 and LIF mRNA synthesis in hepatoma cells was also induced by treatment with IL1, and thus both genes are controlled by similar mechanisms. Secretion of newly synthesized LIF protein by hepatoma cells was also demonstrated by metabolic labeling with ^{35}S-methionine and immunoprecipitation. We have recently cloned the rat IL6 and LIF cDNAs and the corresponding genes and have initiated a comparative investigation of their control elements for inducible expression. We have also shown that the IL6 and LIF response elements of the acute phase rat α_2-macroglobulin reside within the same 1150 bp region, and are currently testing the hypothesis that both cytokines act through signal pathways triggered by distinct cell surface receptors that may converge at common cis-elements in liver acute phase genes and possibly other target genes in leukemic cells.

S 217 APPARENT CLONAL REMISSION IN ACUTE MYELOID LEUKAEMIA.
Rosemary E. GALE and David C. LINCH. Department of Haematology, University College and Middlesex School of Medicine, 98 Chenies Mews, London WC1E 6HX, ENGLAND.

Clinical remission in acute myeloid leukaemia is defined as a normal peripheral blood count with <5% blasts in the bone marrow. It was generally assumed that this situation represented the return of normal polyclonal haemopoiesis but this concept has been challenged by the demonstration of apparent clonal haemopoiesis in ca. 35% of patients in complete remission using X-linked gene products (G6PD) and probes (HPRT and PGK). Bone marrow samples from 40 females who were informative for PGK or HPRT were studied early in remission. 17 (42%) were apparently clonal/oligoclonal in that the ratio of the alleles remaining after digestion with methylation-sensitive enzymes was ≥ 3, as determined by densitometric quantitation. However a ratio ≥3 was also found in 13 of 50 (26%) normal control samples with good agreement on duplicate analysis. This high level of skewing in the normal population suggests that in some AML patients apparent clonal remission may simply represent a return to their constitutive Lyonization pattern and emphasizes the need for simultaneous study of non-haemopoietic tissue. Follow-up of 13 patients with apparent clonal remission is not dissimilar from the overall results obtained in our institution. Samples were also examined from 21 females after chemotherapy for lymphoid malignancies and five (24%) showed an apparent clonal predominance in line with the results from normal controls. Seven patients have been studied before and after very high dose chemotherapy and autologous bone marrow transplantation and the pattern established in the pre-transplant remission marrow has been maintained in the post-transplant granulocytes. This suggests that chemotherapy per se does not sufficiently deplete the stem cell population so as to produce apparent changes in clonal analysis.

S 218 LYMPHOKINE COMBINATION VECTORS: A NEW TOOL FOR TUMOR VACCINATION IN LEUKEMIAS/LYMPHOMAS. B.Gansbacher and E.Gilboa. Department of Hematology/Lymphoma and Molecular Biology, Memorial Sloan Kettering Cancer Center, New York, N.Y., 10021.
We constructed recombinant retroviral vectors carrying both the IL-2 and the IFN-gamma gene to explore the feasibility of their use in the generation an effective antitumor immune response. The constructs are based on the high titer N2 retroviral vector and include the IL-2 and IFN-gamma cDNA which are under the control of the CMV and TK promoter respectively. Virus-containing cell free supernatant was used to infect 38C13 cells, a B cell lymphoma. Infected cells were expanded under G418 selection, appropriate integration verified by Southern blotting and IL-2 and IFN-gamma secretion measured by bioassay and ELISA. Bulk selected 38C13 cells secreted between 10-20 U/ml rIL-2. Upregulation of MHC class I, class II and ICAM was measured using FACS analysis.
We are currently performing in vivo experiments using these lymphokine secreting 38C13 cells in a therapeutic vaccination model in the attempt to protect C3H mice against lethal doses of parental 38C13 cells.

S 219 IL6/LEUKEMIA INHIBITORY FACTOR (LIF) INDUCED TERMINAL DIFFERENTIATION OF MYELOID LEUKEMIA CELLS IS BLOCKED AT AN INTERMEDIATE STAGE BY CONSTITUTIVE C-MYC. Barbara Hoffman-Liebermann and Dan Liebermann, Department of Biochemistry and Biophysics, University of Pennsylvania School of Medicine, Phil., PA 19104-6059
Previously, we have shown that c-myc expression is suppressed in both normal and leukemic cells following induction of terminal differentiation. The cytokines IL6 and leukemia inhibitory factor (LIF), naturally occurring physiological factors, induce terminal differentiation and growth arrest of the myeloid leukemic M1 cell line and down regulate the expression of c-myc. We have transfected vectors containing the c-myc gene under control of the β-actin promoter into M1 cells and have obtained M1myc cell lines which constitutively synthesize c-myc. Our results show that deregulated and continued expression of c-myc inhibits terminal myeloid differentiation in cells treated with physiological inducers of myeloid differentiation. Differentiation is blocked at an intermediate stage in the progression from immature blasts to mature macrophages, shown both by cell morphology and using genetic markers. In addition, some of the antiproliferative effects associated with terminal differentiation are inhibited. The cells are not growth arrested and continue to proliferate; however, the doubling time has increased from 20 to 48 hours, suggesting that growth arrest associated with terminal differentiation consists of multiple components and at least one component is dependent on c-myc suppression. We will show that other mechanisms of growth suppression exist which are independent of c-myc suppression.

S 220 THE PRODUCT OF THE B29 GENE IS ASSOCIATED WITH IgM MOLECULES ON MATURE B LYMPHOCYTES, K. Ishihara, W.J. Wood Jr., R. Wall and P.W. Kincade, Oklahoma Medical Research Foundation, Oklahoma City, OK 73104 and U.C.L.A., Los Angeles, CA 90024
B29 is a B lineage specific gene predicted from sequence information to be a transmembrane member of the Ig superfamily, with a single extracellular Ig-like domain. Its presumptive cytoplasmic region contains a peptide motif present in CD3 and other molecules associated with lymphocyte activation. A polyclonal goat antibody was prepared to a Tryp E fusion protein of B29 and used to study its expression on lymphoid cells. Although early B lineage cells express B29 mRNA, surface expression was only detectable on mature B cell lines (W231, W279, BCL1) and spleen cells, including those from mutant Xid mice. It was not found on thymocytes, T cell lines (EL4, R1.1), or pre-B cell lines (40E, 18-81, 70Z/3). The latter cells contain B29 mRNA and surface B29 was inducible along with sIg on 70Z/3 cells. The density of B29 expression correlated with surface IgM density on positive cells. The antiserum precipitated molecules of 65-75 kDa (unreduced), or 32-35 kDa (reduced) apparent molecular weight by SDS-PAGE analysis. Furthermore, IgM molecules were coprecipitated by the anti-B29 serum from digitonin lysates of W279 cells. We conclude that B29 is intimately, but non-covalently, associated with immunoglobulin molecules on mature B cells. Surface expression is regulated by post-transcriptional mechanisms at discrete stages of B lineage differentiation. Further study may reveal that B29 participates in signal transduction or other functions attributed to B lymphocyte specific receptors.

S 221 THE ENGRAFTMENT OF SCID MICE WITH BONE MARROW TAKEN DIRECTLY FROM PATIENTS WITH PRE-B ALL. S. Kamel-Reid*, M. Letarte, M. Doedens, A. Greaves, B. Murdoch, T. Grunberger, M. H. Freedman and J.E. Dick. The University of Toronto* and The Hospital for Sick Children, Toronto, Ontario, Canada.

We have previously demonstrated the ability to engraft scid mice with a cell line, A-1, derived from a patient with acute lymphoblastic leukemia (Science 246:1597,1989). Dilution experiments with a cell line derived from another patient with ALL revealed the sensitivity of this model, with as few as 100 cells injected IV able to proliferate and cause death of the mice within 4 weeks (Blood 76(10),Suppl 1,1990). In this study we have transplanted bone marrow taken directly from patients with pre-B ALL at initial diagnosis or at relapse into scid mice. The degree of engraftment was measured by flow cytometry, Southern analysis and histochemistry. Bone marrow cells from three patients who had relapsed less than a year after first diagnosis had the most aggressive growth when injected into scid mice. Mice had leukemic infiltrates in the bone marrow, spleen and various other tissues and organs, including the thymic rudiment. In contrast, cells injected from two patients who had relapsed 3 to 4 years after first diagnosis showed less aggressive growth, taking approximately 6 1/2 to 8 months to engraft the mice. The cells from one of these patients grew to a greater extent (>90% in BM) than the cells from the other patient (<10% in BM) and this difference in the rate of growth reflected the clinical outcome of the two patients. The cells that showed the least proliferation in scid mice were ones taken from patients at initial diagnosis. Six and one-half to 9 months after injection mice showed $\leq$5% engraftment of leukemic blasts in the bone marrow. Although this level of engraftment is low, it is nevertheless higher than the degree of growth observed with normal human marrow into scid mice (Science 242:1706,1988). Thus, these data suggest that in scid mice, the growth of bone marrow from patients with pre-B ALL, in relapse or at first diagnosis, may correlate with clinical outcome.

S 222 IN VITRO DIFFERENTIATION OF A HODGKIN'S DISEASE DERIVED CELL LINE

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I. Medizinische Klinik, Universität zu Köln, Joseph Stelzmann Str.9, D-5000 Köln 41, LFI Eb4 R 508, FRG

The cell of origin of Hodgkin's disease is still unknown. Recent data from molecular studies suggest that Hodgkin (H) and Sternberg Reed (SR) cells are derived from immature lymphoid cells. We analyzed the Hodgkin derived cell line Co for the capacity to differentiate in vitro. This cell line shows characteristics of immature T cells, it expresses CD3 molecules only in the cytoplasm. One allele of the TCR β genes is rearranged the other still in germline configuration. Upon activation with 12-O-tetradodecanylphorbol-13-acetate (TPA) Co cells can differentiate in vitro from pre T cells to T cells. After 72 hours of TPA treatment Co cells express CD3 molecules and the TCR αβ on the cell surface. The expression of the IL 2 receptor (CD25) is drastically increased whereas expression of CD4 and CD8 is not altered upon TPA treatment.
These experiments show that a Hodgkin derived cell line can differentiate in vitro from a pre T cell to a T cell like normal T cells. In addition to the molecular studies these experiments suggest a lymphoid cell origin for Hodgkin's disease.

S 223 MOLECULAR ANALYSIS OF THE t(1;19) CHROMOSOMAL TRANSLOCATION FOUND IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA (ALL), A. Thomas Look, Enrica Privitera, Mark P. Kamps, Yasuhide Hayashi, Toshiya Inaba, Linda H. Shapiro, Susana C. Raimondi, Frederick Behm, Linda Hendershot, Andrew J. Carroll, and David Baltimore, St. Jude Children's Research Hospital, Memphis, TN 38105; the Whitehead Institute for Biomedical Research, Nine Cambridge Center, Cambridge, MA 02142; and the University of Alabama, Birmingham, Al 35233

To determine whether *E2A-PBX* fusion genes are a uniform consequence of the t(1;19)(q23;p13.3) chromosomal translocation in childhood ALL, we analyzed leukemic cells from a series of 17 children with either early B-cell precursor (cIg^-, sIg^-) or pre-B (cIg^+, sIg^-) ALL, each of whom had cytogenetic evidence of the t(1;19) in their leukemic cells. In 10 of the 11 patients with t(1;19)-positive pre-B ALL, *E2A-PBX* fusion transcripts with identical junctions were detected by RNA PCR and a characteristic set of five E2A-PBX chimeric proteins were demonstrated by Western blotting. Each of these cases had detectable *E2A* gene rearrangements by Southern blot analysis, including the case in which fusion transcripts were not detected by PCR, suggesting different breakpoint(s) in this case. By contrast, each of the 6 children with t(1;19)-positive early B-cell precursor ALL did not have evidence of rearranged *E2A* genomic restriction fragments, detectable *E2A-PBX* chimeric transcripts, or hybrid E2A-PBX proteins, indicating that in these cases the t(1;19) translocation did not result in typical *E2A-PBX* rearrangements and might involve entirely different loci on chromosomes 1 and 19.

S 224 BIOLOICAL CHARACTERIZATION OF RECOMBINANT RAT AND HUMAN STEM CELL FACTOR, Ian K. McNiece, Neal C. Birkett, Kent A. Smith, Keith Langley, Steve Miles, Cynthia Hartley, and Krisztina M. Zsebo, AMGEN, Thousand Oaks, CA 91320-1789

Recombinant rat and human stem cell factor (SCF), c-kit ligand, has been purified to homogeneity and tested in vitro and in vivo. Rat SCF treatment of steel (Sl/Sl^d) mice leads to a reduction in the severity of their macrocytic anemia, reconstitution of CFU-S_{12} content, and the appearance of mast cells at the injection site. When bone marrow or spleen cells are transplanted from normal mice which have received rat SCF in vivo into W/W^v recipients, the number of cells required for engraftment of donor cells is reduced by 10-100 fold. Lethally irradiated mice treated with rat SCF in conjunction with bone marrow transplant survive long term at bone marrow cell doses which do not result in survival of control animals. Human SCF synergises with CSFs to increase the size and number of colonies in semi-solid cultures. The addition of human SCF to bone marrow and peripheral blood cultures in the presence of CSFs overcomes the inhibition of BFU-E formation by AZT but not inhibition of CFU-GM. Early B cell development is stimulated by rat SCF which synergises with IL-7 to act on a pro B cell population generating pre B cells. These data demonstrate that SCF is an early acting growth factor capable of acting in the myeloid, erythroid and lymphoid lineages possibly directly on the primordial hematopoietic stem cell.

S 225 MOLECULAR STUDY OF THE TARGET CELL FOR LEUKEMOGENESIS IN PH1ALL. Shuki Mizutani, Toshiyuki Miyashita, Kozue Nakamura, Yasuhide Hayashi, Shinpei Nakazawa, Department of Virology, The National Children's Medical Research Center, Tokyo, Japan: A leukemia line KOPN30bi was established from a patient of Ph1ALL with P190BCR-ABL protein. The clonal rearrangement of the IgH gene was identical between KOPN30bi and the predominant clone in the fresh sample , indicating that they are of the same clonal origin. Study of TCR genes including TCRβ , TCRγ and TCR δ loci showed none of these loci was identical between KOPN30bi and the predominant clone in the fresh sample. The result of the TCRδ region analysis which was rearranged on one of the alleles in KOPN30bi and was deleted on both of the alleles in the fresh sample, however, indicated KOPN30bi was not a derivative of the predominant clone in the fresh sample. PCR analysis, using oligonucleotide corresponding to the N region sequence of Vγ -Jγ juncture of KOPN30bi as a probe, indicated that less than one % of the blast cells in the fresh sample was the clone which corresponds to KOPN30bi. These studies indicated the predominant clone in the fresh sample , although it occupied more than 99% of the blasts, did not represent the characteristics of the target cell for leukemogenesis.

S 226 ACTIVITIES OF TUMOUR MARKER ENZYMES DURING INDUCED DIFFERENTIATION IN HUMAN ACUTE MYELOGENOUS LEUKAEMIA(AML)

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AML,notably promyelocytic types(e.g.HL60) possess bipotential ability to induced differentiation along both granulocytic and monocytic lineages.This study concentrates on the measurement of the reported tumour marker enzymes Thymidine kinase (TK)(E.C.2.7.1.21),Thymidine Phosphorylase (TP)(E.C.2.4.2.4) and thymidylate synthetase (TS)(E.C.2.1.1.45) by a radiometric assay based upon High Performance Liquid Chromatography(HPLC).A similar method determined nuclear base,nucleoside and nucleotide levels.Results show distinct changes in TK,TP and TS activities (with accompanying base,nucleoside and nucleotide changes) between differentiated cells and their promyelocytic precursors.Special differentiation markers indicated the degree of maturation.

S 227 NOVEL GENE (LYT-10) RELATED TO THE REL/NFkB FAMILY OF TRANSCRIPTION FACTORS IS INVOLVED IN CHROMOSOMAL TRANSLOCATION AFFECTING BAND 10q24 IN LYMPHOID MALIGNANCIES. Antonino Neri[1,4], Luigia Lombardi[1], Mauro Salina[1], Martino Introna[2], Paolo Ciana[1], Daniele Morpurgo[1], Anna Teresa Maiolo[1], Raju S.K. Chaganti[3] and Riccardo Dalla-Favera[4]. [1]Centro Malattie del Sangue "G. Marcora", Ospedale Maggiore, Milano, Italy; [2]Istituto "Mario Negri", Milano, Italy; [3]Cytogenetics Service, Memorial Sloan-Kettering Cancer Center, New York NY 10021; [4]Department of Pathology and Cancer Center, Columbia University, New York, NY 10032.
Reciprocal translocations involving chromosome 10q24, and T-cell receptor α/δ loci on chromosome 14q11 are associated with T-cell malignancies. More rarely, the same band 10q24 is found juxtaposed to band 14q32 in correspondence of the immunoglobulin heavy-chain (IgH) locus in B-cell malignancies. In an attempt to identify an oncogene at band 10q24, we have now cloned the breakpoints of a t(10;14)(q24;q32) translocation from a case of B-cell non-Hodgkin lymphoma by screening a genomic library made from tumor DNA using IgH probes. A recombinant phage has been isolated which contains chromosome 10 specific sequences linked to the Cα region of the IgH locus on chromosome 14. Probes derived from chromosome 10 sequences in close proximity to the chromosomal breakpoint identified a ~4.0 Kb. mRNA expressed in several lymphoid cell lines which may be derived from the putative 10q24 oncogene (LYT-10 gene). The corresponding cDNA clone has been isolated and sequenced. A complete open reading frame has been identified which predicts a protein of 898 a.a displaying a remarkable homology with members of the rel/NFkB/KBF1 family of transcription factors. As a result of the translocation the 5' portion of LYT-10 coding sequences are juxtaposed to Cα sequences in the same transcriptional orientation. Further structural and functional analysis of the normal and truncated LYT-10 gene products will be presented.

S 228 GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR (GM-CSF) AND GRANULOCYTE COLONY-STIMULATING FACTOR (G-CSF) IN SERUM DURING INDUCTION TREATMENT OF ACUTE LEUKEMIA. Sallerfors B. & Olofsson T. Dept. of Medicine, University of Lund, S-221 85 Lund, Sweden.

GM-CSF measured by a newly developed sandwich ELISA and G-CSF measured by a commersially available kit (Amgen Diagnostics) were used to analyse serum levels in leukemic patients during induction therapy. 18 patients with acute myeloid leukemia (AML) and eight patients with acute lymphoblastic leukemia (ALL)/acute undifferentiated leukemia (AUL) were followed 1-2 times/week during a median interval of 35 and 40 days respectively after the start of chemotherapy. For comparison GM-CSF and G-CSF in serum were also measured in normals; 33/35 had GM-CSF <0.10 ng/ml and 10/10 had G-CSF <0.05 ng/ml. In 15/18 of the patients with AML and in 8/8 patients with ALL/AUL GM-CSF was <0.10 ng/ml in all analysed samples. Two patients had measureable levels of GM-CSF in all samples; median 0.71 (0.26-1.18) ng/ml and the levels successively decreased during and after intravenous chemotherapy and did not increase in response to infections. On the other hand G-CSF >0.05 ng/ml was detected in 54 % of the analysed AML samples; median 0.29 (0.05-2.80) ng/ml and in 40 % of analysed ALL/AUL samples; median 0.09 (0.05-3.00) ng/ml. There was a clear correlation between an elevated concentration of G-CSF and documented infections. Our findings suggest that chemotherapy might interfere with and decrease production of GM-CSF but not that of G-CSF, that severe neuropenia does not cause increased serum levels of GM-CSF even during infection and that serum levels of G-CSF increase during neutropenia mainly as a response to infections.

S 229 NEOPLASTIC TRANSFORMATION OF T CELLS IN IRRADIATED MICE IS MARKED BY INCREASED EXPRESSION OF THE CELL SURFACE MOLECULES 1C11 AND CD3
Anis Sen Majumdar, Cynthia Guidos, Miriam Lieberman and Irving L. Weissman, Cancer Biology Research Laboratory, Departments of Radiation Oncology and Pathology, Stanford University School of Medicine , Stanford, CA 94305.

Thymocytes with the potential to progress to overt neoplasia (preleukemic thymocytes) have been identified through the combined use of a monoclonal antibody , 1C11, which binds to a cell surface heterodimeric glycoprotein on lymphoma cells, and antibodies to the differentiation markers CD4,CD8 and CD3. In the preleukemic thymus, 1C11 recognizes $CD4^-8^-$, $CD4^-8^+$ and $CD4^+8^+$, but not $CD4^+8^-$ cells. By intrathymic transfer to Thy-1 congenic recipients, $1C11^{hi}$ $CD4^-8^-$ cells showed the highest leukemogenic potential, followed by the $1C11^{hi}CD4^-8^+$ and $1C11^{hi}CD4^+8^+$ subsets. $1C11^{hi}$ cells exhibited an unexpected rise in expression of the CD3 marker at an early stage (5 weeks post irradiation) of the disease; this rise was largely confined to the $CD4^-8^-$ population. Intrathymic transfer of $1C11^{hi}$ $CD4^-8^-3^+$ and $1C11^{hi}$ $CD4^-8^-3^-$ cells into congenic recipients resulted in lymphoma induction by the former population only. The lymphomas thus obtained were $1C11^{hi}$ $CD4^-8^+3^+$ and $1C11^{hi}$ $CD4^+8^+3^+$, indicative of further aberrant differentiation.Phenotypic profiles of these lymphomas were similar to those obtained with primary radiation-induced thymic lymphomas. These results define at least one pathway leading from preleukemic $CD4^-8^-3^+$ thymocytes to frank lymphomas.

S 230 **IMPROVED PHARMACOKINETICS AND PHARMACODYNAMICS OF POLYMER CONJUGATED RECOMBINANT HUMAN INTERLEUKIN-3.** Gray D. Shaw, Area G. Bree, Gregg A. Timony, John B. Stoudemire, Marc B. Garnick, Genetics Institute, Inc. Cambridge, MA. 02140
The conjugation of polyethylene glycol (PEG) to a therapeutic protein can markedly prolong its in vivo half-life. Our goal has been to produce a form of Interleukin-3 that can be injected less frequently, therefore allowing a more convenient administration regimen for patients. We have developed a novel variant of human recombinant Interleukin-3 (designated MPC8) which has a single 10,000mw polymer of PEG covalently attached near its amino-terminus. Studies were done to compare the pharmacokinetic and pharmacodynamic properties of MPC8 to those of unmodified recombinant human IL-3 (rhIL-3). I^{131} labeled MPC8 and I^{125} labeled rhIL-3 were co-administered by tail vein injection to rats. A 3.3 fold slower clearance was seen for MPC8 as compared to rhIL-3. Subsequent studies of similar design, but using a subcutaneous route of administration, showed MPC8 having a slower and more sustained release into the circulation relative to rhIL-3. This resulted in an AUC seven fold greater than that observed for rhIL-3. On the basis of these findings, the pharmacodynamics of the two drugs was then compared in cynomolgus monkeys after subcutaneous injections at a dose level of 10ug/kg. Single injections were given every other day for 7 days. Hematological evaluations were done over a fourteen day period. Compared to the rhIL-3 treated groups, the MPC8 treated animals showed a greater increase in their neutrophil, basophil, and eosinophil counts. The mean maximum increases were 4, 6, and 3-fold respectively. These studies indicate that the extended pharmacokinetics of MPC8 increases the white blood cell response at this dose. Further studies are warranted to determine whether the full therapeutic effect of the drug is achieved with as few as three injections per week.

S 231 **EXPRESSION OF BCR-ABL IN HUMAN HEMATOPOIETIC CELLS ABROGATES FACTOR DEPENDENCE,** Christian Sirard, Tsvee Lapidot, Suzanne Kamel-Reid, and John E. Dick, Dept. of Genetics, Research Institute, Hospital for Sick Children and Dept. of Molecular and Medical Genetics, University of Toronto, Ontario Canada.
CML is a stem cell disease involving a translocation between chromosome 9 and 22, joining the bcr locus to the abl proto-oncogene. After a period of 3 to 8 years the affected pluripotential stem cell repopulates both the lymphoid and myeloid compartments. Moreover, bone marrow cells derived from CML patient may be hypersensitive to growth factors. This suggests that the bcr-abl protein provides a proliferative advantage to the affected stem cell and its progeny because of a hyperresponsiveness to growth factor. To address this biological question, we have introduced the bcr-abl cDNA, by retroviral infection, in a human factor-dependent cell line, MO7E. This cell line is derived from an AML patient of megakaryocytic phenotype and responds to IL-3, GM-CSF, in addition we have observed that it also responds to c-kit ligand growth factor. Factor independent populations of MO7E are readily obtained after retroviral infection with bcr-abl. This population is initially polyclonal, expresses very high p210 kinase activity, and has altered differentiation properties based on cytochemical staining. In clonogenic assays, the factor independent population is 2-4 times more responsive to factors than the parental cells and an additive effect is observed when combinations of kit ligand factor and GM-CSF or IL-3 are used. These results suggest that the transduction signal activated by these 3 factors share a common pathway at one point involving bcr-abl or one of its substrates. In liquid culture, the generation time of the factor independent population was considerably shortened and DNA analysis indicates the population becomes progressively clonal during *in vitro* culture. We have found that the parental line grows in the bone marrow of scid mice and progressively invades other peripheral tissues over 3 months. Other cell lines derived from CML patients proliferate preferentially in the bone marrow of scid mice. In order to determine if the bcr-abl expressing MO7E cells show an *in vivo* growth advantage, the infected cells will be introduced into scid mice.

S 232 REARRANGEMENTSOF THE *tal-1* GENE ARE USEFUL CLONAL MARKERS FOR NEARLY 30% OF T-CELL ACUTE LYMPHOBLASTIC LEUKEMIAS, R. Graham Smith, Olafur G. Jonsson, George R. Buchanan and Richard J. Baer, Departments of Internal Medicine, Pediatrics and Microbiology, University of Texas Southwestern Medical Center, Dallas TX 75235.
Despite recent improvements in therapy, at least 40% of children and 65% of adults with T-cell acute lymphoblastic leukemia (T-ALL) still die of drug-resistant disease. Clonal markers are needed to monitor post-remission therapy designed to prevent disease recurrence. The t(1;14)(p34;q11) translocation, found in 3% of T-ALL, interrupts the *tal-1* gene on chromosome 1 (EMBO J. 9:415, 1990). This gene, which encodes a helix-loop-helix domain, is disrupted by site-specific deletion in a much larger subset (25%) of T-ALL (EMBO J. 9:3343, 1990). The translocation and deletion breakpoints are clustered within a 1 kb region in the first known intron of the gene. Sensitive PCR assays can detect 10 rearranged *tal-1* alleles per 10^6 normal genomes. Using internal standards, we can quantitate these rearrangements above a level of 50 per 10^6. We have followed 4 patients with T-ALL whose leukemias contained *tal-1* gene rearrangements. Two of the patients had isolated positive assays in the blood in the 4th and 20th treatment months, followed by multiple negative assays. Both of these patients remain in continuous complete remission in the 16th and 25th treatment months, respectively. The other 2 patients each have developed isolated extramedullary relapses. In one patient, a chest wall mass was noted in the 17th treatment month. At this time, his peripheral blood and bone marrow were morphologically normal, yet by quantitative PCR assay contained 0.05% and 0.35% leukemic cells respectively. In another patient, PCR assay of peripheral leukocytes was positive in the 22nd treatment month; one month later she developed an occult CNS relapse. In conclusion, rearranged *tal-1* alleles are useful clonal markers for monitoring minimal residual disease in a major subset of T-ALL.

S 233 **The Differential Expression of Jun Family Genes and c-Fos is induced after CSFs in a human leukemia cell line with G- and M-CSF receptors**, M. TOWATARI, K. ADACHI, T. KATAOKA, H. KATO, H. SAITO, First Department of Internal Medicine, Nagoya University School of Medicine, Aichi, 466, JAPAN

We had established a novel human leukemia cell line, NKM-1, coexpressing G- and M-CSF receptors. In concordance with the receptors expression, NKM-1 cells proliferated in serum medium responding to exogenous G-CSF or M-CSF in a dose response manner. We examined the expression of immediate early genes, such as c-fos, c-jun, jun-B, and jun-D in this NKM-1 cell line by the stimulation of G-CSF or M-CSF after no serum treatment. A transient and cordinated increase in mRNA levels for c-fos and jun-B occured during the first two hours of G-CSF treatment, but c-jun does not increase significantly. On the other hand, after the addition of fetal bovine serum, the expression of c-jun, jun-B and c-fos was induced clearly. In this human myeloid leukemia cell line, jun-B, not c-jun, might be specifically involved in the G-CSF responding growth. We are now testing the immediate early responding gene expression also by M-CSF in the same way.

S 234 DECREASED INTERCELLULAR ADHESION AND LFA-3 EXPRESSION IN HUMAN AUTOREACTIVE LYMPHOBLASTOID CELLS TRANSFECTED WITH A C-MYC ONCOGENE

Karl Voelkerding, Joni Moore, June Goldman and Leslie Silberstein, Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA 19104

To investigate factors which govern growth, differentiation and neoplastic transformation of autoreactive B cells, we have established several B cell lines, by EBV immortalization, from patients with Cold Hemagglutinin Disease, a B cell proliferation with autoimmune specificity that exhibits a spectrum of phenotpyes ranging from "pre-neoplastic" to malignant clonal B cell expansions. Each cell line is clonal, produces a defined autoantibody of identical specificity as that present in the patient's serum, and represents a novel substrate for determining if oncogenes contribute to autoreactive B cell proliferation and differentiation. The effect of constituitive expression of the c-myc oncogene was evaluated in three autoreactive B cell lines by examining their phenotype prior and subsequent to transformation by a transfected human c-myc oncogene. Prior to transfection, low levels of endogenous c-myc expression were present in each cell line. Phenotypic changes were observed in only two of the three autoreactive B cell lines and correlated with high levels of expression of the exogenous, transfected myc oncogene and included: i) decreased intercellular adhesion evidenced by an altered liquid suspension growth pattern from large, multicellular clumps to small clumps and individual cells; and ii) decreased cell surface expression of the cell adhesion receptor LFA-3. Pertinent negative findings in all c-myc transfected cell lines included: a) no substantial down-regulation of adhesion molecules LFA-1 alpha, LFA-1 beta or ICAM-1; b) no change in the state of cellular differentiation; and c) no increased ability to grow in reduced serum concentrations. These data suggest that c-myc oncogene activation can result in altered expression or activity of molecules which mediate intercellular adhesion in immortalized B cells secreting pathogenic autoantibodies.

S 235 USE OF EPISOMAL VECTORS FOR ANTISENSE RNA-MEDIATED INHIBITION OF GM-CSF IN HUMAN BONE MARROW STROMAL CELLS, Matthew C. Weber, Stanton L. Gerson, and Mark L. Tykocinski, Institute of Pathology, Department of Medicine, and Ireland Cancer Center, Case Western Reserve University, Cleveland, OH 44106

Bone marrow stromal cells regulate hematopoietic stem cell proliferation and differentiation through a complex network of cytokines and cell surface interactions. In order to molecularly dissect these cellular interactions, we have developed stable gene transfer capabilities for the human bone marrow stromal cell line, KM-102 (a kind gift from Dr. K. Harigaya). A hybridization analysis of RNA from KM-102 cells showed significant expression of the colony stimulating factors GM-CSF, M-CSF, and IL-6. GM-CSF mRNA expression dramatically increased following stimulation with IL-1α or IL-1β. Culture supernatants contained GM-CSF, as measured by a sensitive ELISA, and colony stimulating activity (CSA), as detected by a human granulocyte-macrophage colony (GM-CFU) forming assay. An episomal antisense GM-CSF expression construct utilizing Epstein-Barr virus (EBV) replicative signals and the Rous sarcoma virus 3′ long terminal repeat (RSV 3′ LTR) promotor was transfected into the stromal cell line. Stable KM-102 transfectant supernatants contained undetectable levels of GM-CSF and CSA, even following IL-1 stimulation. These data suggest that the major CSA produced by the KM-102 stromal cells is GM-CSF. In addition, inhibition of GM-CSF expression unmasked a hematopoietic progenitor cell inhibitory activity not previously seen in the stromal cell supernatants. Thus, antisense gene transfection technology may permit the unravelling of the hierarchy of positive and negative regulatory molecules involved in controlling normal and malignant hematopoiesis.

S 236 ANALYSIS OF 11Q23 CHROMOSOMAL TRANSLOCATIONS: BREAKPOINTS AND ASSOCIATED TRANSCRIPTS. Sheryl Ziemin van der Poel*^, Heidi Gill*^, Rafael Espinosa III^, Norah McCabe~, Yogesh Patel^, Peter Rubinelli~, Alanna Harden^, Michelle Le Beau^, Charles Rubin~^, Steven D. Smith^, Manuel O. Diaz^ and Janet Rowley*^. *Department of Molecular Genetics and Cell Biology, ^Department of Medicine, ~Department of Pediatrics, University of Chicago, Chicago, IL 60637 11q23 translocations are recurring chromosomal abnormalities in some acute myeloid, lymphoid and acute mixed lymphoid leukemias. Additionally, following epipodophyllotoxin therapy, (ET), patients have been reported to present with a secondary, new acute myeloid leukemia carrying an abnormality in the 11q23 chromosomal region. Breakpoints of four of the translocations involving 11q23 [t(4;11), t(6;11), t(9;11) and t(11;19)] including post-ET patients, t(9;11), are within a 330 kilobase pair yeast artificial chromosome (YAC) which contains the CD3 Gamma gene.(PNAS, Rowley et al, in press). A unique gene probe recognizes rearranged restriction fragments on pulsed-field gel electrophoresis Southern blots of DNA from cell lines and primary leukemia cells with these abnormalities. Human genomic DNA clones isolated from a lambda library of this YAC have been mapped and specific DNA fragments which recognize the various breakpoints are identified, subcloned, and are being characterized. DNA fragments have also been isolated which recognize transcripts on Northern blots of mRNA from primary cells and cell lines with 11q23 abnormalities. A mode of transcriptional control for one of these mRNAs will be described. Clones from cDNA libraries derived from normal, leukemic, and post-ET patient cells are being isolated and characterized.

Growth Factors

S 300 CHANGES INVOLVING T-CELL RECEPTOR, IMMUNOGLOBULIN AND p53 GENES IN MYELOID LEUKEMIAS.
G. Alfaro(1), A. Velázquez(2), R. Ocádiz(3), and V. Ortega(3). 1: Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, 2: Centro Médico La Raza, IMSS. and 3: Instituto Nacional de Cancerología.

Twenty cases of chronic myeloid leukemia (CML) and nine cases of acute myeloid leukemia (AML) were investigated at the genomic and transcription level to determine the existence of cellular heterogeneity. Southern blots were carried out using DNA probes containing sequences for one of the following genes: a) the T-cell receptor subunits: β , γ and δ ; b) the constant region of the heavy (JH) and light chains (κ and λ) of immuno--globulins, and c) p53. We found that genes coding for the β subunit but not for the γ subunit of the T-cell receptor were rearranged in 4/7 cases of AML and in 1/20 cases of CML. However, γ genes were in non-germinal configuration in the cells of two patients (AML and CML). Recombination within heavy chain genes (JH) was found only in CML (3/18). Clearly, changes in this gene family occur independently of each other and may represent one of the following possibilities: recombination errors in myeloid neoplastic cells of the coexistence of lymphoid populations derived from the malignant clone. Another - interesting finding was that cells from patients with CML (19/20) were expressing p53 mRNA whereas acute forms did not (0/9).

S 301 (3H) BROMODEOXYURIDINE USED TO ESTIMATE THE LEUKAEMIA MARKER THYMIDINE KINASE.
Brian Armstrong,Kim O'Neill,Bernadette Hannigan and Gerald McKenna,Biomedical Sciences Research Centre,University of Ulster at Coleraine, Coleraine,Co.Londonderry,Northern Ireland.BT52 1SA.

Deoxythymidine kinase (TK) (EC 2.7.1.21) which catalyses the phosphorylation of deoxythymidine to deooxythymidine monophosphate,provides a salvage pathway for the synthesis of DNA.Of the three TK isozymes,only the cytosolar isozyme TK1 is highly expressed in proliferating cells and in therefore elevated in leukaemic patients.This study measured TK activity in several cell lines using halogenated analogs of thymidine ,the in vivo substrate.Highest affinity was found for 5-bromo-2'-deoxyuridine (BrdU) giving significant sensitivity increase of TK activity.This may be of diagnostic and prognostic use in leukaemic disease

S 302 REGULATION OF C10, A NEW MEMBER OF THE MIP-1 CYTOKINE FAMILY,
Mark S. Berger, Amos Orlofsky, and Michael B. Prystowsky, University of Pennsylvania and Philadelphia VA Medical Center, Philadelphia, PA 19104

Differential screening of a cDNA library made from GM-CSF stimulated mouse bone marrow cells has led to the isolation of C10, a new member of the MIP-1 cytokine family which is only expressed in hematopoietic cells. One of the functions of a closely related protein, MIP-1-alpha, is to inhibit the growth of hematopoietic stem cells. C10 transcripts are increased more than 10 fold within 24 hours of adding GM-CSF to freshly obtained mouse bone marrow cells. The majority of this increase is seen after 6 hours of GM-CSF stimulation. C10 is strongly expressed in $P388D_1$, a murine macrophage cell line derived from a myeloid leukemia but is not expressed in all macrophage cell lines. The 5' flanking region of C10 has been isolated from a mouse genomic library and potential regulatory regions identified. Regulation of C10 may be important in the control of myeloid differentiation.

S 303 *AN NFκB-LIKE PROTEIN IS INVOLVED IN THE TRANSCRIPTIONAL REGULATION OF THE c-JUN GENE IN HUMAN MONOCYTIC LEUKEMIA CELLS,* *Marion A. Brach and Donald W. Kufe, Laboratory of Clinical Pharmacology, Dana-Farber Cancer Institute, Boston, MA 02115*

A novel DNA binding protein has been identified in human HL-60 and THP-1 myeloid leukemia cells that contributes to basal transcriptional activity of the c-jun gene. This protein recognizes a sequence in the c-jun promotor distinct from the AP-1 site which confers autoinduction of the c-jun gene. Binding of the protein is enhanced following induction of monocytic differentiation with TPA, TNF-α and the DNA synthesis inhibitor, Ara-C. As determined by DNAse I protection assay, the factor recognizes a 11bp pallindromic sequence in the c-jun promotor which has similarity to the NFκB binding site. In addition, the binding activity of this protein in myeloid leukemia cells shares certain features with that of the NFκB transcription factor including: 1) activation by treatment with cycloheximide; and 2) enhancement in the presence of GTP. Moreover, binding activity is induced from cytosolic fractions of unstimulated cells by treatment with formamide and sodium deoxycholic acid, and is decreased in the cytosolic fraction of cells treated with TPA, TNF-α or Ara-C. These findings suggest that this protein is released from an inhibitory cytoplasmic protein during induction of monocytic differentiation. A 54bp region of the c-jun promotor containing this element confers basal transcriptional activity as well as the induction of the c-jun gene in these cells after treatment with TPA, TNF-α and Ara-C. Deletion of this fragment abolishes basal transcription and inducibility, whereas introduction of the 54bp fragment is sufficient to confer inducibility to a heterologous herpes thymidine kinase promotor. Further analysis is now under way to determine the size of the binding factor. These results suggest that the c-jun gene is transcriptionally regulated by an NFκB like protein during monocytic differentiation of myeloid leukemia cells.

S 304 EXPRESSION OF ABNORMAL IFNα2 RECEPTORS IN HEMATOLOGIC MALIGNANCIES, OR Colamonici, G Reaman, RA Larson, HM Golomb, MO Diaz, and LC Platanias. University of Chicago, Pritzker School of Medicine, Chicago, IL, and Childrens Hospital, Washington, DC.

We have shown the existence of 2 types of IFNα2 receptors: 1)The IFNα2 receptor expressed in normal lymphocytes, monocytes, and the interferon sensitive cell lines H-929, U-266, and Daudi. This receptor has a multichain structure and is characterized by bands with molecular weights (MWs) of 210, 130, 110, 75 and 55 kD in affinity crosslinking experiments, 2)The abnormal IFNα2 receptor expressed in the monocytic cell line U-937, which is resistant to the antiproliferative effect of IFNα2, characterized by the presence of a novel 180 kD band, loss of the 210 and 110 kD bands, and an enhanced 75 kD band. We studied the structure of IFNα2 receptors in leukemia cells obtained from patients with acute and chronic leukemias by affinity crosslinking of ^{125}I IFNα2 to its receptor. We detected the abnormal receptor complex in 4/6 acute myeloid leukemias (AML), 3/10 acute lymphoblastic leukemias (ALL), 1/2 chronic myelogenous leukemias (CML) in blast crisis, 0/6 hairy cell leukemias (HCL), 0/1 prolymphocytic leukemias (PLL) and 3/26 pediatric acute leukemias (AML/ALL). Studies with the monoclonal antibody IFNαR3 that recognizes the 130 kD protein of the receptor, revealed that the 210 kD band of the normal receptor and the 180 kD band of the abnormal receptor are associations of the 130 kD protein. The novel 180 kD band is homologous to the 210 kD band and is the result of lack of expression of the 55 kD protein in the case of the abnormal receptor. The presence of a unique abnormal IFNα2 receptor, primarily seen in acute leukemias, may be associated with loss of a regulatory mechanism on leukemic cell growth.

S 305 HELIX-LOOP-HELIX TRANSCRIPTIONAL ACTIVATORS BIND TO A SEQUENCE IN GLUCOCORTICOID RESPONSE ELEMENTS OF RETROVIRUS ENHANCERS, B. Corneliussen, A. Thornell, B. Hallberg, C. Grundström and T. Grundström, Department of applied Cell and Molecular Biology, University of Umeå, S-90187 Umeå, Sweden.

An important determinant of the T lymphomagenicity of the murine retrovirus SL3-3 is its enhancer which is preferentially active in T lymphocytes. The enhancer contains, among other domains, a Glucocorticoid Response Element (GRE). However, no or little stimulation of the enhancer activity was seen upon glucocorticoid induction in T lymphocytes, where the basal enhancer activity is high. In contrast, a strong glucocorticoid effect on the enhancer activity was evident in HeLa cells, where the basal activity of the enhancer is much lower. We have investigated whether nuclear factors other than the glucocorticoid receptor interact with the GRE in lymphoid cells. A family of proteins, designated SL3-3 Enhancer Factor 2 (SEF2), were found to interact with a sequence within the GRE. The important nucleotides for binding of SEF2 are conserved within most type C retroviruses. Various cell types displayed differences both in the sets of SEF2-DNA complexes formed, and in their amounts. Mutation of the SEF2 site decreased the basal enhancer activity in different cell types. A cDNA which encoded a protein that interacted specifically with the SEF2 binding sequence was isolated from thymocytes. The protein sequence predicted reveals that SEF2 belongs to the helix-loop-helix class of DNA binding proteins. Several SEF2 mRNA transcripts of different sizes were identified. Molecular analysis of cDNA and genomic clones of SEF2 revealed multiple mRNA species containing alternative coding regions as a result of differential splicing.

S 306 Introduction and Expression of Lac Z gene and FIX gene in Human Leukemia Cell Lines, N.EMI, A.ABE, T.MATUSHITA, Y.MORISHIMA, H.SAITO, LI XU, T.FRIEDMANN, J.K. YEE, First Department of Internal Medicine, Nagoya University School of Medicine, Aichi,466, JAPAN and CMG, UCSD, La Jolla, CA 92093-0634

Human Leukemia cells have proved difficult to infect with MLV derived retroviral vectors. We have developed a retroviral vector, LZRNL that expresses the E.coli lacZ gene from MSV LTR promoter. This marker may prove more sensitive and simple for detection of exogenous gene expression from infected leukemia cells. Using this vector, we have determined the optimal conditions of infection in K562 and Meg01 cells which are derived from leukemia blast cells. We have also characterized the expression of human coagulation factor IX in these cells after infection with L9RNL, a vector derived from LZRNL in which the lacZ gene is replaced by the factor IX cDNA. Results with these vectors in human leukemia cells will be presented.

S 307 PCR ANALYSIS OF CHILDHOOD B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) WITH "GERMLINE" IMMUNOGLOBULIN (Ig) HEAVY (H) CHAIN GENES. C.A. Felix, R. Wasserman, B.A. Reichard, D.G. Poplack, G.H. Reaman, D.E. Cole, & G. Rovera. NCI, Bethesda, MD, Wistar Institute, Philadelphia, PA, Children's Hospital of Philadelphia, Philadelphia, PA & Children's National Medical Center, Washington, DC

Previous studies have suggested a possible association between the germline pattern of Ig and T-cell antigen receptor genes on Southern analysis, and induction failure, in children diagnosed with B-cell precursor ALL (Felix et al. J. Clin. Oncol. 8:431, 1990). In an attempt to determine a possible biological basis for the aggressive clinical behavior of leukemic cells presumably transformed at the earliest stages of B-lymphoid development, lymphoblast DNA was studied by more sensitive PCR methodology. Leukemia-specific CDRIII sequences of VDJ rearrangements were PCR amplified using consensus V and J primers, and the products studied by agarose gel electrophoresis and hybridization with consensus J probes (Yamada et al. Proc. Natl. Acad. Sci. USA 86:5123, 1989). Each of 24 cases showing distinct rearrangements by Southern analysis, showed only 1 or 2 PCR detectable rearrangements. In contrast, each of 4 cases manifesting a germline pattern of Ig H genes on Southern blotting, showed a smear of bands when PCR products were electrophoresed and hybridized. This pattern suggests the presence of many PCR detectable Ig H rearrangements in some cases where a germline pattern is found on Southern blotting. Sequencing studies are now underway to determine more precisely the number of different clones present, and any clonal relationships between the multiple rearrangements detected by PCR. These preliminary investigations suggest that the refractory nature of "germline" cases of childhood B-cell precursor ALL may be related to difficulties in eradication of multiple subclones, present below the threshold of detection of the Southern method. These early data might represent a possible biological basis for differences in clinical behavior.

S 308 CYTOKINE GENE EXPRESSION TESTED BY POLYMERASE CHAIN REACTION IN LEUKEMIC AND NORMAL HEMOPOIETIC CELLS. J. Gabert, M. Lopez, C. Haskovec, L. Da Lio, Y. Toiron, C. Caux and P. Mannoni. Department of Molecular and Cell Biology: Regional Cancer Center and U.119 INSERM, Marseille , FRANCE.

In order to assess the relevance of paracrine and autocrine mecanisms of normal and leukemic hemopoiesis respectively, we applied the PCR technique to the study of cytokine gene expression in a panel of human leukemic cell lines, primary leukemia cells and normal CD34 positive cells isolated from bone marrow. cDNAs obtained by reverse transcription of RNA isolated from the different cells were amplified using pairs of oligonucleotide primers complementary to IL-3, GM-CSF, IL-6, GM-CSF receptor and CD34 sequences. We also tested the sensitivity of the amplification by cDNA dilution experiments which was found variable depending on the set of primers. We are presently checking the reaction conditions in order to obtain similar sensitivity for each primer pairs. Cell lines known to synthesize hemopoietic growth factors either constitutively or after activation were first used to test the validity of the method applied to the analysis of cytokine gene expression. Firstly, we confirmed that cell lines like Mo, MiaPaCa or 5637 constitutively express growth factor genes. On the other hand, human myeloid leukemias cell lines (HL60, THP-1, KG-1, HEL, and K562) were found not to express either IL-3 or GM-CSF. CD34 gene expression was found restricted to the cells expressing the antigen at the membrane. Secondly, we applied this approach to the study of cytokine gene expression in normal CD34 progenitor cells isolated after ficoll gradient and panning selection, to a purity of more than 97% as assessed by FACS analysis. The expression of IL-6 and GM-CSF receptor were routinely detected. Experiments are in progress to analyze the expression of GM-CSF and other hemopoietic growth factors in normal and leukemic progenitors. The results will be discussed in the context of normal and leukemic hemopoiesis.

S 309 UNSTABLE AND REVERSIBLE DIFFERENTIATION IN ACUTE LYMPHOBLASTIC LEUKEMIA, Frank Griesinger*, Burkhard Jansen and John H. Kersey, Department of Laboratory Medicine and Pathology, and Pediatrics, University Minnesota, Minneapolis, MN 55455, *Present address: Department of Hematology, University of Münster, 4400 Münster, FRG

The mature $CD3^+CD7^+TCR\alpha/\beta^+$ T-ALL (MT-ALL) has multilineage differentiation potential in that if can be induced to differentiate in vitro into the myeloid lineage by GM-CSF and IL-3. Identical non-productive TCRγ and δ and a productive TCRβ rearrangements demonstrated a common clonal origin of myeloid and lymphoid leukemic cells. Since productive rearrangements of the TCRα gene are indicative of rather mature T-lymphocytes, the TCRα gene was cloned and sequenced in both myeloid and lymphoid leukemic cells, in order to determine the stage of differentiation at which lineage conversion occurred. An identical productive joint of JαC to a novel Vα region had occurred in all leukemic cell cultures. The highest degree of homology of this novel Vα region is to Vα3.1 and 10.1. Oncogenes mapped in the vicinity of chromosomal breaks of MT-ALL and those associated with lineage conversion (c-myc, c-raf, c-scl) were not found to be rearranged. In conclusion, lineage conversion appears to occur at the level of a genotypically mature T-lymphoid cell as opposed to an uncommitted common T-lymphoid and myeloid "stem" cell. Thus, "terminal" T-lymphoid differentiation in MT-ALL is unstable and reversible to the myeloid lineage.

S 310 IN VIVO EFFECT OF ANTIBODIES TO IL-5 RECEPTOR ON EOSINOPHILIA AND HYPERGAMMAGLOBULINEMIA OF IL-5 TRANSGENIC MICE,

Yasumichi Hitoshi, Naoto Yamaguchi, Eiichiro Sonoda, Seiji Mita, Akira Tominaga, and Kiyoshi Takatsu, Department of Biology, Institute for Medical Immunology, Kumamoto University Medical School, 2-2-1 Honjo, Kumamoto 860, Japan

Murine interleukin-5(IL-5), an inducible glycoprotein mainly synthesized and secreted by T cells following activation with antigen, is known to possess various biological activities on different cell types including B cells and eosinophils. We investigated the in vivo role of IL-5 and its receptor in the activation of IL-5 responsive cells. IL-5 receptor(IL-5R) was recognized on B cells and eosinophils by anti-IL-5R mAbs that inhibit the binding of IL-5 to its receptor. Intriguingly, most of IL-5R-positive B cells in peritoneal cavity expressed Ly-1 antigen that was also expressed by chronic B cell leukemia cells. Binding assay with ^{35}S-labeled IL-5 revealed the presence of two classes of IL-5 binding sites on B cells and eosinophils. Eosinophilia and hypergammaglobulinemia that were observed in IL-5 transgenic mice decreased to nomal level with the passive administration of anti-IL-5R mAbs. The results of the in vivo experiments clearly demonstrated that IL-5 acts on B cells and eosinophils directly through IL-5R and play an essential role in immunoglobulin secretion and eosinophilia in vivo.

S 311 ABERRANT TRANSCRIPTS OF THE C-FPS/FES PROTO-ONCOGENE IN HUMAN LYMPHOID LEUKEMIA AND LYMPHOMA CELL LINES. Manfred Jücker*, Anton Roebroek+, Volker Diehl*, Wim Van de Ven+ and Hans Tesch*, *I. Med. Klinik, Universität Köln, FRG; +Dept. of Biochemistry, University of Nijmegen, NL

Aberrant, short transcripts of the c-fps/fes proto-oncogene were detected in cell lines derived from human lymphoid leukemia, Burkitt's lymphoma and Hodgkin's disease, but not in normal untransformed hematopoietic cells. Northern blotting and S1 nuclease experiments revealed that the short transcripts have a size of about 0.9 kb and start at two distinct sites within exon 16 of the c-fps/fes gene. Sequence analysis of a cDNA clone of the short c-fps/fes transcripts did not reveal any point mutation. A putative open reading frame encompasses the phosphotransfer motif and the autophosphorylation site of the fps/fes kinase domain. In vitro transcription/translation of a cDNA clone corresponding to the short c-fps/fes transcripts revealed a protein of 17 kDa. We could not detect any structural alteration within or around the c-fps/fes gene in the lines which express the aberrant transcripts. This may indicate a novel mechanism for oncogene activation in human lymphoid tumor cells.

S 312 MOLECULAR CLONING AND CHARACTERIZATION OF ABERRANT RETINOIC ACID RECEPTORS FROM A t(15;17) POSITIVE ACUTE PROMYELOCYTIC LEUKEMIA PATIENT

Akira Kakizuka, Wilson H. Miller, Jr.*, Raymond P. Warrell, Jr.*, Ethan Dmitrovsky*, Ronald M. Evans. Gene Expression Laboratory, The Salk Institute, La Jolla, CA.
*Memorial Sloan-Kettering Cancer Institute, New York, NY.

Chromosomal rearrangements of particular loci have often been correlated with certain types of malignancies. The chromosome 17 breakpoint of the t(15;17)(q21-q11-22) translocation, found in human acute promyelocytic leukemia (M3), was recently shown to be within the α retinoic acid receptor (RAR) gene on chromosome 17. Leukemic cells from t(15;17) positive patients contain aberrant forms of α RAR mRNAs which could be fusion transcripts from the RAR gene and the gene on the chromosome 15 breakpoint, reffered to as myl. We have constructed a cDNA library from a t(15;17) positive patient, and isolated several cDNA clones, respectively, coding for myl-RAR, RAR-myl, and myl proteins. These results strongly suggest the involvement of the myl-RAR and/or RAR-myl fusion proteins in oncogenesis. Structural and functional characterization of these proteins are in progress.

S 313 IDENTIFICATION AND CHARACTERIZATION OF THE MURINE CD10 ANTIGEN, Barbara L. Kee Christopher J. Paige and Michelle Letarte, Division of Immunology and Cancer, Hospital for Sick Children, Dept. of Medical Biophysics, Ontario Cancer Institute and Dept of Immunology, University of Toronto, Toronto, Canada M5G 1X8.

CD10 (Common Acute Lymphoblastic Leukemia Antigen) is a 100 kd glycoprotein expressed on >80% of cases of childhood acute lymphoblastic leukemia (ALL),those with a pre-B phenotype. CD10 is also found in other malignancies such as lymphoma and melanoma and on normal pre-B lymphocytes and several tissues including kidney. CD10 is identical to neutral endopeptidase 3.4.24.11 (NEP/enkephalinase), a highly conserved enzyme which hydrolyses numerous biologically active peptides. Other related enzymes, such as aminopeptidases N and A (Look et al, J Clin Invest 83:1299, 1989; Wu Q. PNAS 87:933, 1990), have been identified on lymphoid, myeloid and stromal cells, suggesting that such enzymes play an important role in hematopoiesis. To study the role of CD10/NEP in B cell development we are characterizing the murine equivalent of CD10. Southern blot analysis of murine kidney genomic DNA revealed that a single gene was detected with a human CD10 cDNA. This probe also detected mRNA species of 6.7, 6.2 and 3.4 kb in mouse kidney, liver and lung. Monoclonal antibodies 18B5 and 23B11, which react with the extracellular and cytoplasmic regions of rabbit NEP respectively, both recognized a protein on a Western blot of 100 kd in extracts of murine kidney, liver, lung, bone marrow and the stromal cell line S17. We are currently attempting to identify the subpopulations in murine bone marrow which express CD10/NEP. Abelson transformed pre-B lymphocytes and pre-B lymphocytes from day 15 fetal liver (isolated directly or expanded in IL-7) do not express CD10 at detectable levels. Bone marrow pre-B lymphocytes will be tested. NEP activity will be assessed with and without the specific inhibitor thiorphan in all subpopulations to confirm that CD10 is enzymatically functional and capable of inactivating potential growth and/or differentiation factors.

S 314 THE ROLE OF IMMATURE ERYTHROID CELLS IN LEUKEMIA DEVELOPMENT;

Alexander V.Mitasov, Vladimir A. Kozlov, Irena G. Tsyrlova, Institute of Clinical Immunology, Siberian Branch of the USSR Academy of Medical Sciences

Several models of leukemia development were investigated: I.lymphoid leukemia in AKR mice 2.leukemia induced by IL-3-N-ras-FDCPmix or GM-FDCP mix cell line injection into sublethally irradiated mice. Leukemia induction in all these models was accompanied with an increase of CFUs proliferation level that, in turn, caused an accumulation of immature erythroid cells due to the disturbances in their maturation. We have shown that eryth roid progenitors may have a function of Natural Suppressors, i.e. the cells and soluble factor produced by these erythroid precursors, could inhibit mitogen-stimulated normal B-lymphocyte proliferation and Ig-secretion both in human and in mice. At the same time, erythroid cell factor has an inverse effect on CLL-B-cells, enhancing their proliferative response to mitogen. The difference of normal and CLL-B-cell responsiveness to erythroid cell signals and the dependence on the cell surface phenotype and growth factor presence in culture are discussed.

S 315 DETECTION OF MALIGNANT CELLS IN NON-HODGKIN LYMPHOMA PATIENTS USING PCR, A.C. Lambrechts, P.E. de Ruiter, L.C.J. Dorssers and M.B. v.'t Veer, Dr. Daniel Den Hoed Cancer Center, PO box 5201, Rotterdam, The Netherlands.

The t(14;18) is the most common chromosomal abnormality found in non-Hodgkins lymphoma (NHL). In this translocation an unique DNA segment is created, the bcl-2 oncogene (chr. 18) is placed in juxtaposition of the JH segment of the immunoglobulin heavy chain gene (chr. 14). Two breakpoint clusters (mbr/mcr) are identified within the bcl-2 gene.
In this study the usefulness of the highly sensitive polymerase chain reaction (PCR) as a diagnostic tool for the detection of minimal (residual) disease in NHL patients with a t(14;18) was investigated. High molecular weight DNA was isolated from blood and bone marrow samples of NHL patients, taken somewhere in the course of disease. All samples were subjected to PCR analysis in which we routinely detect one t(14;18) positive cell in 100,000 cells. We have evaluated blood and bone marrow samples from 63 NHL patients for the existence of the mbr- or mcr-t(14;18). Thirty-nine of these patients were diagnosed as NHL with a follicular growth pattern, 20 showing mbr-t(14;18) positive cells, 2 showing mcr-t(14;18) positive cells. From the remaining 24 patients with other types of NHL, samples of 6 patients were positive for a mbr-t(14;18).
The PCR results demonstrated that in 6 patients in clinical complete remission lymphoma cells were detected by PCR. Furthermore, twelve samples of blood or bone-marrow (9 patients) were diagnosed negative on immunological and/or morphological criteria, whereas PCR revealed t(14;18) positive cells. These results clearly demonstrate that this technique is a sensitive and specific technique for the detection of malignant cells in patients who seem to be free of disease by immunological, morphological and clinical criteria.
Now, a prospective study is carried out to further confirm the clinical usefulness of PCR in the detection of minimal disease in t(14;18) positive NHL patients. Further we focus on the amplification of the hypervariable regions of the immunoglobulin heavy chain gene after VDJ rearrangements. This provides a unique marker for lymphoma cells in nearly all patients with B-NHL and is of particular interest in patients with NHL of intermediate or high grade malignancy, lacking a specific chromosomal translocation, whose therapy is aimed at cure.
This project is supported by the Dutch Cancer Society.

S 316 DISSECTION OF THE IMMEDIATE EARLY RESPONSE OF MYELOID LEUKEMIA CELLS TO TERMINAL DIFFERENTIATION AND GROWTH INHIBITION: LIF AND IL6 TRIGGER AN IDENTICAL IMMEDIATE EARLY RESPONSE. Dan A. Liebermann,Barbara Hoffman-Liebermann, Kenneth A. Lord, and Abbas Abdollahi. University. of Pennsylvania School of Medicine, Department of Biochemistry and Biophysics, Philadelphia, PA 19104-6059.

To better understand the immediate early genetic response of myeloid cells to terminal differentiation and growth inhibitory stimuli, cDNA clones of myeloid differentiation primary response (MyD) genes have recently been isolated. In this study, a set of known (JunB, c-Jun, JunD, ICAM-1,$H1^0$ and H3.3 histone variants) and novel (MyD88, MyD116) MyD genes were used as immediate early molecular markers to further dissect the primary genetic response of myeloid leukemic cells to various differentiation and growth inhibitory stimuli. It is shown that the expression of some MyD genes is linked to the developmental program of cell differentiation, whereas expression of others to the inhibition of cell growth. It is indicated also that the immediate early activation of certain MyD genes may not be sufficient by itself to induce terminal differentiation whereas activation of others may play an important role in cell maturation. Also shown are evidence to indicate that leukemia inhibitory factor (LIF) and IL6, two distinct multifunctional cytokines which bind to distinct receptors, directly activate the same set of immediate early response (MyD) genes upon induction of myeloid leukemia terminal differentiation. Thus, MyD genes should provide useful tools to further study the nature of the signalling pathways employed by LIF and IL6, including cis and trans acting control elements that govern the activation of MyD genesby these multifunctional cytokines.

S 317 HUMAN IN VITRO PRE-ACTIVATED T CELLS ENGRAFT IN SCID MICE AND HOME INTO MURINE LYMPHOID TISSUES. Malkovska V, Cigel F, Armstrong N, Borcherding W and Hong R, Department of Medicine and Pediatrics, University of Wisconsin, Madison, WI 53792

It has been shown previously that mice with severe combined immune deficiency (SCID mice) can be engrafted with human lymphoid tumors as well as normal resting T-cells. To investigate the feasibility of a model for adoptive immunotherapy of human lymphoid tumors with T-cells in SCID mice we studied whether T-cells that had been activated in vitro by culture with irradiated normal or malignant allogeneic lymphoid cells can engraft in SCID mice and migrate to the sites of lymphoma growth. $50x10^6$ in vitro pre-activated peripheral blood mononuclear cells were injected i.p. into each mouse. Human cells could be detected using flow cytometry of cells pooled from lymphoid organs and by immunohistochemical staining of frozen tissue sections in 9 of 10 animals sacrificed at 3 or 4 weeks after injection. The percentage of CD45 positive cells ranged from 2% to 45% and the absolute numbers of CD45 cells recovered from lymphoid tissues ranged from $4x10^6$ to $90x10^6$. 2% to 42% cells expressed the CD3 antigen together with either CD4 or CD8. Immunohistochemical staining with biotinylated anti-CD2 and anti-CD3 revealed that in murine spleens the human T-cells were localised in perivascular areas, whereas in the lymph nodes and gut mucosa, they were scattered throughout the tissues showing no site-specific accumulation. Human cells recovered after four weeks from murine splenic tissue and depleted of murine cells displayed moderate proliferative responses in mixed lymphocyte reactions against the original stimulators but did not respond to candida, tetanus or streptococcal antigens. These data suggest that human in vitro activated T-cells can engraft in SCID mice and migrate to sites of lymphoid tumor growth. The response of surviving xenogeneic T-cells to specific antigens is diminished.

S 318 DIFFERENTIAL EXPRESSION OF A SUBSET OF PROTEINS IN ACUTE LYMPHOBLASTIC LEUKEMIA CELLS AND NORMAL PERIPHERAL BLOOD LYMPHOCYTES.
Enrique I. Miranda 1, Araceli Castrejon 1,2, Mario Gutierrez 1, Lilian Hernandez 1, Juan Collazo-Jaloma 1, Alejandro Garcia-Carranca 3 and Patricio Gariglio 2. 1.-Servicio de Hematologia, Hospital General de Mexico SS, Apdo. Postal 7-826 Mexico D.F. C.P.06720; 2.-D.G.B.M. CINVESTAV-IPN. Mexico D.F.; 3.-I.I.B. UNAM. Mexico DF.

Acute lymphoblastic leukemia had been characterized as a disease involving both a defect in the normal maturation of lymphoid cells and loss of proliferation control. Proteins from leukemic cells and normal lymphocytes were subjected to western blot analysis and found to exhibit differential expression of a group of proteins: two (115 kDa and 60 kDa) were found specifically in leukemic cells and one (40 kDa) in normal lymphocytes. Stimulation of normal lymphocytes resulted in the disappearance of p40 and appearance of p115 and p60. On the other hand, the induction of differentiation of leukemic cells resulted in the expression of p40 and disappearance of p115 and p60. Although the origin of these proteins is still largely unknown, this study suggests that these proteins may either be implicated in the control, or are a part of, the differentiation-proliferation program of lymphoid cells.

S 319 MOLECULAR ANALYSIS OF GENES INDUCED DURING MEGAKARYOCYTIC DIFFERENTIATION OF K562 HUMAN MYELOID LEUKEMIC CELLS, Dwight M. Morrow, Robert R. Getty, and Mark L. Tykocinski, Institute of Pathology, Case Western Reserve University, Cleveland, OH 44106
The repertoire of genes induced in the course of human myeloid leukemic cellular differentiation programs is of considerable interest. To date, studies aimed at identifying such induction-specific mRNAs, through differential cDNA cloning, have focused on the bipotential human promyelocytic leukemia cell line HL-60. We have instead focused our differential cloning efforts on the bipotential human erythroleukemic cell line K562. K562 λZAPII cDNA clones were differentially screened with either a single-stranded cDNA probe reverse transcribed from mRNA of uninduced K562 cells, or with a subtracted induced single-stranded cDNA probe. The latter was generated by reverse transcribing mRNA from K562 cells treated with 10^{-7}M phorbol-myristate-acetate, to induce them along the megakaryocytic lineage, and subtracting with a 30-fold excess of photobiotinylated uninduced mRNA. One hundred twenty seven primary differential clones were selected; 14 of 18 subjected to a secondary screen were confirmed to be induction-specific. Following a tertiary screen and partial DNA sequencing, all 14 unexpectedly proved to be independent clones of the same mRNA species, previously identified as erythrocyte-potentiating activity (EPA) and tissue inhibitor of metalloproteinases (TIMP). This finding suggests that EPA/TIMP is highly over-represented among induction-specific mRNAs in this leukemic cell line. Gene transfer experiments are in progress to assess the functional significance of the overexpression of this protein in cells differentiating along the megakaryocytic pathway. Characterization of the remaining induction-specific clones is also in progress.

S 320 THYMOSIN BETA-4 IS A NUCLEAR PROTEIN WITH GROWTH FACTOR ACTIVITY IN VITRO, Lynn C. Moscinski, George Kasnic and Allen L. Goldstein, Department of Pathology, H. Lee Moffitt Cancer Center at the University of South Florida, Tampa, Fl and the Department of Biochemistry and Molecular Biology, George Washington University School of Medicine, Washington DC.
Thymosin beta-4 (TB4) is a 43 amino acid peptide originally thought to be a thymic hormone after its isolation from calf thymic fraction 5. Since then, it has been shown to share a short amino acid sequence homology to tumor necrosis factor alpha and to be present in a variety of tissue types, with high expression of both mRNA and protein in human and murine macrophages and granulocytes. We have recently shown that it is capable of potentiating the proliferative effects of GM-CSF on cultured human bone marrow, and is chemoprotective in mice given lethal doses of cytosine arabinoside. Using immunogold electron microscopy, we now demonstrate localization of TB4 to both the nucleus and cytoplasm of marrow precursor cells of all three hematopoietic lineages. The relative distribution and intensity of labeling varies significantly among cell types. Whereas TB4 protein appears almost exclusively confined to the nucleolus of both normal and neoplastic myeloid blast cells, it distributes in association with the heterochromatin of more mature cells. This distribution is maintained during cell mitosis, where immunologically detectable protein segregates with the chromosomes in a diffuse pattern. Cytoplasmic protein is detected as low-level diffuse labeling in all cell types examined, with the greatest labeling seen in the most immature cells. This data suggests that TB4 may have an important intracellular function during marrow proliferation.

S 321 UNIQUE TRANSFORMING ACTIVITY ASSOCIATED WITH DNAs FROM ADVANCED STAGES OF B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA (B-CLL), E.W. Newcomb, R. Dalla-Favera* and W. Bayona, Department of Pathology and Kaplan Cancer Center, New York University School of Medicine New York, NY 10016; *Department of Pathology, Columbia University, College of Physicians & Surgeons, New York, NY 10032

B-CLL is the most common human leukemia in the United States. The role of transforming genes or oncogenes in a wide variety of human lymphoid malignancies has been well-documented. For example, the c-myc oncogene is implicated in Burkitt's lymphoma, the c-abl oncogene is implicated in chronic myelogenous leukemia, and the N-ras oncogene is implicated in acute myeloblastic leukemia. However, the role of transforming genes in the development of B-CLL has not been well-studied to date. To score for dominant transforming oncogenes, the technique of DNA-mediated-gene-transfer into rodent fibroblasts coupled with injection into nude mice to detect tumor formation was used. Preliminary results obtained from screening DNAs from 10 cases of advanced stages of B-CLL disease for a dominant oncogene in the nude mouse tumorigenicity assay showed transforming activity was readily detectable in 6 of 10 cases. Southern blot analysis of DNAs from secondary nude mouse tumors 1) displayed a similar pattern of Alu positive bands in 3 cases, suggesting that they may share the same transforming DNA sequences and 2) were negative for several transforming oncogenes known to be activated in vitro (dbl, mas, raf and trk). The further characterization of the unique B-CLL-associated transforming activity will be presented.

S 322 DETECTION OF t(14;18) TRANSLOCATION IN SALIVARY GLAND BIOPSIES FROM PATIENTS WITH SJÖGREN'S SYNDROME, Pavel Pisa, Eva Pisa and Robert I.Fox, Department of Immunology, Karolinska Institute, Stockholm, Sweden and Department of Immunology, Scripps Clinic, La Jolla, CA 92037

Sjögren's syndrome (SS) is a chronic autoimmune disorder characterized by lymphocytic infiltration of salivary and lacrimal glands. These patients have a markedly increased frequency of developing non-Hodgkin's lymphoma. Among 200 SS patients followed for more than 5 years, 14 developed non-Hodgkin's lymphoma. Pre-lymphoma biopsies were available on 7 of these patients and provided the opportunity to detect pre-neoplastic cells. Using primers specific for chromosome 14 and 18, translocation of the protooncogene BCL-2 was detected by polymerase chain reaction (PCR) in 5 out of 7 SS lymphomas. In the pre-lymphoma biopsies of these patients, BCL-2 translocation was not observed, using a PCR that would detect a single cell with t(14;18) translocation among 100,000 normal cells. These results demonstrate that lymphoma arising in SS develops as a multistep process. We conclude that the great sensitivity of PCR can help us in detecting early onset of lymphoma in SS patients and serve as a prognostic factor for the course of the disease.

S 323 BIOCHEMICAL CHARACTERIZATION AND MODE OF SYNTHESIS OF THE *pim*-1 ONCOGENE PRODUCTS, Chris Saris, Jos Domen, Hanneke van der Gulden and Anton Berns, Division of Molecular Genetics, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands.

The *pim*-1 gene is frequently found activated by proviral insertion in murine T cell lymphomas. Overexpression of *pim*-1 in lymphoid cells by transgenesis formally proved its oncogenic potential. The *pim*-1 cDNA sequence predicts that both murine and human *pim*-1 encode a 34 kd protein with homology to protein kinases. In this study, we show that the murine *pim*-1 gene encodes a 44 kd protein in addition to the predicted 34 kd protein. The 44 kd protein is an amino-terminal extension of the 34 kd protein and is synthesized by alternative translation initiation at an upstream CUG codon. Contrary to previous findings by others, we provide evidence that both murine and human *pim*-1 gene products are protein-serine/threonine kinases. Murine 44 kd and 34 kd *pim*-1 proteins exhibit comparable *in vitro* kinase activity and are both mainly cytoplasmic, but they differ in *in vivo* association state and half-life.

In vivo activity of the Pim-1 protein is currently studied in transgenic mice overexpressing either one of these proteins.

The results of these studies and of experiments dealing with *in vivo* regulation of Pim-1 kinase activity will be discussed.

S 324 CLONING OF LAK CELLS AND THEIR POTENTIAL IN TREATMENT OF LEUKEMIA, Peihsien Tang, Ning Mao, Feizi Jiang, Minwei Zhang, Yongzi Xi, Yinling Lu. Institute of Basic Medical Sciences, PO Box 130, Beijing 100850, PR China.

A series of T cell clones were developed from PBMNC of healthy adults and patients with leukemia by means of semisolid cultures containing rhIL-2. The clones were transferred into 96-well plates with liquid medium and rhIL-2. 10-20% of clones showed high LAK activity against NK-sensitive K562 human leukemic cell lines and some NK-resistant tumor cell lines. Each could expand up to 10 with proliferation and died soon afterwards. FCM showed their phenotypes were CD 3,8,25 and DR positive and CD4 negative while CD16(+)or(-) that were subject to T cell lineage. After recloning, 90% of subclones showed completely the same phenotypes and activities as the original clone, that demonstrated good clonality rhIFN- alone or along with rhIL-3 well synergized with rhIL2 to strengthen LAK activity but rhIL-3 alone did not. Clone contact of LAK cells with target cells brought about tiny pores formation over tumorcells and cytoplasmic collapes after ½ to 4 hrs. LAK cells could also be successfully cloned from PBMNC of patients of acute and chronic myelogenous and acute lymphoblastic leukemias during their relapsing, acute or even crisis phases and had high cytocidal effects on leukemic K562,HL60,U937 and H7402 in vitro. LAK cells could be successfuly cloned and expanded to large amount enough for clinical therapy despite very low frequency of LAK precursors in PBMNC of leukemic patients. These cloned LAK cells alone or in combination with IL-2 or some other cytokines are likely to be feasible means in treating leukemias clinically or purging in vitro for autologous bone marrow transplantation.

S 325 ISOLATION AND CHARACTERIZATION OF A MYELOID SPECIFIC ACTIVATING ELEMENT IN THE CD11b PROMOTER, Daniel G. Tenen and Heike L. Pahl, Hematology-Oncology Division, Department of Medicine, Harvard Medical School and Beth Israel Hospital, Boston, MA 02215.

CD11b is the alpha chain of the Mol adhesion heterodimer, which is specifically expressed in myeloid (granulocyte/monocyte) lineages and NK cells, but not in other cell types. CD11b mRNA is specifically expressed in myeloid cells and up-regulated in myeloid cell lines induced to differentiate in vitro; nuclear run-on experiments demonstrate that the up-regulation is predominantly transcriptional. We have isolated the 5' upstream CD11b genomic region including a 1.7 kb sequence which directs myeloid-specific transcription of a reporter luciferase gene in transfected HL-60 and U937 cells, but not in the Jurkat T cell line. Luciferase expression in these constructs is increased when the cells are induced to differentiate with the phorbol ester TPA. Preliminary experiments suggest the presence of a silencer element which represses transcription in T cells. Future experiments shall be directed at localizing the precise sequences responsible for myeloid specific expression, and attempting to identify and isolate myeloid specific transcription factors. Such factors may play a role in normal myeloid differentiation, and could potentially be involved in the pathogenesis of acute myelogenous leukemia.

S 326 IMPROVEMENT OF LEUKEMIC LTBMC ESTABLISHMENT BY USING SPECIFIC INHIBITOR OF HEMATOPOIETIC STEM CELL PROLIFERATION; Irena G. Tsyrlova, Elena V. Shklovskaja, Department of Molecular Immunohematology, Institute of Clinical Immunology, Siberian Branch of the USSR Academy of Medical Sci.

The combination of AraC treatment with the Inhibitor of stem cell (SCI) proliferation was shown to protect normal hemopoiesis and to kill all leukemic cells (Tsyrlova I.G., Lord B., 1989). Based on these results we tried to use such combination of drugs for "purging" of CML patients bone marrow but it was noticed that SCI alone was able to improve the establishment of LTBMC from CML patients up to the level of cell production and CFU-GM formation of LTBMC from healthy donors. The same was observed in model system: LTBMC from leukemic AKR mice, cultivated in the presence of SCI developed intensive cell production and higher level of CFU-GM, comparable with LTBMC from young nonleukemic mice. The mechanism of such effect could be due to the preferential ability of quiescent CFUs to attach stroma layer in Dexter culture. After 8-20 hs of cultivation of murine BMC both on heavily irradiated stroma from LTBMC or 3T3 cell line, there were more CFUs-I2 in the adherent layer and less in suspension when incubated with SCI as compared with control. Taking into account the disappearance of leukemic cells in Dexter cultures, it might be a very promising step for BMT.

S 327 Detection of tumor-specific bcr-abl junctions in Philadelphia chromosome positive leukemias.

Janneke van Denderen, Paulien ten Hacken, Wim Boersma, Gerard Grosveld and Willem van Ewijk.
Dept. of Immunology, Erasmus University, Rotterdam, the Netherlands.

In leukemic cells of patients with Chronic Myeloid Leukemia (CML) and Acute Lymphoblastic Leukemia (ALL) the Philadelphia (Ph') chromosome can be frequently detected. The Ph' chromosome is the result of a reciprocal translocation between chromosome 9 and chromosome 22. As a consequence of the translocation two normal genes, bcr and abl, are fused, resulting in the formation of a hybrid bcr-abl gene and corresponding mRNA and protein. The hybrid protein is by definition tumor-specific, since the molecule is only expressed in cells carrying the Philadelphia chromosome. Depending on the localization of the breakpoint in the bcr gene different types of chimeric proteins have been revealed: b2-a2P210$^{bcr\text{-}abl}$, b3-a2P210$^{bcr\text{-}abl}$, and e1-a2P190$^{bcr\text{-}abl}$. The hybrid molecules comprise large parts of the normal, non-tumor-specific proteins bcr and abl. However, the amino acids in the respective junctions between bcr and abl do not occur in the normal bcr and abl molecules and therefore form new, tumor-specific epitopes on P210$^{bcr\text{-}abl}$ and P190$^{bcr\text{-}abl}$.
The aim of our study is the specific detection of leukemic cells of CML and ALL patients by virtue of their unique expression of bcr-abl junctional epitopes. To this purpose rabbits were immunized with synthetic peptides consisting of 10 amino acids corresponding to the respective bcr-abl junctions. At present, we have isolated three types of antisera, each type specifically recognizing one bcr-abl junctional epitope. From the reactivity of these antisera we conclude that the amino acids on all three bcr-abl junctions in the respective proteins are expressed in an antigenic fashion. These tumor-specific determinants are therefore unique immunologic markers for diagnosis of Philadelphia positive leukemias.

S 328 DEFICIENT PRODUCTION OF MONOCYTE-DERIVED CYTOKINES IN CLL,

H.W. Lõms Ziegler-Heitbrock, Elke Leinisch, Berthold Emmerich, Jürgen G. Haas, Institute for Immunology and Department of Internal Medicine, University of Munich, W8000 Munich 2, Germany

Isolated mononuclear cells from patients with chronic lymphocytic leukemia (CLL) exhibit a strongly reduced expression of tumor necrosis factor (TNF) when stimulated with LPS (Int. J. Cancer, 45: 280, 1990). Direct ex vivo stimulation from CLL patients of whole blood with LPS or with staphylococci also gave severely depressed values for TNF and IL-6 secretion into plasma. Thus suggesting that cytokine production by monocytes might be defective in CLL, also in vivo. We are currently studying the impact of either IFNα therapy (Blood 73: 1426, 1989) or of standard chemotherapy on the ex vivo cytokine production in this disease.
As shown by two chamber experiments, we can demonstrate that the deficiency is caused by the leukemic B cells in the sample, which produce a low MW soluble factor. Candidate soluble factors are gangliosides, and we can in fact demonstrate that different types of purified gangliosides can suppress production of TNF mRNA and protein by mononuclear cells and by the monocytic cell line Mono Mac 6 in vitro.

Therapy-Experimental and Clinical

S 400 VINCRISTINE AND VERAPAMIL IN RESISTANT RELAPSES AND IN A CONDITIONING REGIMEN FOR BMT, M.Andolina E. Agosti, A. de Manzini, D. Recanati, M. Ferrari, R. Padrini, D. Piovan
Istituto per l'Infanzia, Trieste
Istituto di Farmacologia Clinica, Padova. Italy.
In advanced leukemia the blast cells express a glycoprotein (gp170) on their membranes and therefore achieve a resistance to a series of chemotherapic agents (multi drug resistance). The MDR could be circumvented after verapamil incubation or in vivo continuous infusion. Suitable blood levels are achieved only with extremely high doses (16-80 mg/sqm/hr) of verapamil that causes a 2nd grade cardiac block. We think that the failures in most trials could be explained with too low dosages.
We treated with verapamil plus vincristine 7 patients with end stage leukemia resistant to chemotherapy. A partial or a complete remission was achieved in 5 patients that soon underwent a mismatched BMT.
A patients in CR2 after an early relapse of ALL was submitted to an autologous BMT conditioned with vincristine in continuous infusion plus verapamil, TBI and cytoxan. His bone marrow was purged with vincristine plus verapamil. The boy is in remission after one year.
This form of therapy is probably too toxic for a wide use. We forcast future use of D-Verapamil far less toxic than L-Verapamil.

S 401 FRACTIONATED TOTAL-BODY IRRADIATION PRECEDING HIGH DOSE CYTOSINE ARABINOSIDE AS A PREPARATIVE REGIMEN FOR BONE MARROW TRANSPLANTATION IN CHILDREN WITH ACUTE LEUKEMIA: A PILOT STUDY FROM THE CHILDREN'S HOSPITAL OF PHILADELPHIA . Charles S. August, Naynesh Kamani, Eliel Bayever, Joel W.Goldwein, Giulio J. D'Angio, Children's Hospital of Philadelphia, and Departments of Pediatrics and Radiation Oncology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104

Sixteen children with acute leukemia between 4 and 19 years of age underwent allogeneic bone marrow transplantation (BMT) at the Children's Hospital of Philadelphia. Nine patients had acute lymphoblastic leukemia (ALL) in 2nd remission; one, ALL/lymphoma in first remission; two, ALL in 2nd relapse; 3, acute non-lymphocytic leukemia in first remission and one with acute mixed lineage leukemia in first remission. Treatment consisted of fractionated total body irradiation (1200 cGy in six fractions of 200 cGy twice daily for 3 days), followed by high dose cytosine arabinoside (12 doses of 3 g/m^2 given every 12 hours), then BMT from HLA-matched sibling donors. One patient died of Candida sepsis and acute renal failure 10 days post-BMT, and another on day +78 of cardiorespiratory failure after a successful resuscitation for a cardiac arrest suffered 28 days following transplantation. Eight patients developed acute and/or chronic graft versus host disease. One patient developed idiopathic interstitial pneumonia 5 months post BMT. With a median follow-up of 27 months (range 6 to 54 months), fourteen children are alive and in remission with Karnofsky scores of 90-100%. No leukemic relapses have occurred thus far. Despite the relatively short follow-up, our results suggest that this pre-conditioning regimen is associated with tolerable toxicity and may be highly effective in controlling leukemia resistant to conventional chemotherapy.

S 402 INTERMEDIATE DOSE CCNU WITH A 3+7 AML REMISSION INDUCTION SCHEDULE - EFFECT ON HAEMATOLOGICAL RECOVERY AND DISEASE FREE SURVIVAL.
Barrett AJ, Tighe J, Samson DM; Riverside Haematology Group and Department of Haematology, Hammersmith Hospital, London W12 0NN, UK.
Treatment with high dose of alkylating agents or total body irradiation is effective in the cure of acute myeloblastic leukaemia (AML) by autologous or allogeneic marrrow transplantation in a proportion of cases. Such treatments may owe their success in part to the eradication of non-cycling leukaemia stem cells. We used the nitrosourea CCNU as an addition to a standard remission induction schedule in an attempt to increase the cytoreduction on non-cycling leukaemic stem cells and thereby improve disease free survival.
49 patients with AML (39 de novo "standard risk" and 10 secondary to myelodysplasia or chemotherapy "high risk") were treated using a multi-institutional protocol in 12 hospitals in the UK. Median age was 55 (range 23 - 81). Patients were treated between Jan 1987 and Nov 1990. Remission induction used CCNU 200 mg/m^2 day 1, Daunorubicin 45 mg/m^2 day 1, 2 and 3, and Ara-C 100 mg/m^2 IV over 12 hours days 1 - 7.
The overall remission induction rate (which was achieved in 1 course in 72% of patients) was 85% (87% standard risk and 70% high risk). Actuarial disease free survival from presentation was 40% at 4.5 years (44% standard risk and 0% high risk) with a relapse probability of 53% standard and 100% for high risk. These results suggest that CCNU can reduce the relapse risk after successful remission induction without prejudicing normal haematopoietic recovery but emphasize differences in outcome for patients with high risk AML. A randomised study to further evaluate this approach is now in progress.

S 403 GROWTH INHIBITORY AND CYTOTOXIC EFFECT OF ANTISENSE OLIGONUCLEOTIDES IN CHRONIC PHASE OF CHRONIC MYELOGENOUS LEUKEMIA (CML), Albert Deisseroth, Tianying Yuan, Charles V. Herst, Hanchun Chen and Christopher Reading, Department of Hematology, University of Texas M.D. Anderson Cancer Center, Houston, TX 77030

In CML, the formation of the bcr-abl chimeric mRNA and the p210 tyrosine-specific protein kinase emancipates late myeloid progenitor cells from dependence on extracellular growth factors. This results in an expansion of the late myeloid progenitor pool which leads to death of the patients through bleeding and infection. In order to develop autologous marrow transplantation therapy for this disease, we have established conditions under which antisense oligonucleotides inhibit the growth of and induce the death of CML cells in vitro. Antisense oligonucleotides of 17 and 28 nucleotides length were designed to bind to the region of the translation initiation codon of the bcr mRNA. These oligonucleotides were added at a concentration of 50 micromolar to cultures of the CML cell lines BV173, EM2, and K562. The in vitro growth of BV173 and EM2 were markedly inhibited by antisense but not sense oligonucleotides in the presence of serum-free as well as serum-containing cultures. The growth of the K562 cell was not inhibited by these antisense oligonucleotides. Growth of bcr-abl negative myeloid cell lines (Josk) were not inhibited by these oligonucleotides. Antisense oligonucleotides to p53 mRNA did not inhibit the growth of CML cells. 95% of the cells died in the presence of the bcr antisense oligonucleotide and in the absence of serum in chemically-defined culture conditions. Thus, the use of antisense oligonucleotides not only arrest cell growth but also results in CML cell death in serum-free conditions. The use of such antisense oligonucleotides in vitro to destroy CML cells under conditions which would permit the continued growth of normal cells have now been defined. These data are leading to in vitro methods of treating autologous marrow for autologous transplantation of CML.

S 404 **THERAPY OF ACUTE PROMYELOCTYIC LEUKEMIA (APL) WITH ALL-TRANS RETINOIC ACID (RA).** Stanley R. Frankel, Wilson H. Miller Jr., David A. Scheinberg, Ethan Dmitrovsky, Raymond P. Warrell, Jr. Memorial Sloan-Kettering Cancer Center, New York, NY 10021.

The retinoic acid receptor-alpha (RAR-α) gene located on chromosome 17 at the breakpoint of t(15;17) seen in APL may serve as a target for non-cytotoxic therapy. As part of an ongoing study, 12 patients (pts) with APL were treated with oral RA at a dose of 45mg/m^2/d. Of 10 evaluable cases, 8 pts (4 *de novo* and 4 relapsed) have achieved complete remission (CR). Two pts (1 *de novo*, 1 relapsed) were withdrawn after developing marked leukocytosis; however, 2 subsequent pts were continued on RA through the period of leukocytosis and achieved CR. One patient in 2nd relapse achieved a CR by day 28 but relapsed while on maintenance RA at day 79. Ten days of additional RA treatment at 90 mg/m^2/d failed to reinduce remission. The principal adverse effect was headache with intracranial hypertension in 2 pts. Morphologic and immunophenotypic studies were consistent with differentiation as a primary mechanism of response. Response was characterized by the transient appearance of an "intermediate" population of cells that expressed both CD16 (late myeloid) and CD33 (early myeloid) surface antigens. mRNA expression of aberrant RAR-α transcripts detected by Northern blot was positively correlated with clinical response to RA and was a more sensitive predictor than either conventional cytogenetics or Southern blot analysis. No patient had an abnormally low basal serum retinol level; however, plasma [RA] $T_{1/2}$ during treatment was unexpectedly short (< 1 hr). These data suggest that pharmacologic provision of a RAR ligand may correct a disrupted program of myeloid differentiation in APL.

S 405 IMMUNE RESPONSES OF CATS TO INFECTION WITH FELINE LEUKAEMIA VIRUS O Jarrett, J Christie, A Pacitti and B Willett, Department of Veterinary Pathology, University of Glasgow, Bearsden, Scotland G61 1QH.

Our longterm aim is to cure cats of FeLV infection by adoptive transfer of FeLV-specific T cells during the asymptomatic phase of the infection. To this end we are studying the cellular immune response of cats to FeLV, and investigating means to identify histocompatible cats. Cats exposed to the virus either develop a persistent viraemia (PV) or recover. Recovered cats mount a neutralizing antibody response directed at virion envelope glycoproteins and are resistant to reinfection. The cellular basis of this immune response will be described. Cats with PV do not have antibodies to the viral glycoproteins and are at high risk of developing leukaemia. However, evidence will be presented that these cats too produce T cells which are FeLV-specific.

Polymorphism in the major histocompatibility complex (MHC) is being investigated. Using a mixed lymphocyte reaction histocompatible cats have been identified. Experiments to define the MHC restriction of cat T cell responses to FeLV will be described.

S 406 EFFECT OF DIETHYLCARBAMAZINE IN LEUKEMIA VIRUS MODELS: Lynn W. Kitchen, M.D., Marshall University School of Medicilne, Huntington, WV.

Oral administration of diethylcarbamazine (N,N-diethyl-4-methyl-1-piperazine carboxamide [DEC]), a biological response modifier agent used in the prevention and treatment of the filariases, has been shown to prolong survival in newborn mice inoculated with murine leukemia virus. Six (10.7%) of 56 DEC-treated Cas-Br-M inoculated mice died at an age of less than 125 days, compared to 19 (34.5%) of 55 untreated control mice (Fisher's exact test 1-sided p=.0024). Reductions in the frequency/severity of brain and splenic pathologic lesions consistent with untreated Cas-Br-M infection were also noted in the mice that received oral DEC in comparison to untreated Cas-inoculated mice. These data support the findings of earlier preliminary studies of DEC in feline leukemia virus (FeLV) models: (1) Serum viral infectivity was lowered in 12 of 14 FeLV-infected cats following one month of treatment with oral DEC (10 mg/kg/day as a single dose per day; Kitchen LW and Cotter SM, Journal of Clinical and Laboratory Immunology, 25:101-103, 1988). (2) DEC treatment of 2 kittens shortly after evidence of infection with FeLV significantly reduced the rate of decline of circulating lymphocytes in comparison to 2 untreated littermates (Kitchen LW, Mather FJ, and Cotter SM, JCLI 27:179-181, 1988). DEC is an inexpensive, orally bioavailable, and relatively nontoxic drug, and all available data indicate that it can be given safely during pregnancy.

S 407 MATURE B-CELL TARGETED TOXIN GENE EXPRESSION, Ian H. Maxwell, L. Michael Glode, and Françoise Maxwell, University of Colorado Cancer Center, Denver, CO 80262.

Towards ablating B-cell malignancies, we have constructed a plasmid, designated pTHA71, which expresses the A-chain of diphtheria toxin (DT-A) with high efficiency and specificity in transfected, mature B-lymphoid cells. This construct, incorporating an immunoglobulin (Ig) kappa light chain promoter, small intron, partial constant region exon, and 3'-flanking sequence (but lacking a known enhancer), conferred substantially more efficient expression of DT-A in mature B-cells than was seen from previous constructs that included only Ig promoters and enhancers. Characterization of the sequences responsible for high level expression is in progress. When transfected into the 70Z/3 murine pre-B-cell line, pTHA71 was not detectably expressed unless the cells had previously been exposed to lipopolysaccharide (which induces expression of their endogenous, rearranged Ig kappa gene). DT-A expression constructs similar to pTHA71 show promise for the possible therapeutic ablation of malignant B-cells of mature stages, while sparing normal progenitor cells.

S 408 ABERRANT STRUCTURE AND EXPRESSION OF RETINOIC ACID RECEPTOR ALPHA (RAR-α) AND EXPRESSION OF RELATED RECEPTORS IN PATIENTS (PTS) WITH ACUTE PROMYELOCYTIC LEUKEMIA (APL) TREATED WITH ALL-TRANS RETINOIC ACID (RA). Wilson H. Miller, Jr., S. Frankel, A. Jakubowski, J. Gabrilove, R. Warrell, and E. Dmitrovsky. Memorial Sloan-Kettering Cancer Center, New York, NY. 10021

The RAR-α gene is rearranged in the characteristic translocation t(15;17) of APL. RA induces maturation and clinical remission in APL. During our clinical trial of RA in APL, we evaluated RAR-α mRNA expression in 10 pts. Aberrant mRNA transcripts were seen by Northern analysis of total cellular RNA isolated from bone marrow mononuclear cells in 9 pts, all of whom achieved complete remission (CR) after RA treatment. One pt lacked the t(15;17) by classical cytogenetics but expressed an abnormal transcript and achieved CR. One pt with morphologic APL expressed only the two normal RAR-α transcripts and failed to respond to RA. Two predominant patterns of aberrant RAR-α mRNA expression were seen. Although both normal and aberrant RAR-α mRNA expression were initially augmented in vivo, the abnormal RAR-α mRNA declined as pts achieved CR. To search for a related receptor that might mediate the clinical response to pharmacologic doses of RA, we probed APL Northern blots to RXR-α, and abundant expression of the 4.8kb RXR-α mRNA was seen in 4/4 samples tested. Rearrangement of the RAR-α gene was confirmed by Southern analysis in several pts. Different DNA rearrangement patterns were found among samples with the same mRNA expression pattern. However, Southern analysis with exonic RAR-α cDNA probes and several restriction enzymes did not detect the rearrangement in all cases expressing abnormal mRNA. Thus, altered RAR-α expression is implicated in the blocked differentiation seen in APL, and predicts the clinical response to RA.

S 409 IDARUBICIN VERSUS DAUNORUBICIN AS TREATMENT OF ACUTE MYELOID LEUKEMIA IN ELDERLY PATIENTS WITH ACUTE LEUKEMIA. J. Reiffers, F. Rigal-Huguet, A.M. Stoppa, M. Michallet, G. Marit, M. Attal, J.A. Gastaut, B. Corront, G. Lepeu, M. Routy, P. Cony-Makhoul, J. Pris, D. Hollard, D. Maraninchi, M. Mercier, P. Hurteloup for the BGMT Group.

We have conducted a prospective study comparing Idarubicin (IDA) or Daunorubicin (DNR) in elderly patients (age between 55 and 75 years) with acute myeloïd leukemia (AML) without a preleukemic phase. For induction chemotherapy, the patients were given Cytosine-arabinoside (Ara-C : 100 mg/m2/day, continuous infusion, 7 days) associated with either IDA (8 mg/m2/day, IV, 5 days) or DNR (50 mg/m2/day, IV, 3 days). Once a complete remission was achieved, the patients received a consolidation chemotherapy with Ara-C (100 mg/m2/day, 5 days), combined with either IDA (8 mg/m2/day x 3 days) or DNR (30 mg/m2/day, 3 days), then a continuous maintenance chemotherapy with or without androgens for two years. The main characteristics of patients were similar in the IDA and DNR Groups. The complete remission rate was higher in the IDA Group (69.4 %) than in the DNR Group (61.6 %) but the difference was not statistically significant ($p = 0.3$). Cardiac toxicity was more frequently seen in the DNR Group than in the IDA Group (p = N.S). There was a trend for a longer disease-free survival in the IDA Group than in the DNR Group ($p < 0.097$) but this needs to be confirmed with a longer follow-up. However, these results confirm that IDA is at least equivalent and probably superior to DNR for the treatment of AML in the elderly.

S 410 TREATMENT OF ADULT ACUTE LYMPHOBLASTIC (ALL) AND UNDIFFERENTIATED (AUL) LEUKEMIA IN SWEDEN. REPORT FROM THE NATIONAL STUDY GROUP. Smedmyr B, Simonsson B, Björkholm M, Carneskog J, Gahrton G, Grimfors G, Hast R, Järnmark M, Killander A, Kimby E, Lerner R, Löfvenberg E, Malm C, Nilsson PG, Paul C, Rödjer S, Stahlfeldt AM, Sundström C, Turesson I, Udén AM, Wahlin A, Willén L, Westin J, Vikrot O, Winqvist I, Zettervall, Öst Å. The Swedish ALL-group.

All Swedish adults with ALL/AUL are since june 1986 treated according to one protocol. Remission induction consists for all patients of Vcr 2 mg days 1,8,15,22, cyclophosph. 600 mg/m^2 day 1, dauno 30 mg/m^2 days 1,2,15,16, pred 60 mg/m^2 days 1-28, L-asp 15000 E/m^2 days 15-28. Consolidation therapy is Vcr 2 mg day 1, dauno 30 mg/m^2 day 1, VP-16 100 mg/m^2 days 1-5, ara-C 100 mg/m^2 x2 iv days 1-5 and pred 60 mg/m^2 days 1-5. For CNS prophylaxis mtx 15 mg it x6 and irradiation 24 Gy is given. After CR patients are considered to have a standard- (SR) or high-risk (HR)(Lpk>30x10^9/l, B-ALL, AUL, L3, Ph'+, CNS-leuk, time to CR>d.28) leukemia. After one consolidation course SR patients receive maintenance therapy with 6-MP daily and Mtx for 2,5 y, with doses adjusted to keep LPK 2-3x10^9/l. HR-patients get two consolidation courses (from jan 89 also ara-C 3 g/m^{2}x1 days 1-4, AMSA 200 mg/m^2 days 1-3) followed by BMT (<45y), ABMT (<60y) or maintenance therapy (>60y).

Until jan 31 -90, 126 consequetive patients were diagnosed. Eleven patients, median age 69 (31-85) y, were excluded from the treatment protocol due to complicating disease (7) or initially wrong diagnosis (4) or protocol violation (2). Median age of the remaining patients was 38 (16-79) y. CR rate is 87/113 (77%), 86% in patients <60y and 43% in patients >60y. Remission failure was due to resistant leukemia, infections and other complications in 1/3 each. The most frequent complication during induction treatment was infections (46%). With minimal follow up of 9 mo CCR for SR and HR is 0.62 and 0.42, respectively.

S 411 FUNCTIONAL INTEGRITY OF HUMAN NEUTROPHILS AFTER MEROCYANINE 540-SENSITIZED PHOTOIRRADIATION, Orla M. Smith, Donald L. Traul and Fritz Sieber, Department of Pediatrics, Medical College of Wisconsin, Milwaukee, WI 53226

The photosensitizing dye, merocyanine 540 (MC 540), is currently undergoing phase I clinical testing as a purging agent for autologous bone marrow grafts from leukemia and lymphoma patients. Normal human neutrophils were used as a model system to study the mechanism of the anti-leukemic effect elicited by MC 540-sensitized photoirradiation. Treatment with MC 540 and white light (35 W/m^2) for 90 minutes (which achieves a >5 log reduction in clonogenic HL60 and K562 leukemia cells) reduced the number of neutrophils phagocytosing $\geq$1 *S. aureus* bacteria (after 30 minutes) from 94% $\pm$ 9% to 75% $\pm$ 12% ($\bar{x} \pm$ sd, n=3). The phagocytic index was reduced from 40.7 $\pm$ 4.2 bacteria/neutrophil for untreated cells to 18.9 $\pm$ 6.3 (n=3) for treated cells. While MC 540 obviously affected phagocytosis, a function mediated by the plasma membrane, intracellular activities of neutrophils were not disrupted. After 30 minutes of phagocytosis, treated neutrophils killed 96.2% $\pm$ 1.5% of ingested bacteria compared to 97.4% $\pm$ 0.7% (n=3) for untreated neutrophils. The nitroblue tetrazolium and 2',7'-dichlorofluorescin diacetate tests showed that MC 540 treatment did not inhibit generation of superoxide radical or hydrogen peroxide, respectively. We conclude that the primary site of MC 540 attack is the plasma membrane. Supported by CA42734 and the MACC Fund.

S 412 MEROCYANINE 540-SENSITIZED PHOTOIRRADIATION FOR THE PROPHYLAXIS OF GRAFT-VERSUS-HOST DISEASE? Donald L. Traul, Laura McOlash, Chiu-Yang Shih and Fritz Sieber, Department of Pediatrics, Medical College of Wiscon sin, Milwaukee, WI 53226

The ability of merocyanine 540 (MC 540) to photosensitize leukemia and lymphoma cells but spare normal pluripotent hematopoietic stem cells is well documented and is already being exploited clinically for the purging of autologous bone marrow grafts. Recent investigations have shown that MC 540-treated human marrow and peripheral blood cells are deficient in a broad range of immune functions (Lum et al, Exp Hematol 18:560, 1990), prompting speculations on a potential role of MC 540 in the prophylaxis of graft-versus-host disease (GVHD). In this study, we examined the effects of MC 540-sensitized photoirradiation on the functional integrity of mouse spleen cells and the ability of murine marrow grafts to cause GVHD in recipients that were mismatched at minor histocompatibility antigens. Exposure to MC 540 (15 μg/ml) and white light (35 W/m^2) for 90 min abrogated the proliferative response of spleens cells to PHA, ConA, LPS, IL-2, or allogeneic spleen cells. NK activity and T cell-mediated responses were greatly reduced, even if cells were stimulated with IL-2 before or after exposure to MC 540 and light. When mixtures of marrow cells (1 x 10^7) and spleen cells (5 x 10^7) from C57BL6 (H-2^b) or B10.BR (H-2^k) mice were exposed to dye and light for 90 min prior to injection into lethally-irradiatied LP (H-2^b) or AKR (H-2^k) mice, all surviving recipients developed signs of GVHD (dermatitis, persistent weight loss). The incidence of fatal GVHD was reduced from 65% to 30% when grafts from C57BL6 donors were treated with MC 540 and light prior to injection into LP recipients. By contrast, the incidence of fatal GVHD in AKR recipients increased from 25% to 55% when grafts from B10.BR donors were treated with MC 540. These data suggest that the ability of MC 540-sensitized photoirradiation to prevent GVHD may be limited. The opposing effects of MC 540 on the incidence of fatal GVHD in the two bone marrow transplantation models may have been caused by minor differences in the conditioning regimens or a strain-specific response of immune cells to photodynamic damages. *Supported by CA42734 and the MACC Fund.*

S 413 NEW PRECLINICAL MODELS FOR BONE MARROW TRANSPLANTATION IN HEMATOLOGICAL MALIGNANCIES. L. Uharek, B. Glass, W. Gassmann, H. Loeffler, W. Mueller-Ruchholtz. Depts. of Immunology and Internal Medicine II, University of Kiel, D-2300 Kiel, F.R.G.

Relapse is still the leading cause of death after BMT in hematological malignancies. For the development of more effective treatment strategies appropriate preclinical models are required. To determine the value of different murine leukemia and lymphoma cell lines, we studied (1) in vivo tumor growth characteristics after injection of increasing cell numbers obtained from different sources (culture, spleen, marrow, fresh, frozen), (2) the effect of prior immunization with irradiated tumor cells, (3) the influence of total body irradiation followed by syngeneic BMT at various stages of the disease, (4) the in vitro sensitivity against NK and LAK cells of different origin, and (5) the expression of MHC antigens. Until now two cell lines of Balb/c origin have been fully characterised this way: the myolomonocytic leukemia WEHI-3 and the B-cell neoplasm A-20. Our data indicate that cells obtained from tumor bearing animals result in more reliable death rates than cultured cells, irrespective of whether they had been frozen or not. The injection of 10^4 and more spleen or BM cells from animals bearing A20 or WEHI-3 resulted in 100% mortality within 14-36 days (A-20) or 12-17 days (WEHI-3). Marked hepatosplenomegaly was always observed and allowed a definite diagnosis after death. Both neoplasms express H-2d and proved to be nonimmonogenic in Balb/c. Syngeneic BMT (performed at day 2, 5, 10, or 15 after tumor inoculation) prolonged survival significantly. After injection of WEHI-3, lasting curative effects were achieved with BMT at day 2 or 5. If syngeneic grafts were used, no long-term survivors were observed after injection of A-20. WEHI-3 was shown to be more sensitive against NK-mediated lysis than A-20. We conclude that WEHI-3 and A-20 provide useful tools to investigate factors that are supposed to infuence leukemic relapse after autologous or allogeneic BMT.

S 414 THE CLINICAL RELEVANCE OF THE DETECTION OF MINIMAL RESIDUAL DISEASE BY PCR IN CHILDHOOD ALL. C.Ellen van der Schoot[1], Mieke R. Wester[1], H.Theo Cuypers[1], Eleonore N. van Leeuwen[2], Ineke C.M. Slaper-Cortenbach[1], Albert E.G. Kr.von dem Borne[1,2]. [1]Central Laboratory of the Blood Transfusion Service, Amsterdam and [2]the Academical Medical Centre/Emma Children Hospital, Amsterdam.

Using primers complementary to consensus sequences in the Jh and Vh region of the IgH gene-locus, we are able to amplify and to sequence the VDJ region of ALL cell DNA. Based on this sequence a tumorspecific oligonucleotide (ONT) can be synthesized. These ONT's are radiolabeled and used as probe to detect tumor-VDJ in amplified VDJ-DNA, isolated from cells during remission. We determined the detection-limit of this technique to be 0.01-0.001%. In order to evaluate the clinical relevance of such low numbers of malignant cells a longitudinal study is being performed. During the past 10 years we have collected bone marrow samples at regular intervals from 100 ALL patients at diagnosis, during remission and at relapse. From three patients a longitudinal study has been completed. In one patient that relapsed twice, increasing numbers of malignant cells could be detected from 4 months after initial treatment and at least 9 months before relapse. In the other 2 patients which seem to be cured no leukemic cells could be demonstrated from 6 months till 5 years after initial treatment. The results of a retrospective study will be presented.

Late Abstracts

The t(15;17) translocation of acute promyelocytic leukemia generates a structuraly altered retinoic acid receptor. H de Thé, C. Lavau, C. Chomienne*, A. Marchio, L. Degos*, A. Dejean, UREG, Institut Pasteur and *Hopital St Louis, Paris, France.

A specific translocation t(15,17) has been reported in every patient with acute promyelocytic leukemia (APL). We have previously reported that, in an APL-derived cell-line (NB4), this translocation fused the retinoic acid receptor α (RAR α) gene to a previously unknown locus: *myl* (Nature, 347,558-56, 1990). Moreover, genomic alterations of either RAR α or *myl* loci were demonstrated in most patients, suggesting that the fusion of these two genes might be a general situation in APL.

We now report the molecular cloning of the wild type *myl* and *myl*/RAR α transcripts. No striking homology was found between the *myl* gene product and any known protein. Two hybrid cDNAs, that differ by an alternatively spliced coding exon of *myl*, were isolated from the NB4 cell-line and shown to encode proteins containing the 500 first AA of *myl* fused to the B to E domains of RAR α. This chimeric RAR, which retains the DNA- and hormone- binding domains, could be biologicaly active. A functional analysis of these mutant receptors will be presented.

ACUTE PROMYELOCYTIC LEUKEMIA (APL) CHROMOSOME 17 TRANSLOCATION BREAK-POINTS CLUSTER WITHIN RARÁ INTRON I. Myriam Alcalay*, Pier Paolo Pandolfi*,Daniela Zangrilli&, Amedea Mencarelli*, Andrea Biondi+, Alessandro Rambaldi^,Vincenzo Rossi+,Francesco LoCoco@,Daniela Diverio@, Fausto Grignani* and PierGiuseppe Pelicci*, * Istituto Clinica MedicaI,Perugia University, 06100 Perugia; &Dipartimento Medicina Interna,Rome University,00100 Rome;+Clinica Pediatrica,Milan University,20052 Monza,^Divisione Ematologia, 24100 Bergamo; @Dipartimento Biopatologia,Rome University 00161 Rome, Italy. APLs are characterized by a translocation that involves chromosomes 15 and 17.We demostrated that the chromosome 17 breakpoint lies within the RARÁ locus.Nucleotide sequencing of the 15;17 junction showed that it occurred at the 3' end of the RARÁ intronI,370 bp upstream from the splicing donor site of exon II.In such a recombination the translocated RARÁ gene would not be expected to generate a normal mRNA and the finding of aberrant RARÁ mRNA transcripts in all APLs analysed supported this prediction.Isolation of cDNAs representative of the APL RARÁ aberrant transcripts from one case showed that they resulted from the synthesis of a fusion mRNA composed of chromosome 15 sequences (myl gene) and a RARÁ segment,from exon II to the 3' end.The translocated RARÁ gene could still contain the exons encoding the DNA and retinol binding domains. Southern blot analysis of 26 APL cases and cDNA cloning from 3 showed that the chromosome 17 breakpoint is consistently located within the RARÁ intron I and that the translocated RARÁ gene is always transcribed as part of a myl/RARÁ transcript.

RECRUITMENT OF LEUKEMIA TO CYTOTOXICITY WITH BIOMODULATION COMBINED WITH TIMED SEQUENTIAL THERAPY, P.J.Burke,P.D.Nicholls, K.Cowan,L.Morrall,E.M.Bonnem,K.Hall,The Johns Hopkins Oncology Center, Baltimore,MD 21205

Pharmacologic amounts of granulocytic-macrophage colong stimulating factor (hGM-CSF)given throughout therapy were added to a sequence of drugs timed to coincide with tumor growth recruited by host stimulating factor (HSA). To define an effective dose of GM-CSF, 10 patients with relapsed AML received 1 course of induction therapy and 1 course in remission with GM-CSF at either 1(4),5(5) or 10(1) ug/kgm/d given by continuous infusion beginning 3 d prior to TST with AC-D-VP16 and continuing through d 13. Measurements of changes in bone marrow morphology,growth by flow cytometry, and serum activities by DNA synthesis assays made during the initial period defined a doubling of normal and leukemic cells,serum stimulation and leukemic cell "S" phase.Prior to VP16 on d 11, the serum stimulating activity doubled,reflecting the additive effect of GM-CSF and HSA,as predicted in in-vitro pretreatment assays.Similar results were obtained with assays using remission bone marrow.Toxicities included pericarditis (1,d13) and DIC(1,d3).There was no evidence of prolongation of bone marrow aplasia or uncontrolled recruitment of leukemia.Pharyngitis was similar in intensity to controls.The dose of 5 ug/kgm/d for 13 d seems reasonable and effective.

EPIDEMIOLOGICAL STUDIES OF RADIATION-INDUCED LEUKEMIA, John D. Boice, Jr., Radiation Epidemiology Branch, National Cancer Institute, Bethesda, Maryland 20892. Leukemia is the most commonly identified malignancy following irradiation, probably because of its short appearance time, relatively low natural incidence, and high radiation sensitivity. However, not all types of leukemia are radiation-induced and chronic lymphocytic leukemia has never been found excessive in exposed populations.

Only a few studies provide dose-response information, specifically atomic bomb survivors, and patients treated for spondylitis, cervical cancer, and benign gynecologic disorders. Other populations showing increased rates of leukemia include patients irradiated for breast cancer, endometrial cancer and tinea capitis; patients given Thorotrast; children prenatally exposed to x-rays; USA and Chinese x-ray workers. Conversely, little or no excess has been noted following radiotherapy for childhood cancers, multiple chest fluoroscopies during lung collapse for TB, general diagnostic x-ray exposure, I-131 treatment for hyperthyroidism, radium dial painters, underground miners exposed to radon, UK radiologists, and x-ray technologists. Enhanced environmental radiation from nuclear weapons testing, nuclear facility operations, and natural background has been suggested as a leukemogenic factor.

NCI studies will be discussed in detail. The quantitative description of risk continues to present unique opportunities for research that may lead to a better understanding of the pathogenesis of leukemia in man, with implications to public health and standard setting.