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Robert Peter Gale and David W. Golde, Organizers

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Keynote Address

0176 THE MOLECULAR BASIS OF CANCER, David Baltimore, Whitehead Institute for Biomedical Resesarch, Nine Cambridge Center, Cambridge, MA 02142

The discovery that oncogene activation underlies much of human and animal cancer has raised a myriad of focused questions in a field where the opportunity for focused thinking previously did not exist. We now wish to know which oncogenes are active in which tumors, how they got activated, and what role each oncogene plays in the overall malignant phenotype. These are not easy questions and may not lend themselves to easy generalizations. It is likely that any one tumor type can be produced by many subsets of the constellation of potentially active oncogenes. In fact, the subtle differences between manifestations of the apparently same tumor may have their origin in the specific set of oncogenes active in those tumor cells. Malignancy may ultimately be categorized both by cell type and by active oncogene. To utilize such information we must have an explicit idea of how each oncogene acts and what are its effects on cells. Our laboratory has been concentrating its attention on a single oncogene, that of the Abelson murine leukemia virus. This virus carries a protein-tyrosine kinase activity as part of its oncogene product and therefore might appear to be interchangeable with the many other oncogenes that encode such an activity. We have found, however, that this protein-tyrosine kinase has specific properties both in the range of cells it will transform and in the biochemical parameters of its kinase. Thus, even within a category of oncogenes, the specific gene may have specific transformation properties.

Oncogenes

0177 ras ONCOGENES AND THE INITIATION OF CARCINOGENESIS, Mariano Barbacid, Saraswati Sukumar, Helmut Zarbl, Anne Arthur, and Dionisio Martin-Zanca, Developmental Oncology Section, NCI-Frederick Cancer Research Facility, P.O. Box B, Frederick, Maryland 21701

We have utilized animal tumor model systems to investigate the role of ras oncogenes in tumor development. Administration of a single dose of carcinogen to female rats during their sexual development led to the efficient and reproducible induction of mammary carcinomas. DNAs isolated from these tumors were tested in gene transfer assays in order to identify oncogenes capable of transforming NIH/3T3 cells. To date, transforming genes have been detected in fifty-one of seventy-two tumor DNAs tested. In each case, these transforming genes were identified as the H-ras oncogene. The frequency of H-ras activation appears to be determined by the type of carcinogen utilized. H-ras oncogenes were present in 83% of the mammary carcinomas induced by nitroso-methyl-urea (NMU), but only in 21% of tumors induced by 7-12-dimethylbenz(a)anthracene (DMBA). The reason for this difference is, as yet, unknown. Interestingly, Gullino and co-workers have shown that the majority of the NMU-induced tumors are hormone independent. In contrast, only a small fraction of those induced by DMBA will reach full neoplastic growth if the rats are ovariectomized during tumor development. Thus, it is possible that oncogene activation may influence the need of hormonal stimulus during tumor progression.

NMU is a direct acting alkylating agent that preferentially methylates the N^7 and the O^6 positions of deoxyguanosine residues. Whereas N^7 methylations induce repair systems, O^6Me-deoxyguanosine can be efficiently utilized as a template. However, O^6Me-deoxyguanosines direct the incorporation of thymidine instead of deoxycytosine residues leading to the creation of G → A missense mutations, unless methylation is repaired before DNA synthesis. The nature of the mutations responsible for the malignant activation of the H-ras locus in this animal model system has been investigated. Thirty-six NMU-induced tumors known to contain activated H-ras oncogenes were examined. Each H-ras oncogene exhibited the same activating mutation, a G → A transition in the second deoxyguanosine residue of its twelfth codon. In contrast, none of the three H-ras oncogenes identified in DMBA-induced tumors contained any mutations affecting this critical codon. The striking specificity of these findings strongly suggests that the G → A mutations responsible for the malignant activation of the H-ras locus in these tumors are the direct consequence of the mutagenic activity of NMU. Considering that these mammary carcinomas were induced by a single injection of NMU, a carcinogen of very short half life, the above results imply that malignant activation of H-ras oncogenes is concomitant with, and presumably responsible for, the onset of carcinogenesis in this animal model system.
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0178 MOLECULAR GENETICS OF B CELL NEOPLASMS OF ADULTS, Carlo M. Croce, The Wistar Institute, 36th and Spruce Street, Philadelphia, PA 19104

We have used recombinant DNA technologies to clone the chromosomal joinings between human chromosome 11 and 14 and between chromosomes 14 and 18 which are involved in chronic lymphocytic leukemia, diffuse B cell lymphoma, multiple myeloma and follicular lymphoma in man. Two different loci, residing on band 11q13 and 13q21 have been identified. These loci are involved in the pathogenesis of the majority of human B cell neoplasms.

Retroviruses, Oncogenes, Chromosomes and Leukemia

0179 AMPLIFIED ONCOGENES AND MYC PROTEIN IN TUMOR CELLS

Kari Alitalo, Kalle Saksela and Robert Winqvist: Department of Virology and Recombinant DNA Laboratory, University of Helsinki, Haartmaninkatu 3, 00290 Helsinki 29, FINLAND We have discovered several cases of oncogene amplification in human tumor cell lines and human tumors. The c-myb gene is expressed only in immature hematopoietic cells and in the COLO 201/205 cells where it is amplified. The amplified copies of c-myb reside in two marker chromosomes that may have evolved from chromosome 6 by complex chromosomal rearrangements. This suggests that c-myb was amplified in situ in a segment that became translocated to the marker chromosome without the extrachromosomal intermediate form of double minute chromosomes. There is an enhanced frequency of sister chromatid exchanges at the site of c-myb amplification. The c-myc oncogene is amplified in sporadic cases of colon carcinoma and in several human small-cell lung cancers. The amplified copies of c-myc often reside in double minute chromosomes or in homogeneously staining chromosomal regions of marker chromosomes. The amplification of c-myc is accompanied by adundant exression of c-myc RNA and protein. The protein products of cellular and viral myc oncogenes are located in nuclei by immunofluorescence. No myc fluorescence is found in nucleoli. In mitotic cells, the myc antigens are not found associated with metaphase chromosomes, but are diffusely distributed throughout the cytoplasm. Cytoplasmic myc fluorescence is first observed when chromatin begins to condense in early pophase. Granular nuclear myc fluorescence is again discerned in telophase cells, when the nuclear envelope is formed and becomes more prominenet upon cytokinesis, when the diffuse cytoplasmic myc staining is lost.

0180 LEUKEMIA INDUCTION BY A TYPE-B RETROVIRUS, Larry O. Arthur, Judith K. Ball, and Gregory A. Dekaban, (PRI) NCI-Frederick Cancer Research Facility, Frederick, MD 21701 and University of Western Ontario, London, Ontario, CA N6A 51

A highly leukemogenic Type-B retrovirus was isolated from a DMBA-induced thymic lymphoma of CFW/D mice. The biological behavior of this Type-B retrovirus differs markedly from that of Type-B retroviruses that induce mammary tumors. This leukemogenic virus induces thymic lymphomas in 100% of intrathymically inoculated newborn mice within 35 to 40 days post injection. Tumor induction is completely and specifically inhibited by monoclonal antibodies prepared against the major envelope protein of C3H-mouse mammary tumor virus (MMTV) at dilutions of greater than 1:1000. All thymic lymphomas induced by this virus have newly integrated Type-B proviral sequences integrated in an apparent nonrandom fashion. Site of inoculation was not the determining factor in type of tumor induced by this Type-B virus because 100% of the female mice inoculated intrathymically with C3H- and C3Hf-MMTV developed mammary tumors 4 to 12 months post injection with no lymphomas. This leukemogenic Type-B virus is antigenically and genetically distinct from MMTV isolated from C3H, GR, C3Hf, and RIII mice as revealed by binding of monoclonal antibodies to the major envelope protein, peptide maps of the major internal protein, and restriction endonuclease analysis. Low levels of Type-B endogenous sequences in the mouse genome make this lymphotropic Type-B retrovirus an excellent model for studying genetic interaction in the induction of leukemias by retroviruses.

0181 C-ABL AND BCR REARRANGEMENTS IN PH[1]-NEGATIVE CML, Claus R. Bartram[1], Annelies de Klein[2], Gerard Grosveld[2]; University Childrens Hospital, D-7900 Ulm, W.Germany[1], Department of Cell Biology and Genetics, Erasmus University Rotterdam, The Netherlands 2.

Chromosomal analysis of a patient with chronic myelocytic leukemia (CML) revealed a translocation (9;12) without a detectable Philadelphia chromosome (Ph^1). Using molecular approaches we demonstrate

1) a rearrangement within the CML breakpoint cluster region (bcr) on chromosome 22 and

2) a joint translocation of bcr and c-abl oncogene sequences to the derivative chromosome 12.

These observations support the view that sequences residing on both, chromosome 9 (c-abl) and 22 (bcr), are involved in the generation of CML and suggest that a subset of Ph^1-negative patients may in fact belong to the clinical entity of Ph^1-positive CML associated with a better prognosis. Detection of c-abl/bcr rearrangements thus seems to be of value in the classification of human leukemias.

0182 SIZE ANALYSIS OF SENDAI VIRUS PROTEIN VESICLES CONTAINING EPSTEIN-BARR VIRUS DNA Ronald Bartzatt *, Charles Kuszynski, Pathology, UNMC, Omaha, Ne. 68105. * U.C.S.D. Cancer Center T-011, La Jolla, Ca. 92093.
Gene transfer by reconstituted Sendai Virus envelopes (RSVE) is a well known procedure of studying carcinogenesis of Epstein-Barr Virus. Previous studies showed that the internal volume (represented as the percent of total volume) of vesicles correlated with the amount of protein present. Volumes of 2.1 %, 4.2 %, and 17.6 % correlated with 0.7, 1.4, and 5.6 mg protein/ml respectively. Now its found that a size distribution of RSVE exists. The amount of solubilized Sendai Virus protein used in this study is what we consider a standard system, that is 1.4 mg protein/ml. The majority of entrapped DNA is contained within vesicles with a diameter in the range of 0.1 micron to 0.35 micron. The vesicles can be fractionated by size on a Sephacryl 1000 column of dimensions 0.9 X 25 cm and flow rate 0.33 ml/minute. The column is calibrated with monodispersed polystyrene beads from Polysciences with UV detection. By using P^{32} labeled DNA we found that the DNA is entrapped in quantities roughly gaussian in distribution by vesicles falling within the size range previously described. In addition, with protein content held constant (1.4 mg protein/ml) the amount of DNA entrapped increases as the amount of DNA per solubilization increases up to 20 microgram total DNA/solubilization. Adding a greater amount of DNA after this point saturates the solubilization system and lower entrapment results. These results suggest that more efficient gene transfer by RSVE can be obtained by using proper experimental parameters.

0183 MOUSE FIBROBLASTS EXPRESSING A NEGATIVE-SENSE v-abl MESSAGE ARE PROTECTED FROM A-MuLV TRANSFORMATION. Yinon Ben-Neriah and David Baltimore. Whitehead Institute, Cambridge, MA 02142

Abelson murine leukemia virus transforms murine fibroblasts and lymphoid cells in vitro. We have established NIH 3T3 fibroblast cell lines which express v-abl antisense mRNA by DNA transfection and selection for the antisense mRNA expression. Cells were cotransfected with the plasmid pSV2-Neo and with a plasmid containing the V-Abl gene coding for P100 protein protein in reverse orientation to a MuLV LTR. NIH 3T3 cell clones selected for G418 (Neo) resistance were screened for expression of v-abl, mRNA and 3 clones expressing high levels were selected for further studies. The three cell clones were exposed to infection with various transforming retroviruses in vitro and were found to be highly resistant specifically to A-MuLV transformation. The antisense expressing cells were 100-1000 fold more resistant than normal NIH 3T3 or NIH cells transfected with a control plasmid. We have found no significant protection of these cells against other transforming viruses like Harvey-Murine Sarcoma virus and Moloney Sarcoma virus. We are currently investigating the mechanism for the transformation protection effect.

0184 MODULATION OF HTLV-I EXPRESSION BY SELECT VITAMINS. James R. Blakeslee, The Ohio State University, Columbus, Ohio, N. Yamamoto and Y. Hinuma, Institute for Virus Research Kyoto University, Kyoto, Japan.

Human T-Cell Leukemia Virus Type I (HTLV-1) was induced by MNNG and IUdR in the MT-1 cell line. Virus expression was monitored by immunofluorescence microscopy with GIN-14, mouse monoclonal antibodies directed toward p19 and p24-specific virus polypeptides. MNNG (0.1 μg/ml) and IUdR (50 μg/ml) both induced virus synthesis in MT-1 cells. MNNG-induced virus expression peaked between 24 and 48 hr, whereas IUdR induced maximum virus expression between 48 and 72 hr incubation. Superinduction resulted when MNNG was added to cells induced 48 hr previously ith IUdR, but not with concomitant treatment. Thirteen-cis retinoic acid, retinol, retinol aldehyde and retinol acetate (10^{-6} M-10^{9}M) were concomitantly added with IUdR to MT-1 cells for 24, 48 and 72 hr incubation. All inhibited virus induction to various degrees. The retinoids were ranked as to inhibitory activity: Retinol>retinoic acid>retinol aldehyde>retinol acetate. The most sensitive period for inhibiting IUdR induction by retinoic acid was 24 hr post-induction or with concomitant treatment. Vitamin C and vitamin E inhibited IUdR induction most effectively with 48 hr incubation. Retinol and vitamin C also inhibited virus induction by MNNG. None of the retinoids, vitamin C or E significantly inhibited virus expression in non-induced cells, nor were toxic to the cells at the concentrations used in these experiments.

0185 IN VITRO AND IN VIVO EFFECTS OF A MuLV RECOMBINANT CONTAINING V-MYC, B. Kay Brightman and Hung Fan, University of California, Irvine, California 92714

A recombinant retroviral genome containing the v-myc gene from myelocytomatosis virus 29 inserted into the genome of Moloney murine leukemia virus was constructed by molecular cloning. The recombinant clone, pM-MuLV(myc), was designed to encode a gag-myc fusion protein. pM-MuLV(myc) DNA was introduced into NIH-3T3 cells by contransfer with a dominant selectable drug-resistance plasmid. pM-MuLV(myc)-containing cell colonies (Mo-myc) had a morphology similar to parental NIH-3T3 cells. However, infection of Mo-myc cells with amphotropic or Moloney murine leukemia virus (Am-MuLV or Mo-MuLV) resulted in morphological transformation and formation of foci of densely packed, highly refractile cells overgrowing the monolayer. Virus rescued by Am-MuLV superinfection of Mo-myc cells transformed and immortalized fibroblasts and macrophages upon in vitro infection of mouse embryo cell cultures, parallel to the effects of MC29 in chick embryo cells. However, inoculation of neonatal NIH-Swiss mice with M-MuLV(myc)/Am-MuLV resulted in rapid development (6-12 weeks) of lymphoblastic leukemia instead of myeloid disease. Previous reports indicate that Am-MuLV alone causes lymphoblastic leukemia in inoculated mice at six to twelve months (Rasheed, S., et al., Virology 130, 439-451 (1983)). Thus, while M-MuLV(myc) demonstrated capacity to transform fibroblasts and myeloid cells in vitro, it appeared to accelerate the weak leukemogenic potential of the helper MuLV in vivo.

0186 ISOLATION AND CHROMOSOMAL LOCALIZATION OF HUMAN CELLULAR REL SEQUENCES, Elise Brownell[1], Stephen O'Brien[2], William Nash[2], and Nancy Rice[1], [1]Laboratory of Molecular Virology and Carcinogenesis, LBI-Basic Research Program, NCI-Frederick Cancer Research Facility, Frederick MD 21701, [2]Section of Genetics, NCI, Frederick, MD 21701

Reticuloendotheliosis virus (strain T) (REV-T) is a replication-defective, acutely transforming type C retrovirus that induces leukemia in young turkeys and chickens. REV-T carries a 1.42 kilobase (Kb) sequence, termed v-rel, that is derived from a turkey cellular genome. The presence of v-rel in REV-T is necessary for cellular transformation by this retrovirus. We used a subcloned portion of v-rel to screen a human genomic DNA library. We have characterized a portion of one recombinant clone that is highly related to v-rel on both the nucleotide and predicted amino acid sequence levels. In addition, we used rodent x human somatic cell hybrid lines to localize these cellular rel sequences to human chromosome 2. (Supported by NCI Contract NO1-CO-23909)

0187 EXPRESSION OF HUMAN T CELL LEUKEMIA VIRUS TYPE III (HTLV-III) env AND LOR REGION IN E. coli, Nancy T. Chang, Pranab Chanda, James Huang, John Ghrayeb and Dale Barone, Centocor, Inc., Malvern, PA 19355

Human T Cell Leukemia Virus, type III (HLTV-III) has been identified as the causative agent of Acquired Immunodeficiency Syndrome (AIDS). The genome of HTLV-III has recently been cloned and was found to be approximately 10 Kb in length. DNA sequencing analysis of HTLV-III genome has just been completed. Several open reading frames were identified encoding the viral gag, polymerase and envelope proteins. Similar to HTLV-I and HTLV-II an extra long open reading frame (LOR) was identified at the 3' terminus of the HTLV-III genome. The DNA sequences coding for HTLV-III envelope protein and the LOR region was placed under the control of a lactose promoter. E. coli transformants containing these plasmid expressed HTLV-III antigens are immunologically reactive with the AIDS patients serum and not with normal human serum. Thus, these bacterial expressed HTLV-III polypeptides are recognized by antibodies specific to HTLV-III present in the AIDS patient blood. Experiments are in progress to inject these bacterial expressed polypeptides into laboratory animals for generating neutralizing antibodies for HTLV-III and also to develop valuable immunodiagnostic or therapeutic reagents using the bacterial expressed proteins for diagnosis and treatment of the infectious AIDS.

0188 A TEMPERATURE-SENSITIVE MUTANT OF AVIAN ERYTHROBLASTOSIS VIRUS AS AN ERYTHROID-SPECIFIC CLONING VECTOR, Ok-Ryun Choi, Cecelia Trainor and James Douglas Engel, Northwestern University, Evanston, IL 60201.

In order to study the expression of modified chicken red cell genes we have developed an avian retrovirus vector. The provirus loci of Avian Erythroblastosis Virus (AEV) have been isolated from a genomic recombinant DNA library of HD6 cells (a producer cell line containing three integrated copies of a temperature-sensitive transforming allele [ts 167] of AEV; Beug et al. Cell, 28:907, 1982). The theoretical advantage of ts AEV over that of wild type AEV is that we may be able to generate transformed early erythroid lineage cells whose differentiation block by the viral onc gene is released synchronously by thermal induction. Since the v-erbB gene product has been shown to be the transforming agent of AEV, we can insert modified cellular genes into the v-erbA locus without alteration of the transforming ability of AEV. The chicken-erythroid specific histone H5 gene has been chosen as a test gene, and a series of mutants of H5 have been generated.

We have sequenced the erbB gene from the ts 167 allele of AEV in order to identify the temperature sensitive lesion. This ts allele has two deduced amino acid substitutions relative to the wild type gene; we are currently constructing substitution mutants with the wild type v-erbB gene to determine which change confers temperature sensitivity in the ts allele. Such an analysis may yield important structural information about the physical properties of the cellular homolog of v-erbB (the epidermal growth factor receptor).

0189 Expression and Biological activity of the human myc gene in rat embryo fibroblasts, François DAUTRY and Nathalie NICOLAIEW, Institut Gustave Roussy, 94805 VILLEJUIF.

As measured by low density cloning and long term culture, a normal myc gene can stimulate growth of rat primary fibroblasts. However, replacement of the normal promotor by an heterologous promotor (e.g. mouse metallothionein promotor) gives a large increase in mRNA accumulation and a 5-15 fold enhancement of biological activity. This indicates a tight regulation of the normal myc promotor. We are currently investigating the role of the level of expression for cellular proliferation, using the metallothionein promotor.

0190 HUMAN T-CELL LEUKEMIA VIRUS TYPE II: PRIMARY STRUCTURE ANALYSIS OF THE MAJOR INTERNAL PROTEIN, p24, AND THE NUCLEIC ACID BINDING PROTEIN, p15, S. G. Devare, Y. Kim, J. L. Fox, J. Getchell*, C. Cabradilla* and V. S. Kalyanaraman*. Department of Molecular Biology, Abbott Laboratories, Abbott Park, North Chicago, Illinois 60064 and *Molecular Virology Branch, Centers for Disease Control, Atlanta, Georgia 30333.
The 24,000 molecular weight major internal protein (p24) and the 15,000 molecular weight nucleic acid binding protein, p15 of human T-cell leukemia virus type II (HTLV-II) were subjected to amino acid composition and amino-terminal amino acid sequence analysis. A comparison of amino aicd composition of p24 and p15 of HTLV-II with those of the analogous proteins of HTLV-I revealed that these two proteins share overall similarity. Further, alignment of amino-terminal amino acid sequence of the first 27 residues of p24 and 34 residues of p15 from HTLV-II showed extensive sequence homology with analogous proteins of HTLV-I. These data suggest that though disease associated with HTLV-I is malignant T-cell leukemia whereas that associated with HTLV-II is a relatively benign variant of hairy-cell leukemia, HTLV-I and HTLV-II are very closely related to each other

0191 THE HUMAN METALLOTHIONEIN (MT) GENES ARE INVOLVED IN REARRANGEMENTS OF CHROMOSOME No.16 IN ACUTE MYELOMONOCYTIC LEUKEMIA (AMMoL). Manuel O.Diaz, Michelle M.Le Beau, Heidi Holtgreve,* Michael Karin*and Janet D.Rowley. The University of Chicago, Chicago, IL 60637 and*The University of Southern California, Los Angeles, CA 90033.

The malignant hematologic diseases are characterized by nonrandom chromosomal abnormalities which are often associated with specific morphologic and clinical subsets. The inv(16)(p13 q22) is present in 25% of AMMoL patients. The inv(16) as well as the t(16;16)(p13;q22) and a del(16)(q22) are associated with unique morphologic and cytochemical abnormalities of marrow eosinophils. Using in situ chromosomal hybridization, we have regionally localized the MT genes to chromosome No.16 at band q22. In cells from two AMMoL patients with an inv (16) this same probe, which hybridizes to all the MT genes, shows labelling of equal intensity on both arms of the inv(16), indicating that the break at 16q22 splits the MT gene cluster. In cells from two AMMoL patients with a t(16;16) labelling was observed on both No.16 homologs, clustered around the translocation junctions. These results indicate that in the t(16;16) the MT cluster is also split by the break at 16q22. Southern blot analysis of DNA samples prepared from leukemic cells of four patients with a inv(16) and one patient with a t(16;16), revealed new unique restriction fragments hybridizing to the MT probe. Although the particular MT genes adjacent to the breakpoint have not yet been identified, these results suggest that the MT genes or their regulatory regions may play a role in the development of AMMoL with abnormal eosinophils by functioning as an "activating" sequence for an as yet unidentified cellular gene located at 16p13 analogous to an oncogene.

0192 ANALYSIS OF THE ACUTE MYELOGENOUS LEUKEMIA (AML) 8;21 TRANSLOCATION, Harry A. Drabkin[1,2], Manuel Diaz[3], Cynthia M. Bradley[1], Michelle M. Le Beau[3], Janet D. Rowley[3], Jose Trujillo[4], Ann Cork[4], and David Patterson[1,2], [1]The Eleanor Roosevelt Institute for Cancer Research and [2]The University of Colorado Health Sciences Center, Denver CO; [3]The University of Chicago, Chicago IL; [4]M.D. Anderson Hospital, Houston TX

The 8;21 translocation, t(8;21)(q22.1;q22.3), is specific for the M2 subtype of AML. We have isolated the 21q+ chromosome as the only identifiable human material in a somatic cell hybrid. As expected, c-myc (8q24) is present in the 21q+. However, c-mos (8q22), located near the site of rearrangement on no. 8, is not translocated. A restriction enzyme analysis of the original t(8;21) leukemic cell DNA probed with c-mos demonstrated no rearrangements in a 12.4 kb region, suggesting that the breakpoint must occur outside this region. A similar analysis of the c-mos region in 3 additional patients with t(8;21) AML shows the same results. The 21q+ hybrid has been useful in mapping several chromosome 21 specific DNA sequences. We have identified a sequence, CP21G1, located in region 21q22.3→qter that is translocated to the 8q- chromosome. Importantly, persons with Down Syndrome (trisomy 21) have a markedly higher incidence of acute leukemia and the smallest region of chromosome 21, when trisomic, produces the Down phenotype 21q22. We propose that genes present on chromosome 21 may play a role in the etiology of the t(8;21) AML. If c-mos is important in this disease, then our evidence suggests that some mechanism such as an enhancer acting at a distance may be involved.

0193 MURINE RETROVIRUSES CONTROL EXPRESSION OF CLASS I MHC ANTIGENS, Douglas V. Faller, David C. Flyer, and Steven J. Burakoff, Harvard Medical School, Boston, MA. 02115
Recognition and killing of cells infected with murine leukemia viruses by virus-specific or tumor-specific cytotoxic T lymphocytes (CTL) require that both retroviral antigens and class I major histocompatability (MHC) antigens be present on the surface of the infected cell. Using mouse cells which have been transfected with isolated single retroviral genes and which are expressing single retroviral proteins, we have determined that the viral envelope glycoprotein (gp70) is the dominant antigen recognized by virus-specific or tumor-specific CTL. Core viral antigens expressed on the surface of infected or transfected cells do not appear to play a major role in the cellular immune response. High levels of murine MHC gene products are required for efficient CTL recognition of infected cells. We demonstrate that retroviral infection of mouse cells induces a rapid and marked increase in cell surface levels of class I MHC gene products (H-2K, D and L) as shown by fluorescent antibody labeling and by susceptibility to lysis by allogeneic CTL. This induction of MHC protein expression is mediated at the genomic level, because higher levels of H-2 mRNA and B-2 microglobulin mRNA are found in infected cells. Under conditions which prevent this increase in MHC gene expression, CTL recognition and killing of infected cells is severely impaired. Transformation of a leukemia virus-infected cell line with a murine sarcoma virus abolishes the leukemia virus-induced increase in MHC expression. Such transformed cells are recognized poorly or not at all by virus-specific or tumor-specific CTL.

0194 GENETIC DETERMINANTS OF PATHOGENICITY IN AKR MCF MuLV: ROLE OF p15(E). Nancy G. Famulari[1], Christie A. Holland[2], Nancy H. Hopkins[2] and Paul V. O'Donnell[1],[1]Memorial Sloan-Kettering Cancer Center, New York, NY 10021, [2]Center for Cancer Research and Department of Biology, MIT, Boston, MA.
One region of the viral genome which distinguishes nonpathogenic, endogenous ecotropic MuLV of AKR mice from pathogenic, MCF MuLV of that strain is the carboxy terminal coding region of p15(E). To determine whether p15(E) sequences contribute to the pathogenic phenotype of MCF MuLV, viruses were constructed which contain p15(E) sequences from ecotropic virus. These constructs displayed diminished pathogenicity when assayed in AKR mice. A detailed analysis of their replication in thymus demonstrated that the p15(E) construct spread through the thymocyte population with a slower kinetics than that of its wild type counterpart, but eventually infected all cells. The rate of synthesis of the virus PrENV protein was equivalent in thymocytes infected by the construct or by wild type MCF virus. However, thymocytes infected by the p15(E) construct exhibited approximately 10-fold lower gp70 on their cell surface than did wild type infected cells. These results suggest a block in intracellular processing of PrENV protein encoded by the p15(E) construct. Comparison of env gene expression of the construct with its wild type counterpart indicated that this processing defect occurs in fibroblasts, but is less pronounced than in thymocytes. The leukemias that do arise in mice infected by the p15(E) construct express gp70 on their cell surface at wild type levels, a result which suggests that regeneration of the wild type virus in vivo may be required for transformation to occur.

0195

Effect of enhancer alterations on murine leukemia virus pathogenicity. Hung Fan and Brian Davis. Department of Molecular Biology and Biochemistry, University of California, Irvine, Ca. 92717. Two variants of Moloney murine leukemia virus (M-MuLV) were generated by molecular cloning. Transcriptional enhancers from polyoma virus (the F101 mutant) were inserted into the wild-type M-MuLV LTR, or into an M-MuLV LTR lacking the M-MuLV enhancers. Both variants grew well in NIH-3T3 fibroblasts, although with a slightly decreased infectivity-to-particle ratio. However, when inoculated into newborn NIH Swiss mice, the variant viruses were completely non-leukemogenic; in contrast, animals inoculated with wild-type M-MuLV developed typical thymic leukemia in 3-4 months. The variant viruses could be recovered from inoculated animals even months after injection, indicating that they could establish infection. This suggests that insertion of the polyoma enhancers into the M-MuLV LTR altered the ability of the virus to replicate in a tissue or cell type necessary for leukemogenesis.

0196 BIOLOGICAL ACTIVITY OF A RETROVIRUS CARRYING AN ACTIVATED N-ras GENE OF HUMAN ORIGIN, Erwin Fleissner and Michèle Souyri, Memorial Sloan-Kettering Cancer Center, New York, NY 10021

Activated forms of the N-ras oncogene have been isolated from a variety of neoplasms, particularly of the hemopoietic system. We have reported N-ras oncogenes active in transfection analysis from human T-cell leukemias. To explore the oncogenic potential of this gene we constructed a retroviral vector in which a human leukemia-derived N-ras sequence is transcribed from a Moloney leukemia virus LTR and can be packaged as a retroviral particle. This construction was used to infect newborn mice, as well as mouse bone marrow and spleen cells cultured under various conditions. We found that, in distinction to the H-ras and K-ras members of the ras gene family, N-ras does not markedly stimulate proliferation of erythroid cell precursors in vivo or in vitro. The N-ras virus did stimulate limited, factor-independent growth of granulocyte-macrophage precursors. Investigations of the effect of this virus on lymphoid cells are in progress.

0197 DISTRIBUTION OF PH' BREAKPOINTS IN THE BCR REGION ON CHROMOSOME 22, Nora Heisterkamp[1], John Groffen[1], Annelies de Klein[2] and Gerard Grosveld[2]. [1]Oncogene Science Inc., Mineola, New York; [2]Erasmus University, Rotterdam, The Netherlands.

Chronic myelocytic leukemia (CML) is a pluripotent stem cell disease characterized by the presence of the Philadelphia chromosome (Ph'). The latter chromosome consist of chromosome 22 sequences and a region of chromosome 9 encompassing the c-abl oncogene. We have previously demonstrated that on chromosome 22 the breakpoint in Ph' positive CML occurs in region of up to 5.8 kb, the breakpoint cluster region (bcr). Bcr is part of a protein-encoding region; by restriction enzyme and hybridization analysis we have determined the exon-intron organization of this gene on chromosome 22, thus enabling to redefine the breakpoints within bcr of a number of previously described CML patients. This information, in combination with data derived from the studies of a number of additional CML patients, contribute to elucidate the role of bcr in CML on a molecular level.

0198 CHROMOSOME ABERRATIONS AND GENE AMPLIFICATION INDUCED BY HYDROXYUREA IN MOUSE LYMPHOMA CELLS. Anna B. Hill and Robert T. Schimke, Biological Sciences, Stanford, CA. 94305. L5178Y mouse lymphoma cells were pretreated with hydroxyurea (HU) and effects on chromosome rearrangements and gene amplification were studied. DNA synthesis was blocked with either 1mM or 10mM HU for 6 hours, HU was removed and cells were placed into medium containing bromodeoxyuridine. HU induced a 2 to 4 fold increase in sister chromatid exchanges (SCEs); however, the increase in SCEs was transient. If bromodeoxyuridine was added 6 hours after the removal of HU, the number of SCEs was the same as control cells. 25% of all metaphases observed after the HU treatments had one or more chromosome abnormalities, including endoreduplication, polyploidy, fragmented chromosomes and the presence of extrachromosomal DNA. Control and HU treated cells formed colonies in soft agar; when cells were selected in methotrexate (MTX), a 5 to 8 fold increase in the number of MTX resistant colonies was induced by the HU pretreatment. Many HU pretreated cells had amplified the dihydrofolate reductase (DHFR) gene. The fluorescence-activated cell sorter was used to analyze cells for DNA content and DHFR content. 24 hours after HU was removed, a subpopulation emerged from the HU treated cells which was larger than control cells, contained increased DHFR and increased DNA content per cell. This aberrant subpopulation was transient; it was 72 hours after the HU was removed. These abnormal cells had an increased frequency of resistance to MTX compared to control cells and had a large number of chromosome aberrations. We propose that transient inhibition of DNA synthesis causes overreplication of DNA in the recovery period which leads to chromosome aberrations and an increased frequency of MTX resistance.

0199 VARIANT CHROMOSOMAL TRANSLOCATIONS IN BURKITT LYMPHOMA CAN OCCUR 3' TO THE C-MYC ONCOGENE AND ALTER ITS TRANSCRIPTION, Gregory F. Hollis, Christopher Denny, Ian Magrath, J. Whang-Peng, and Ilan R. Kirsch, NCI-Navy Medical Oncology Branch, National Naval Medical Center, Bethesda MD 20814

Burkitt lymphoma cells have characteristic translocations involving chromosome 8 and one of the immunoglobulin gene containing chromosomes 2, 14, and 22. Lenoir described a correlation in Burkitt lymphoma between the chromosome involved in variant translocations and the immunoglobulin light chain produced, that is, cells with translocations involving chromosome 2 produced kappa immunoglobulin light chains, while those Burkitt lymphomas with chromosome 22 translocations produced lambda immunoglobulin light chains. We have described an exception to this correlation, BL 37, a kappa producing Burkitt lymphoma with an t(8;22) where the translocation breakpoint on chromosome 8 is close to the 3' end of the c-myc oncogene. We have now investigated another kappa immunoglobulin producing Burkitt lymphoma, KK, with an t(8;22). The translocation breakpoint on chromosome 8 occurs several kilobases 3' to the c-myc gene, yet the cell line produces high levels of c-myc mRNA. The translocation breakpoint on chromosome 22 appears to have occured 5' to a V-J recombined lambda constant gene. This result is surprising because in the ordered mechanism of rearrangement of human light chain genes, lambda immunoglobulin genes remain in th germline configuration in kappa producers. Our studies on these Burkitt lymphoma cell lines indicate that variant translocations may not correlate with the immunoglobulin light chain produced and that chromosomal translocations that occur 3' to genes can effect gene expression at a distance.

0200 CONFIGURATION AND EXPRESSION OF THE GENE ENCODING THE β-CHAIN OF THE T-CELL RECEPTOR IN HTLV-I INFECTED CELLS, Ruth F. Jarrett, Huroaki Mitsuya, Samuel Broder, Dimitrios T. Boumpas, Dean L. Mann, Robert C. Gallo and Marvin S. Reitz, Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, MD 20205

The configuration of the gene encoding the β-chain of the T-cell receptor (TCR) was studied in several cell lines infected by the human T-cell leukemia virus type I (HTLV-I). Infection of T-cells by HTLV-I results in immunological dysfunction including impairment of antigen recognition and loss of the requirement for antigen presenting cells. Most HTLV-I infected cell lines that were analyzed show rearrangement of the TCR gene. Infection of cloned T-cells, with known antigenic specificities, by HTLV-I *in vitro* does not appear to result in any change in the genomic organization of the TCR gene. One cloned cell line infected with HTLV-I *in vivo* express surface immunoglobulin and shows rearrangement of the TCR gene. We have also studied expression of the TCR β-chain gene by RNA dot blot and Northern blot analyses. Cell lines infected by HTLV-I express low levels of the TCR gene; expression is approximately 2-fold less than in PHA-stimulated lymphocytes and 8 to 16-fold less than in a T-cell line phenotypically similar to the HTLV-I infected cell lines examined. Furthermore, expression of this gene, at the mRNA level, decreases following *in vitro* infection by HTLV-I. This may help explain some of the changes in immunological function that follow HTLV-I infection.

0201 CHARACTERIZATION OF THE MURINE-SPECIFIC LEUKEMIA VIRUS RECEPTOR FROM L CELLS, Pegram A. Johnson and Marsha R. Rosner, Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, MA 02139

The host receptor for Moloney murine leukemia virus was solubilized from murine L cell membranes and characterized. In initial studies designed to identify a receptor-rich cell line, different mouse cells were screened for binding to Moloney gp70, the viral envelope glycoprotein which determines host cell binding specificity. Gp70 binding to murine L cells was specific and saturable with an apparent Ka of 4×10^8 M^{-1}, and the number of receptors per cell (6×10^5/cell) exceeded that of other receptor-containing cells several fold. Characterization of the gp70 receptor with regard to extractibility by detergents or chaotropic agents, protease sensitivity and heat denaturation suggests that the receptor is an intrinsic membrane protein. Upon extraction of L cell membranes with 0.2% deoxycholic acid and precipitation with acetone, specific and saturable binding of gp70 could be detected. The solubilized gp70 receptor fraction was further resolved by column chromatography.

0202 REGULATED EXPRESSION OF THE C-MYB AND C-MYC ONCOGENES DURING ERYTHROID DIFFERENTIATION Ilan R. Kirsch, Virginia Bertness, Jonathan Silver, and Gregory F. Hollis, NCI-Navy Medical Oncology Branch, National Naval Medical Center, Bethesda, MD 20814

We have investigated the expression of the genes c-myb, c-myc, and alpha globin in murine erythroid cells at different stages of development as well as in two mouse erythroleukemia cell lines which can be induced to terminally differentiate when exposed to dimethylsulfoxide. We find that there is a reciprocal correlation between the cells production of messenger RNA for c-myb and globin. C-myc message shows a similar but less dramatic decrease coincident with globin production. Coincident to the administation of an inducing agent, dimethylsulfoxide, there is a rapid turnover of myc and myb message subsequent to which the cells of the induced culture begin to manifest signs of differentiation. We conclude that the normal function of these oncogenes involves a role in the early maturational stages of development of the cells. In the erythroleukemic state the genes are down regulated by forced differentiation. These genes may play a role in influencing the state of differentiation of these cells.

0203 VARIABLE POSITION OF THE BREAKPOINT OF THE PH^1 TRANSLOCATION IN CHRONIC MYELOGENOUS LEUKEMIA(CML), D. Leibowitz, K. Schaefer-Rego, D. Popenoe, J. G. Mears, and A. Bank, Columbia Univ., Depts. of Medicine, and Human Genetics, New York, NY 10032

We have isolated a series of clones from a human recombinant DNA library which extends from 13 kilobases(kb)5' to 11 kb 3' to the *v-abl* homologous region. These cloned human *c-abl* fragments have been used to examine the DNA structure and RNA expression of the *abl* locus in patients with CML. We used these probes to examine Southern blots of 15 patients with CML and found new restriction fragments in 2 patients. By restriction mapping, the breakpoints in both these patients are closer to the *v-abl* homologous region than any previously described. Restriction mapping has also shown that the breakpoint location in chromosome(ch) 22 is different in 1 patient from the region previously described to encompass these translocations. A clone containing the unique *c-abl* hybridizing fragment from 1 of the patients has been obtained. Using this clone to probe a cellular DNA blot fragments from both ch 9 and ch 22 have been detected indicating that the ch 9-ch 22 junction has been isolated. The cloned human *c-abl* probes have also been used on Northern blots of CML RNAs. Using these probes an abnormal 9 kb *c-abl* transcript is present in all CML patients examined to date. This abnormal transcript is absent in the RNA of patients without CML. Two normal *c-abl* transcripts, which measure $\sim$ 6 and 7 kb, are detectable in both normal and CML RNA. Taken together our results suggest that while the position of the translocation of *c-abl* in the DNA in different CML patients varies, a similar sized abnormal RNA is consistently detected in all these patients. This abnormal RNA is presumably transcribed from the Ph^1 ch and may be closely related to the pathogenesis of CML.

0204 HUMAN c-fps PROTEINS IDENTIFIED IN NORMAL AND LEUKEMIC LEUKOCYTES, Ian A. MacDonald, Julia G. Levy, Anthony J. Pawson; Department of Microbiology, University of British Columbia, Vancouver, B.C., Canada, V6T 1W5

The retroviral v-fps(fes) oncogene encodes a protein with tyrosine kinase activity. We have identified proteins apparently encoded by mouse and human fps proto-oncogenes in a number of hemopoietic cells, both normal and malignant. Rat sera originally raised against the Fujinami Sarcoma Virus $P140^{gag-fps}$ protein immunoprecipitates a 92 kd protein from human leukemic cell lines and from peripheral leukoyctes of normal donors and patients with A.M.L. This protein undergoes autophosphorylation an an in vitro kinase reaction and is able to phosphorylate exogenous substrates. Analysis of tryptic peptides obtained from the protein phosphorylated in vitro reveals two major phosphopeptides. We have examined peripheral leukocytes from normal donors and from patients with A.M.L. and in all cases have been able to demonstrate the presence of the same 92 kd protein; additionally, this protein is present in the leukemic cell lines HL-60 and KG-1. On the basis of immunological cross-reactivity, in vitro kinase activity, and similarity of M.W. with known c-fps products, we conclude that this protein is the human homologue of the fps oncogene proteins. This protein is present in differentiated normal human leukocytes as well as normal and neoplastic cells of more immature stages of development. Lineage specific expression of this protein is being investigated as well as its role in hemopoietic differentiation.

0205 EFFECT OF RETROVIRUS PROTEIN ON INTERLEUKIN PRODUCTION AND FUNCTION. Lawrence E. Mathes,[a] Charles Orosz,[b] Edward Copelan,[c] and Richard G. Olsen.[a] [a]Department of Veterinary Pathobiology, [b]Department of Surgery, and [c]Department of Medicine, The Ohio State University, Columbus, Ohio 43210.

The in vitro immunosuppressive effect of retrovirus protein was studied using ultraviolet light-inactivated feline leukemia virus (UV-FeLV). UV-FeLV inhibited antigen- and lectin-driven blastogenesis of feline, human and murine lymphocytes. Inhibition was independent of accessory cells and appeared to be directed at T-lymphocyte functions. UV-FeLV inhibited the production of interleukin 2 (IL-2) by lymphocytes. This inhibition was not due to cytotoxicity or to interaction of viral protein with lectin or antigen. UV-FeLV also inhibited the IL-2-dependent proliferation of resting lymphocytes or CTLL cells both in the presence of excess IL-2. However, UV-FeLV did not interact directly with IL-2, nor did it occlude the IL-2 receptor. These data suggest that retrovirus protein inhibit blastogenesis of lymphocytes at one or both of the following stages of proliferation: first, following lectin or antigen stimulation, UV-FeLV prevents IL-2 production by activated lymphocytes (T-helper cells). Second, lymphocytes having developed IL-2 receptors following activation are prevented from proliferating even in the presence of excess IL-2.

0206 SELECTIVE EXPRESSION OF ENDOGENOUS RETROVIRAL GENES IN HUMAN LEUKEMIA, Kenneth McClain and Chris Wilkowski, University of Minnesota, Minneapolis, MN 55455

Genomic DNA of humans contains DNA sequences homologous to baboon endogenous virus (BaEV), Molony leukemia virus, and mouse mammary tumor virus. Activation of avian and murine endogenous retroviral genes is a well established event in the development of leukemias by those animals. As many as 40% of acute leukemia patients have surface antigens related to BaEV gp30 only on leukemic blasts. Thus retroviral genes may play some part in originating or maintaining the transformed state. A BaEV gag-pol gene probe was hybridized to a human genomic library and several clones were identified which contained retroviral sequences ("provirus clones"). A panel of mRNA dot blots from normal and leukemic patients was screened with ^{32}P-labeled DNA from the provirus clones. Several clones were identified which hybridized more strongly to leukemic mRNA. Conversely, labeled cDNA was made from normal and leukemic messenger RNAs. Provirus clone inserts had essentially no hybridization with normal cDNA. However, leukemic cDNA hybridized strongly to a few clone inserts ranging in size from 1-13 kb. Restriction enzyme maps of these clones illustrated conservation of several restriction enzyme sites seen in other proviruses. Low stringency hybridization of erb, ras, myc, and abl probes demonstrated distantly related oncogene sequences on the same inserts as hybridized to leukemic cDNA. A 13 kb insert with highly expressed sequences in acute lymphoblastic leukemia which was hybridized to Eco RI digests of normal and leukemic cell DNAs identified sequence rearrangements. Normal DNAs had bands at 5.9 and 5.0 kb, whereas leukemic DNAs had bands at 22.0, 14.0, 5.4 and 4.6 kb. These provirus probes are being studied for DNA sequence homology to oncogenes and growth factors as well as for being possible markers for leukemic cells.

0207 DNAse 1 HYPERSENSITIVE SITES 5' TO V-ABL LOCUS IN PH^1 K562 CELLS, J. Gregory Mears, David Leibowitz, Kim Schaefer-Rego, Columbia University, New York, NY 10032

C-abl resides on the long arm of chromosome 9 and is involved in a reciprocal translocation between chromosomes 9 and 22(Ph^1), seen in 90% of patients with chronic myelogenous leukemia (CML). The K562 cell line, derived from a patient with Ph^1 CML, is a useful model to study the expression of c-abl since it is amplified 4-8 fold and accumulates an abnormal c-abl RNA species similar in size to that observed in patients with CML. Nuclei obtained from K562 cells and WBCs from normal and CML patients were partially digested with increasing concentrations of DNAse 1. The extracted DNAs were digested with various restriction endonucleases, Southern blotted, and hybridized to various c-abl related cloned DNA sequences 5' to the v-abl region. Our results indicate the presence of a major DNAse 1 hypersensitive (HS) site residing 7 kb 5' to the v-abl region in K562 cells. This area on chromosome 9 also is the translocation site of 2/15 CML subjects. We have identified at least 2 other less intense DNAse 1 HS sites in the 18 kb region 5' to the v-abl region. Hemin induced K562 cells possess the same HS sites. HS sites were not generated in similar experiments using normal human WBCs and HL60 cells. We were unable to detect comparable HS sites in WBCs from CML subjects. This apparent difference from K562 cells may be related to the lack of c-abl amplification in fresh CML cells limiting detection of HS sites or to the greater extent of differentiation of the CML WBCs. The HS sites identified in K562 cells may denote transcriptionally active regions of DNA causally related to the genesis of CML or may be a more general feature of K562 cells.

0208 ANALYSIS OF THE CELLULAR HOMOLOG TO THE AVIAN LEUKAMIA VIRUS E26 ETS ONCOGENE, Michael F. Nunn, Peter H. Duesberg, and Tony Hunter, The University of California, Berkeley, CA 94720 and The Salk Institute, La Jolla, CA 92037

E26 is an avian retrovirus which transforms erythroblasts and myeloblasts in vivo and in vitro. Analysis of a molecular clone of the E26 genome has shown that it contains two oncogenic elements: myb and ets. The myb oncogene is also found in Avian Myeloblastosis Virus. The ets element is unique to E26 and may determine the erythroid specificty of the virus. A DNA subclone of the ets sequence has been used to identify and isolate its normal chicken cellular homolog. This proto-ets sequence spans more than 30 kilobases in cellular DNA.

Synthetic peptides have been made which correspond to the amino acid sequence predicted by the DNA sequence of the viral ets. These peptides have been used to generate antibodies which recognize specific determinants of the E26 oncogenic product. These antibodies have been used, in turn, to identify the proto-ets gene product in normal chicken cells.

0209 KINETICS OF ACTIVATION OF THE c-myc ONCOGENE DURING AKR LEUKEMOGENESIS. Paul V. O'Donnell, Erwin Fleissner, Herinder Lonial and Charles F. Koehne, Memorial Sloan-Kettering Cancer Center, New York, NY 10021

Using flow cytometric techniques, transplantation bioassays, and Southern blot analysis of DNA we have defined several discrete stages in development of MCF virus-accelerated leukemia in AKR mice. Stage I represents spread of infection to the major subpopulations of thymocytes; stage II, the emergence of clonal populations of proliferating cells; stage III, progression to the fully transformed phenotype of primary leukemia cells. Activation of the c-myc oncogene in thymocytes due to proviral insertion was studied as a function of time post-injection. Southern analysis showed rearrangements at the c-myc locus in 3/20 DNAs from cells at stage II of leukemogenesis but in 18/32 DNAs from cells at stage III or from frankly leukemic cells. Preliminary evidence indicates that the pim-1 locus is altered much less frequently in these leukemias.

Progression of stage II cells to a fully transformed phenotype was analyzed by transplantation. DNA from transplants of stage II cells into AKR recipients frequently possessed new clonal proviral integrations in addition to those present in the original stage II cells. Furthermore, c-myc rearrangements were found in leukemias that arose after transplantation of stage II cells which initially did not contain a c-myc rearrangement. These results suggest that leukemogenesis in this system requires at least two selectable genetic changes in target cells and that c-myc alteration need not be an early event.

0210 FELINE RETROVIRUS VACCINE. Richard G. Olsen, Lawrence E. Mathes, John Mastro. Department of Veterinary Pathobiology and Comprehensive Cancer Center, The Ohio State University, Columbus, Ohio 43210.

A feline leukemia vaccine has been the subject of intense interest following the recognition that human T-lymphocyte virus and the human lymphadenopathy virus share many properties in common with the feline retrovirus. Earlier, we demonstrated that feline retrovirus peptides shed from persistently-infected cells protect cats from retrovirus disease. Efficacy studies demonstrated that greater than 80% of vaccinated cats resisted viremia, the acquired immune deficiency syndrome, anemia and lymphoma when challenged with oncogenic feline retrovirus. Inactivated feline retrovirus, as well as the glycosylated envelope protein (FeLVgp70), fails to protect cats from retrovirus disease. Recent studies in our laboratory found that vaccinated cats respond within 14 days to at least peptides in the STAV vaccine of $\geqq$70,000 molecular weight. Antibody response to gag proteins appeared late in the vaccine regimen. It is hypothesized that STAV contains membrane-associated feline retrovirus precursor peptides of env origin and that these peptides contain immunoprotective epitopes not found in the virion envelope peptides.

0211 ABELSON-MuLV INDUCED MALIGNANT CONVERSION OF MAST CELLS: ELIMINATION OF DEPENDENCE ON IL-3 FOR GROWTH, J. H. Pierce, S. A. Aaronson, M. Potter and J. Ihle, National Cancer Institute, Bethesda, MD 20205

Although pre-B cells are preferential hematopoietic targets for transformation by Abelson-murine leukemia virus (Ab-MuLV) in vitro and in vivo, there have been reports that Ab-MuLV induces rare mastocytomas in vivo. Normal basophil/mast cells can be grown in long term culture when medium is supplemented with interleukin-3 (IL-3). Ab-MuLV infection of normal IL-3 dependent fetal liver cultures led to the conversion of mast cells from dependence on IL-3 for growth to factor independence. Although other acute transforming retroviruses, including Harvey, BALB and Moloney-MSV, were capable of productively infecting mast cell cultures, they were unable to release them from dependence on IL-3. Ab-MuLV-induced mast cell lines expressed abl-specific transforming proteins, were tumorigenic in nude mice, and had high cloning efficiencies in soft agar containing no exogenous factors. Analysis of Ab-MuLV-derived lines for characteristics associated with IL-3 revealed that they did not autonomously produce IL-3 nor were they stimulated to proliferate by IL-3. However, all lines tested retained receptors capable of binding iodinated IL-3. These results indicate that Ab-MuLV complementation of the IL-3 requirement was not the result of an autocrine mechanism involving direct or indirect induction of an IL-3-like growth factor. Moreover, release of normal mast cells from dependence on IL-3 for growth by Ab-MuLV infection correlated with the conversion of these cells to a transformed phenotype, defining a new hematopoietic target cell for transformation by Ab-MuLV.

0212 ISOLATION OF V-ONC GENE RELATED SEQUENCES FROM LOWER EUKARYOTES
K.Prakash and V.L.Seligy,Division of Biological Sciences,
National Research Council,Ottawa,Canada KIA OR6.

In order to elucidate the role of cellular onc genes in normal growth and differentiation,we have initiated studies on the characterisation of v-bas, v-kis and v-abl related DNA sequences in Schwanniomyces alluvius, Saccharomyces cerevisiae and Candida.

After hybridization under very relaxed conditions,homologous v-onc related fragments were identified in all the three yeasts. An approximately 2.1 kbp Hind III fragment,a 1.6 kbp Eco RI fragment and 20-22 kbp plus 9.4 kbp Bam HI pieces were detected in S.alluvius using the v-kis probe. Similarly, different restriction fragments were identified in S.cerevisiae and Candida with all the three probes. Some of them were cloned in pBR322,restriction maps worked out and compared with known viral and cellular onc genes.Utilising techniques of mutagenesis,we intend to find out the functional sites in these sequences after hooking them onto appropriate expression vectors.

0213 THE ENVELOPE gp70 OF SOME PRIMATE RETROVIRUSES CLOSELY RESEMBLES THE NORMAL HUMAN CELL SURFACE ANTIGEN, HuLy-m5. Damian F.J. Purcell, R.L. Sparrow, N.J. Deacon, I.F.C. McKenzie. University of Melbourne, Parkville, Melbourne, Australia, 3052

The monoclonal antibody, E4.3, detects the human cell surface antigen, HuLy-m5, which has a close physical association with HLA class I antigens on the cell surface. HuLy-m5 molecules consist of two acidic, non-disulphide linked glycoproteins with a molecular weight of approx. 60 and 69 kd. Pure preparations of Mason Pfizer Monkey Virus (MPMV) and Gibbon Ape Leukemia Virus (GALV), but not Simian Sarcoma Virus (SSV) or Baboon Endogenous Virus (BaEV) absorb E4.3, while antisera to MPMV and GALV block the binding of E4.3 to human target cells, showing that HuLy-m5 shares cross-reactive determinants with the envelope glycoprotein of MPMV and GALV. Sequential immunoprecipitation and two dimensional gel analysis shows that the anti-retrovirus antisera detect the same molecular entity as E4.3. Enzymatic removal of the carbohydrate from the glycoprotein does not affect the cross-reactivity, and the cross-reacting antisera all precipitate a similar protein from cell free translations of human thymus mRNA, showing that the cross-reaction is not due to a carbohydrate moiety. The close relationship between the gp70 of MPMV and GALV retroviruses and the HuLy-m5 antigen is further suggested by the strong reaction of E4.3 monoclonal antibody with the gibbon ape cell line UCD-144-MLA, chronically infected with GALV, compared to the negative reaction with normal uninfected gibbon ape leukocytes. There is, therefore, a close relationship (? identity) between a normal human cellular glycoprotein and primate retrovirusal gp 70.

0214 TOPOLOGY AND FUNCTION OF THE TRANSMEMBRANE GLYCOPROTEIN ENCODED BY THE v-fms ONCOGENE. Carl W. Rettenmier, Martine F. Roussel and Charles J. Sherr. St. Jude Children's Research Hospital, Memphis, TN. 38105.

The v-fms oncogene encodes an integral transmembrane glycoprotein which is expressed at the cell surface and required for transformation. A membrane anchor sequence of 26 hydrophobic amino acids is located near the middle of the glycoprotein and positions it in membranes. Subcellular fractionation and proteolytic digestions demonstrate that the molecule is oriented such that sequences aminoterminal to the transmembrane anchor are within the lumen of the endoplasmic reticulum. This orientation is maintained during intracellular transport of the protein so that the glycosylated aminoterminal sequences are exposed at the cell surface. Amino acid sequences carboxylterminal to the membrane anchor are positioned in the cytoplasm and show homology to other oncogene products that are tyrosine-specific protein kinases. Immune complexes containing v-fms-coded glycoproteins exhibit a tyrosine kinase activity which phosphorylates the transforming gene product itself as well as exogenously added protein substrates. The role of this kinase activity in vivo is uncertain since the carboxylterminal domain of the glycoprotein is phosphorylated only at serine and threonine residues in transformed cells. The topology of the v-fms-coded gene product and the presence of in vitro tyrosine kinase activity support the hypothesis that this transforming glycoprotein is an analog of a c-fms-coded growth factor receptor. Expression of c-fms RNA in bone marrow cells suggests that the putative receptor has a function in hematopoiesis.

0215 EFFECT OF MNU ON AGE-RELATED APPEARANCE OF UNINTEGRATED MuLV IN PRELEUKEMIC AKR MICE, Ellen R. Richie & Rodney S. Nairn, Univ. of Texas Science Park, Smithville, Tx, 78957

The high incidence of spontaneous thymic lymphoma in old AKR mice is thought to be due to dualtropic MuLVs that arise by recombination between endogenous proviruses. AKR mice also are suseptible to MNU induction of thymic lymphomas prior to the age-related appearance of spontaneous tumors. AKR mice have a higher incidence and shorter latent period for MNU-induced lymphomas than do low leukemia incidence strains suggesting that MuLVs play a role in chemically induced lymphomagenesis in AKR mice. Although we observed amplified expression of MuLV gp70 on MNU induced lymphomas, we found that DNA from MNU-induced AKR lymphomas does not contain the BclI/XbaI restriction linkage generated during spontaneous lymphomagenesis by recombination between env genes of endogenous MuLVs. However, our data do not rule out MuLV participation in MNU-induced lymphomagenesis, since various recombination events occur among MuLV sequences during spontaneous lymphoma development. Therefore, we studied the appearance of unintegrated MuLV DNA during the preleukemic period in untreated and MNU-treated AKR mice. Herr and Gilbert have shown that thymocytes from 5 mo old preleukemic AKR mice contain unintegrated recombinant MuLV DNA. To determine if MNU treatment affects the appearance of free MuLV DNA, Southern blots of undigested DNA were hybridized with a probe that detects ecotropic and nonecotropic MuLVs. Thymic DNA from the majority of 5 mo old untreated mice showed unintegrated MuLV DNA. In contrast, unintegrated MuLV DNA was not routinely found in thymus tissue from MNU-treated mice suggesting that MNU interferes with the age related expression of free MuLVs in preleukemic AKR thymic tissue.

0216 DETECTION OF NEW CELL SURFACE DETERMINANTS ON CELLS TRANSFORMED BY HUMAN ONCOGENES Jack A. Roth, Robert S. Ames, Carlos Restrepo, Stuart Aaronson, Shiv Srivastava, Philip Scuderi, and Eric Westin. National Cancer Institute, National Institutes of Health, Bethesda, MD 20205.

To determine if cells transfected with human oncogenes express new tumor-associated cell surface antigens, monoclonal antibodies (MoAb) were prepared to NIH 3T3 cells transfected with either an oncogene from a human acute lymphocytic leukemia (ALL, N-ras) or a human small cell lung carcinoma (c-bas/has). MoAb were screened for binding to transfectants but not 3T3 cells in an enzyme-linked assay. Cell surface binding of MoAb 17-9H3, produced by immunizing with the N-ras tertiary transfectant, was observed to the parental ALL, 3 of 5 N-ras tertiary transfectants, but not 3T3 cells or other murine tumors by immunoperoxidase staining of unfixed cells. Antigen expression was restricted to null cell leukemias, CML in blast crisis, and T-cell leukemia lines. Myelogenous leukemias, B-cell lines, and lymphomas were negative. Expression did not correlate with HTLV infection. A second antibody (45-2D9) prepared by immunizing with a c-bas/has tertiary transfectant demonstrated a different binding distribution. The antigen was expressed by some human tumors (primarily colon) but not by 3T3 cells, non c-bas/has transfectants, murine tumors, normal human tissues, or human leukemias. 45-2D9 immunoprecipitated a 74 K M_r glyco-phosphoprotein distinct from the p21 oncogene product. Thus, oncogenes appear closely linked with other genes responsible for tumor antigen expression. MoAb to oncogene-linked antigens may be potentially useful for cancer diagnosis and therapy.

0217 OCCURANCE OF ANTIBODY TO HTLV-III AND IMMUNOLOGIC ABNORMALITIES IN HEMOPHILIA, C. Scheffel, D. Norton, J. Casey, J. Gill*, J. Menitove*, R. Montgomery*, C. Wood, S. Devare, P. Andersen. *Blood Center of Southeastern Wisconsin, Milwaukee, WI and Abbott Laboratories, North Chicago, IL.

A human T-cell tropic retrovirus, HTLV-III is currently regarded as a possible etiologic agent of acquired immune deficiency syndrome (AIDS). We investigated the relationship of the presence of antibody to HTLV-III and immunologic abnormalities in patients with hemophilia, a group known to be at risk for AIDS. Antibody to HTLV-III was detected in serum utilizing solubilized HTLV-III antigens in an ELISA assay. Results will be presented which indicate that the presence of antibody to HTLV-III is associated with immunologic abnormalities in patients with hemophilia.

0218 HOST CELL RANGE OF ADULT-T CELL LEUKEMIA VIRUS (ATLV): VIRUS BINDING, PENETRATION AND INFECTIVITY IN VARIOUS MAMMALIAN CELLS, F. Sinangil and D. J. Volsky, Dept. of Pathology, U.N.M.C., Omaha, NE 68105

Adult T-cell leukemia virus (ATLV) is the member of a human type-C retrovirus family (HTLV) found to be asociated with adult T-cell leukemia in Japan. In our study, ATLV was isolated from MT-2 cell line, purified on Dextran gradient and labelled with either fluorescein-isothiocyanate (FITC-ATLV) or with ^{125}iodine (^{125}I-ATLV). Fresh human lymphocytes, separated B and T cells, mouse and rabbit lymphocytes, mouse fibroblasts, and 12 different tumor cell lines were tested for the binding of FITC-ATLV, penetration by ^{125}I-ATLV and infectability by the virus. Virus-cell receptor binding was assayed by flow cytometry. Virus penetration was determined by following the association of ^{125}I-ATLV with trypsinized cells 4 h after exposure to the virus and culture at 37°C. Successful infection was monitored by following the expression of ATLV-determined antigen (ATLA) by immunofluroescence 2 and 7 days after infection. Most of the cells bound FITC-ATLV at the levels ranging from 5% to 130% of the MT-2 cell binding. Virus-binding cells were also penetrable to ^{125}I-ATLV. However, only fresh human T, mouse and rabbit lymphocytes and one EBV genome-positive human T cell line expressed ATLA after exposure to cell-free virus preparation. The results demonstrate that ATLV can bind to and penetrate into many different types of cells. Expression of the virus biological activity is restricted to fewer host cells but not limited to a specific class of human lymphocytes. Thus, the biological host range of ATLV is determined at the cytoplasmic/nuclear rather than at the membrane receptor level.

0219 THE EFFECTS OF V-MYB AND V-MYC ON CELLULAR PHENOTYPE, Geoff Symonds[1], Karl-Heinz Klempnauer[1], Giovannella Moscovici[2], Carlo Moscovici[2] and J. Michael Bishop[1], [1]University of California, San Francisco, CA 94143;[2]Veterans Administration Hospital, Gainesville, FL 32602

v-myb and v-myc are the oncogenes of avian myeloblastosis virus (AMV) and avian myelocytomatosis virus,respectively. Both encode nuclear-located proteins and transform cells of the myelomonocytic series. During induced differentiation of AMV-transformed myeloblasts, the expression of v-myb did not alter but the location of the v-myb encoded protein ($p45^{v\text{-}myb}$) changed from predominantly nuclear to cytoplasmic. This was in contrast to myeloblasts transformed by a temperature-sensitive mutant of AMV where the shift to the non-permissive temperature was accompanied by lowered expression of v-myb. Thus reversion of the transformed phenotype may be associated with either redistribution of $p45^{v\text{-}myb}$ within the cell or decreased synthesis of the protein. The phenotype of hematopoietic cells transformed by v-myb is that of relatively immature cells of the myeloid lineage. By contrast, hematopoietic cells transformed by v-myc are more like mature macrophages. When the action of v-myc was superimposed on cells previously transformed by v-myb, certain properties of the neoplastic cells "progressed" to those of a more mature phenotype. These findings illustrate the countervailing effects of v-myb and v-myc on cellular phenotype and call into doubt the view that v-myb and v-myc elicit leukemia by simply arresting otherwise normal myelomonocytic differentiation.

0220 INFLUENCE OF NON-p21 CODING SEQUENCES ON THE ONCOGENIC ACTIVITY OF HARVEY MURINE SARCOMA VIRUS, Pierre E. Tambourin, Sisir K. Chattopadhyay and Douglas R. Lowy, Laboratory of Cellular Oncology, National Cancer Institute, Bethesda, MD 20205

Harvey murine sarcoma virus (Ha-MuSV) induces fibrosarcomas and erythroleukemia in susceptible mice. The p21 transforming protein of Ha-MuSV, which is encoded by the v-ras^H gene, is the only known product encoded by the virus. We have created specific deletions and substitutions of sequences located outside the p21 coding region (and outside the viral LTR, which is already known to contain an enhancer element) to study the influence of noncoding sequences located outside the LTR on the in vivo oncogenic activity of Ha-MuSV.

Deletion of specific sequences located downstream from v-ras^H markedly lowered the DNA transforming activity on NIH 3T3 cells. Rescued virus specified low levels of wild type viral p21, did not induce fibrosarcomas or erythroleukemia, but did induce lymphoid leukemia. Substituting 0.1 kb of 5' non-coding sequences from c-ras^H for 0.1 kb of 5' viral non-coding sequences increased the transforming activity of the viral DNA in vitro and shortened the latent period of injected virus. When the normal (non-mutated) rat c-ras^H gene was substituted for the v-ras^H gene, the presence or absence of these non-coding sequences had a marked influence on the transforming activity of the viral DNA in vitro and on the oncogenicity of the rescued virus. These biological changes correlated with alterations in levels of viral RNA and p21 protein. We conclude that noncoding sequences outside the LTR can significantly affect the oncogenic activity of Ha-MuSV.

0221 THE LEUKEMOGENIC SL3-3 MURINE LEUKEMIA VIRUS (MuLV) OF AKR MICE FORMS ENVELOPE GENE RECOMBINANTS IN VIVO, Christopher Y. Thomas, University of Virginia.

Recent studies have concluded that the gene enhancer element within the non-coding U3 region of the retroviral genome mediates the leukemogenic potential of the AKR ecotropic MuLV SL3-3. The results of these experiments have led several authors to propose that the U3 region of MuLVs also regulate the replication, tissue tropism, and target specificity of these viruses. To test if other viral sequences might be involved in the induction of thymic leukemia by the SL3-3 virus, this isolate was injected into newborn CBA/J mice. Lymphoblastic lymphomas developed in 9/11 animals by 5 months of age. Viruses were recovered from lymphoma cells by cocultivation of the cells on NIH3T3 fibroblasts. Genetic analysis of the viral RNA genomes by T_1-oligonucleotide fingerprinting revealed that all four isolates were mixtures of the injected SL3-3 virus and recombinant viruses. The recombinant viral genomes contained substitutions by endogenous CBA/J viral sequences within the 5'-portion of the gp70 gene. Moreover, analysis of tumor DNAs by Southern blotting techniques demonstrated a large number of integrated proviruses of this genotype.

Thus, the leukemogenicity of the ecotropic SL3-3 virus may depend on the formation of specific envelope gene recombinant MuLVs in vivo. The results are consistent with earlier observations that portions of the retroviral genome outside the U3 region, including the envelope genes, are also important in conferring the oncogenic phenotype to MuLVs.

0222 MONOCLONAL ANTIBODIES SPECIFIC TO HUMAN T-CELL LEUKEMIA VIRUS III GAG PROTEINS, F. Veronese diMarzo[1], M.G. Sarngadharan[1], R. Rahman[1], P.D. Markham[1], M. Popovic[2] and R.C. Gallo[2], [1]Litton Bionetics, Inc., Kensington, MD 20895; [2]Laboratory of Tumor Cell Biology, NCI, Bethesda, MD 20205

A T-lymphotropic virus, designated HTLV-III, was isolated from patients with acquired immunodeficiency syndrome (AIDS) or lymphadenopathy syndrome, and has been indicated as the causative agent for the disease. Since to date the only reagent available for immunological studies is a polyclonal rabbit antiserum, we have undertaken the development of monoclonal antibodies directed against HTLV-III proteins. Hybridomas secreting antibodies to the HTLV-III major core protein and to p15 have been isolated. They belong to several immunoglobulin subclasses, including IgM, IgG_1 and IgG_2. The specificity of the reaction was analyzed by several methods, including radioimmunoassay on Western blotted nitrocellulose, immunoprecipitation of iodinated, purified proteins and indirect immunofluorescence. All of them specifically recognize p15 or p24 in cells infected with the virus and are negative against the same cells before viral infection. The anti-p24 antibodies have also been tested for crossreactive epitopes with HTLV-I and -II and found to be type-specific. Because of this, they are extremely valuable reagents for identifying cultures and tissues expressing HTLV-III.

0223

A NEW HUMAN DNA SEQUENCE RELATED TO THE CELLULAR ONCOGENE c-abl

Harry White, Brian Young & John M Goldman. MRC Leukaemia Unit, Hammersmith Hospital and Royal Postgradute Medical School and the Imperial Cancer Research Fund, London, UK.

By screening a human genetic genomic library at medium stringency with a v-abl probe (corresponding to the 3' most exon of c-abl), we have isolated a recombinant phage with an insert of 10 kb, designated hwl. Two HindIII fragments of the insert have been subcloned in pUC8 and have been designated hwl2 and hwl3, being 1.7 and 3.0 kb respectively. From an analysis of several different enzyme digests, we infer that hwl2 and hwl3 are contiguous in the genome and that hwl2 has the homology to c-abl and one or two other loci. This homology lies over a region of less than 0.6 kb, and will be sequenced. The hwl2 genomic blot bands are present as minor bands on a genomic blot probed with v-abl. Conversely, hwl3 sequence appears to be single copy within the genome. hwl maps to human chromosome 9. Further work is underway to determine its location more precisely. It is not amplified in K562 cells suggesting that it is not in the immediate proximity of c-abl. Preliminary analysis suggests that transcripts with homology to hwl3 may be present in buffy coat (leucocyte) total RNA. This is also being followed up with analysis of polyA+ RNA. Completion of work will determine the nature of the sequence within hwl. It could be one of many things, including a pseudogene, a provirus or even another functional gene related to c-abl.

0224

A NOVEL HUMAN DNA SEQUENCE RELATED TO THE CELLULAR ONCOGENE C-ABL.

WHITE, H.N.; YOUNG, B.D.; GOLDMAN, J.M. MRC LEUKAEMIA UNIT, LONDON

By screening a human genomic library at medium stringency using a v-abl probe, we have isolated a recombinant phage with an insert of 10kb, designated λhwI.

Two HindIII fragments of the insert have been subcloned, and designated λhwl2 and λhwl3 being 1.7 and 3.0kb respectively.

From an analysis of several different enzyme digests, we infer that λhwl2 and λhwl3 are contiguous in the human genome. Also, λhwl2 has the homology to v-abl and to one or two other loci. This homology lies over a region of less than 0.6kb. and is being sequenced. The λhwl2 genomic blot bands are present as minor bands on a genomic blot probed with v-abl. Conversely the hwl3 sequence appears to be single copy within the genome.

λhwl maps to human chromosome 9. Further work is under way to determine its location more precisely. It is not amplified in K562 cells suggesting that it is not in the immediate proximity of c-abl.

Preliminary analysis suggests that transcripts with homology to λhwl3 may be present in buffy coat (leucocyte) total RNA. This is also being followed up with analysis of polyA mRNA.

Completion of work will determine the nature of the sequence within λhwl. It could be one of many things, including a pseudogene, part of a provirus or even another functional gene related to c-abl.

0225

TRANSFORMING ACTIVITY OF THE p21 V-RAS^H GENE PRODUCT: INFLUENCE OF MUTATIONS IN THE REGION OF AMINO ACID HETEROGENEITY.

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The human cellular ras^H-1 gene and its counterpart in rodents encode an identical product of 189 amino acids. There is a high degree of homology between this gene and the other known biologically active members (ras^K and ras^N) of the mammalian ras gene family, with the notable exception of the segment between residues 166 and 185, which shows virtually no homology among the three genes. Previous results have suggested that the extreme carboxyterminal amino acids of p21 are required for membrane localization of the v-ras^H gene product (Willumsen et al., Nature 310: 583-586, 1984); N-terminal amino acids probably serve a catalytic function.

We have constructed duplication and deletion mutants of the v-ras^H gene in the 20 amino acid region of heterogeneity in order to study the effects of changes in size on the transforming activity of the gene. Mutants containing a heterogeneous domain varying from 5 to 48 amino acids (the largest duplication tested thus far) can still transform NIH 3T3 cells. Since extensive deletion or duplication in the region of heterogeneity has little influence on the transforming activity of the gene, we conclude that this segment functions in transformation principally as a hinge region to link the C-terminal membrane anchoring structure of the protein to its N-terminal domain.

0226 CONTROL OF RETROVIRUS EXPRESSION BY GENE(S) LINKED TO H-2 IN CULTURED TUMOR CELLS. J.H. Wolfe and K.J. Blank, Univ. Pennsylvania Sch. Med., Phila., Pa. 19104

Certain H-2 haplotypes, notably $H\text{-}2^k$, are associated with susceptibility to endogenous and some exogenous (e.g. Gross and Friend) retrovirus-induced leukemias, whereas others, primarily $H\text{-}2^b$, are associated with resistance. In vivo, both immune responses to viral antigens and levels of virus replication have been shown to be influenced by genes linked to H-2. In order to examine the possible effects of H-2 on virus replication in the absence of any interaction with the host, e.g. immunoselection, we have studied 26 cultured cell lines which were established from tumors induced by Gross or Friend virus in mice congenic at H-2. Most of the cell lines derived from mice expressing the $H\text{-}2^k$ haplotype continuously produce infectious, oncogenic input-type virus in tissue culture, while most of the cell lines of the $H\text{-}2^b$ and $H\text{-}2^d$ genotypes become non-producers after passage in tissue culture. All of these non-producers cell lines continue to express MuLV-encoded proteins, but harbor various defects in gag and env gene protein expression compared to producer cell lines. This block is not at the cell surface because some non-producers can be superinfected with homologous virus. The exogenous inducing-type virus has been induced out of some non-producer cell lines with IUdR indicateing that the provirus is intact in these lines subsequent to the cessation of virus production. Thus the block appears to be at the level of transcription, translation, or post-translational modification.

0227 GENERATION AND CHARACTERIZATION OF HEMOPOIETIC CELL LINES ARISING FROM ABELSON VIRUS INFECTION OF ISOLATED MULTI-LINEAGE COLONIES, Peter M.C. Wong and Connie J. Eaves, Terry Fox Laboratory, B.C. Cancer Research Centre, 601 West 10th Avenue, Vancouver, B.C. V5Z 1L3, Canada

Previous studies have shown that Abelson murine leukemia virus (A-MuLV) can transform both lymphoid and myeloid cells although the range of target cells susceptible to malignant transformation and the steps they undergo still remain poorly defined. We have recently shown that exposure of single multi-lineage colonies generated in standard methylcellulose assays to A-MuLV results in the development of continuously growing factor-independent cell lines. The possibility that these arise from other cells co-existing in the assays was ruled out by subsequent experiments in which the multi-lineage colonies used were generated in cultures seeded with single stem cells. Critical to the development of these cell lines was the presence during exposure to the virus of irradiated NIH-3T3 cells. The transformed cells contain metachromatic granules typical of "p" or mast cells. They can be maintained in medium supplemented with 10-20% FCS in which they proliferate with a doubling time of 48-96 hrs, although more rapidly dividing sublines have recently been obtained by subcloning one of the lines in methylcellulose. The lines and sublines produce high titre virus and when injected subcutaneously into nude mice (10^6 cells per mouse) give rise to rapidly growing tumours apparent 2 weeks later at the site of injection. These lines and the methodology developed for their generation may provide new tools for the analysis of malignant transformation of myeloid cells.

0228 MOLECULAR STUDIES ON HUMAN T-CELL LEUKEMIA VIRUS-III, C. Wood, M. Kieselburg, P. Andersen* and S. Devare*, Depts. of Cancer Research and Molecular Biology*, Abbott Laboratories, North Chicago, IL 60064.

Human T-cell leukemia virus-III is implicated as an etiological agent for acquired immuno deficiency syndrome. On the basis of its T-cell tropism and for T-helper cell subset. The requirement of Mg of its reverse transcriptase as well as retroviral morphology, HTLV-III was classified as a member of HTLV family. In order to compare HTLV-III with the other members of the family at genomic level, Southern hybridizations were performed with specific probes. HTLV-I and HTLV-II probes did not hybridize to HTLV-III infected HT-9 cell DNA. However, sequence homology could be detected at the transcription level. In order to further characterize this similarity between HTLV isolates we have attempted to derive molecular cDNA clone of the HTLV-III specific transcript in HT-9 cells. Data will be presented on characterization of this cDNA clone and its comparison to other members of HTLV family.

0229 Ig CHAIN GENE EXPRESSION IN BURKITT LYMPHOMA DERIVED CELL LINES
J.J.M. van Dongen, M.A. Versnel, A.H.M. Geurts van Kessel, E.M.E. Smit, H. Hooijkaas and A. Hagemeijer, Erasmus University, Rotterdam, The Netherlands.

Burkitt lymphomas and Burkitt lymphoma derived cell lines are characterized by the presence of specific chromosome translocations: t(8;14) or one of the variant translocations t(2;8) and t(8;22). The breakpoint areas of chromosome 14, 2 and 22 carry the genes coding for the Ig heavy chain, κ Ig light chain and λ Ig light chain respectively, while the breakpoint area of chromosome 8 carries the cellular oncogene c-myc. Erikson et al. (1982; 1983) demonstrated that in the t(8;14) positive Daudi and P3HR-1 Burkitt cell lines the expressed μ chain gene is on the normal 14. According to Lenoir et al. (1982) the expression of the κ and λ Ig light chains correlates with the variant translocations t(2;8) and t(8;22), respectively, suggesting that the Ig light chain genes that are involved in the translocations, are the expressed genes.

We isolated the Burkitt lymphoma derived cell lines ROS-1, ROS-5 and ROS-16 with the translocations t(8;14), t(8;22) and t(2;8), respectively. Using somatic cell hybridization techniques it appeared that in ROS-1 the expressed μ heavy chain gene is on chromosome 14q+. Immunological characterization revealed that ROS-5 expresses the κ light chain and ROS-16 the λ light chain. These data are in contrast with the literature and illustrate that, although the Burkitt translocations and especially the c-myc oncogene may play an important role in the oncogenesis, the expression of the Ig heavy and light chain seems to be regulated independently.

Erikson et al., 1982, Proc. Natl. Acad. Sci. USA 79: 5611-5615; Lenoir et al., 1982, Nature, 298: 474-476; Erikson et al., 1983, Proc. Natl. Acad. Sci. USA 80: 820-824.

Viral Leukemogenesis

0230 HEMATOPOIETIC CELL TARGETS FOR TRANSFORMATION BY RETROVIRAL ONCOGENES, S. A. Aaronson, J. Pierce, and A. Eva, National Cancer Institute, Bethesda, MD 20205.

Investigations of acute transforming retroviruses have led to important insights concerning a small group of cellular genes with transforming potential. Such transduced cellular onc genes confer to the virus properties essential for the induction and maintenance of the transformed state. Accumulating evidence indicates that these cellular genes can also be activated as transforming genes in human tumors by mechanisms completely independent of retrovirus involvement. We have demonstrated activation of members of the ras family in a wide variety of human hematopoietic tumors. N-ras oncogenes appear to be preferentially activated. More than 50% of AMLs tested, as well as diverse lymphomas and leukemias, have been shown to contain this oncogene. By transfection analysis, we have also detected an oncogene in a non Hodgkin's lymphoma, which does not appear to be related to any previously studied retroviral transforming gene. Our characterization of this new oncogene will be described.

In efforts to investigate normal hematopoietic targets for transformation by ras and other oncogenes, we have utilized primary murine bone marrow cell cultures as well as populations of hematopoietic cells proliferating in response to specific growth factors. We demonstrate that some hematopoietic cell types appear to undergo conversion to the malignant phenotype directly in response to a single retroviral onc gene, while the same cell population can remain unaffected by other transforming genes. Our studies establish a wide array of cell targets whose growth can be altered by oncogenes at specific stages of hematopoietic cell differentiation.

0231 TRANSFORMING FUNCTIONS OF EPSTEIN-BARR VIRUS, Bill Sugden, John Yates, and David Reisman, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, WI 53706

Epstein-Barr virus (EBV) infects human B-lymphocytes in vitro and efficiently induces and maintains cell proliferation in the infected cell. The viral DNA is usually detected in the proliferating or "transformed" cells as complete plasmids which are maintained through serial cultivation of these transformed cells. We wish to identify and characterize viral functions that are required by EBV to transform B-lymphocytes. We have taken one path towards this goal by first identifying viral genetic elements that are required to maintain DNA as plasmids in mammalian cells. There are two such viral elements: one acts in cis and is required for replication of plasmid DNAs; the second encodes the nuclear antigen, EBNA-1, which interacts with the cis-acting element to permit replication of plasmid DNAs. We term the cis-acting element oriP for origin of plasmid replication. We have not shown that DNA synthesis begins within oriP, only that oriP is required in cis for DNA replication. oriP itself is composed of two cis-acting elements; one contains 20 tandem copies of a 30 bp sequence; the other, about 1 kbp away, contains a 65 bp inverted repeat with symmetrically positioned, partial copies of the 30 bp sequence. Derivatives of oriP have been generated in vitro which when tested in vivo indicate that the distance between its two elements can be eliminated or increased to 2.5 kbp without loss of function. Neither element serves as an enhancer of transcription. The gene for the nuclear antigen, EBNA-1, maps 100 kbp away from oriP on the viral genome. When this gene is linked to oriP and to a marker that is selectable in eukaryotic cells, such a recombinant plasmid can replicate stably in established dog, monkey, and human cell lines that neither contain EBV nor are infectable by this virus. These plasmids are usually maintained at the level of 1-20 copies per cell in the presence of the selective agent. These results indicate that we have identified the genetic elements of EBV that are necessary to maintain DNA as plasmids in a variety of cells. We are now adding to this mini-replicon other viral DNA in order to identify the minimal viral information required to transform human B-lymphocytes.

Human T-Leukemia Virus (HTLV)

0232 STRUCTURAL AND BIOLOGICAL FEATURES OF HUMAN T-CELL LEUKEMIA VIRUSES, Irvin S.Y. Chen[1], David W. Golde[1], Kunitada Shimotohno[2], Dennis J. Slamon[1], and William Wachsman[1]. [1]Division of Hematology-Oncology, Department of Medicine, UCLA School of Medicine, Los Angeles, CA 90024; [2]National Cancer Center, Tokyo, Japan

Human T-cell leukemia viruses type I and II are human retroviruses associated with specific T-cell malignancies. Aside from their importance as the likely etiologic agents of some forms of human cancer, these viruses have a number of structural and biological features which distinguish them from most other animal retroviruses. The complete sequence of both HTLV-I and HTLV-II has been determined (1-3). A comparison of these sequences reveals several unique features, particularly 1) the long terminal repeats (LTRs) have an unusual organization of transcriptional control elements and 2) the 3' region of the genome between the env and the 3' LTR contains sequences sufficient to encode a protein(s) of as yet poorly defined function.

These structural features are likely to be at least partly responsible for some unusual biological properties of the viruses. HTLV replicates primarily in T cells and B cells and transforms exclusively T cells. We have shown that this property of HTLV-II is due partly to a limited transcriptional activity of the LTR in different cells (4).

The so-called X region of HTLV was originally defined only by sequence analysis. By identification of protein products (5) encoded by specific spliced mRNA transcripts (6), we demonstrated that a novel gene is present in the 3' region of HTLV. We believe that the X gene of HTLV is necessary for both virus replication and transformation of T cells. A genetic analysis is underway to analyze the effect of mutations in the X gene as to their effect on replication and transformation by HTLV.

1. Seiki et al.: Proc Natl Acad Sci USA 80:3618, 1983
2. Haseltine et al.: Science 225:419, 1984
3. Shimotohno et al.: Proc Natl Acad Sci USA, in press, 1984
4. Chen et al.: Nature 309:276, 1984
5. Slamon et al.: Science 226:61, 1984
6. Wachsman et al.: Science 226, in press, 1984

0233 ADULT T-CELL LEUKEMIA IN JAPAN, Yorio Hinuma, Institute for Virus Research Kyoto University, Kyoto 606, Japan:

A retrovirus, adult T-cell leukemia (ATL) virus (ATLV) was shown to associated with ATL, which is an endemic disease in southwestern parts of Japan. ATLV, which is similar to HTLV isolated from patients with cutaneous T-cell lymphoma-leukemia in the USA was consistently detected in fresh leukemic cells after short-term culture of peripheral blood from ATL patients. The proviral DNA was detected in the fresh leukemic cells. Antibodies to ATLV-specific antigens, previously called ATL-associated antigen (ATLA), were found in sera from almost all ATL patients and also in sera of a considerable proportion of healthy adults in ATL-endemic areas. ATLA and/or ATLV particles were consistently demonstrated in fresh peripheral leukocytes of anti-ATLA-positive healthy, or asymptomatic, individuals after short-term culture, especially by a clonal culture technique. These findings indicate that all anti-ATLA positive individuals, including both ATL patients and healthy donors, had ATLV-bearing cells in their blood. Thus, it could be predicted that areas in which the incidence of anti-ATLA-positive residents is high are ATLV-endemic areas. This was confirmed in a nation-wide, seroepidemiologic study, which demonstrated unequivocally that ATLV (virus)-endemic areas coincide with ATL (disease)-endemic areas.

This seroepidemiologic survey of ATLV, detected as anti-ATLA, showed that the incidences of antibody-positive donors were high mainly in southwestern parts of Japan, but in some northern parts of Japan. Result of the familial distribution of anti-ATLA positive individuals (ATLV-carriers) suggested that routes of natural transmission of ATLV might be from mother to child and from husband to wife. Furthermore, it was found that ATLV could be transmitted by blood transfusion. Retrospective studies on transmission of ATLV by blood transfusion strongly suggested that cells of ATLV-carriers were highly infectious. More than 60% of the recipients of whole blood or blood components containing cells from donors having antibodies to ATLA produced anti-ATLA.

Since ATL was reported in Japan in 1977, more than 500 cases of patients with ATL have been found. Majority of ATL patients took acute course with rapid progression, but fewer chronic, lymphoma or smoldering types were also identified.

Chromosomes and Leukemia

0234 THE INVOLVEMENT OF ONCOGENES IN CML, John Groffen[1], Kees Stam[1], Nora Heisterkamp[1], Annelies de Klein[2], and Gerard Grosveld[2]. [1]Oncogene Science Inc., Mineola, New York; [2]Erasmus University, Rotterdam, The Netherlands.

The Philadelphia (Ph')translocation is characteristic for chronic myelocytic leukemia (CML). By somatic cell hybrid analysis we have localized c-abl to human chromosome 9 band q34 and have demonstrated that this oncogene is consistently translocated to the Ph' chromosome in CML (1). The possible involvement of human c-sis is unlikely in this type of leukemia as the chromosomal breakpoint in CML (band 22 q11) is not near the chromosomal location of c-sis (22 q12.3-q13.1) (2). Chimeric fragments of chromosome 9 and 22 which contain the Ph' chromosomal breakpoint have been isolated. In two CML patients, the Ph' breakpoints have been localized within 14kb and 30kb, respectively, of the most 5' v-abl homologous region (3). The possibility that these breakpoints are located within the human c-abl locus cannot be excluded, since the position of the most 5' exon in human c-abl has not been determined (1). These results establish that the breakpoints on chromosome 9 occur at variable sites and that the orientation of human c-abl is with its 5' toward the centromere. We have used the chromosome 22 specific region of a chimeric breakpoint fragment to isolate an extended region of chromosome 22 of non-CML human DNA. The genomic organization of this region of chromosome 22 was investigated in DNAs of a number of CML patients (4). In all (over 30) Ph'-positive CML patients studied to date chromosomal breakpoints were detected within a limited region of up to 5.8 kb, a breakpoint cluster region (bcr). We have been able to isolate cDNAs with homology to bcr, establishing that this region encodes a protein. By nucleic acid hybridization experiments the orientation of this protein-encoding region on chromosome 22 could be determined; the 5' end is toward the centromere of the chromosome. The cDNA clones also enabled us to do a comparative nucleotide sequence analysis and to determine if in Ph'-positive CML cells aberrant mRNAs are produced which are possibly chimerics of human c-abl and bcr. Evidence that such mRNAs are present in the CML cell line K562 and that in this cell line the breakpoint has occurred in the bcr region will be presented.

1) de Klein, A. et al. 1982. A cellular oncogene is translocated to the Philadelphia chromosome in chronic myelocytic leukaemia. Nature, 300:765 2) Bartram, C.R. et al. 1983. Localization of the human c-sis oncogene in Ph' positive and Ph' negative chronic myelocytic leukemia by in situ hybridization. Blood, 63:223 3) Heisterkamp, N. et al. 1983. Localization of the c-abl oncogene adjacent to a translocation breakpoint in chronic myelocytic leukemia. Nature, 306:239 4) Groffen, J. et al. 1984. Philadelphia Chromosomal Breakpoints Are Clustered within a Limited Region, bcr, on Chromosome 22. Cell, 36:93

0235 AN ALTERED C-ABL PROTEIN IS DETECTED IN Ph[1]-POSITIVE CML CELL LINES AND PATIENTS. Owen N. Witte[1], James B. Konopka[1] and Susan M. Watanabe[2], [1]Department of Microbiology and Molecular Biology Institute, University of California, Los Angeles, CA 90024, [2]Genetic Systems, Seattle, WA 98112

The Philadelphia chromosome (Ph[1]) results from a specific chromosomal translocation [t(9:22)] that is observed in greater than 90% of chronic myelogenous leukemia (CML) patients. The translocation breakpoint occurs near the c-abl gene and correlates with the synthesis of an altered c-abl mRNA. We have detected a structurally altered c-abl protein (P210) in Ph[1]-positive CML cell lines but not in control Ph[1]-negative cell lines. P210 was also detected in bone marrow cells from Ph[1]-positive CML patients. P210 has in vitro tyrosine kinase activity similar to the v-abl protein. However, the normal c-abl protein lacks detectable tyrosine kinase activity. Structural alteration of P210 may have unmasked its tyrosine kinase activity. Partial proteolytic analysis of P210 suggests that the alteration is likely to be N-terminal. Two-dimensional analysis of tryptic peptides generated from P210 and the normal human c-abl protein (P145) indicates that additional polypeptide sequences are present in P210. Possibly, the generation of Ph[1] results in the creation of a chimeric gene which directs the synthesis of an altered c-abl protein (P210). Abnormal expression of c-abl tyrosine kinase activity by P210 may have a growth stimulating effect similar to the v-abl protein. These results strongly implicate P210 in the pathogenesis of CML.

Growth Regulation and Differentiation

0236 REGULATION OF HEMATOPOIESIS BY NATURAL AND BIOSYNTHETIC (RECOMBINANT) HUMAN LYMPHOKINES, Judith C. Gasson[1], Richard H. Weisbart[2], Rodney M. Hewick[3], Gordon G. Wong[3], Stephen C. Clark[3], and David W. Golde[1], [1]Division of Hematology-Oncology, Department of Medicine, UCLA School of Medicine, Los Angeles, CA 90024, [2]Veterans Administration Medical Center, Sepulveda, CA 91343, [3]Genetics Institute, Boston, MA 02115

Stimulated human T lymphocytes and HTLV-infected T-lymphoblast cell lines produce a number of factors which promote the growth and differentiation of hematopoietic cells and activate mature blood elements. In order to study the biochemical and biological properties of these mediators, purified proteins are required. Molecular cloning of the genes for these factors makes it possible to study the genomic organization and regulation of gene expression, as well as facilitating the production of large quantities of pure protein for research and therapeutic use.

We have described the purification of human erythroid-potentiating activity (EPA) from medium conditioned by the Mo cell line (1). This 28,000 molecular weight protein stimulates the growth of human and murine erythroid colonies (BFU-E and CFU-E) but not myeloid colonies (CFU-GM). N-terminal amino acid sequence has been used to isolate cDNA clones encoding EPA. These cDNA clones have been used to identify and characterize genomic clones from a human library.

We have also purified a 22,000 molecular weight lymphokine from Mo-conditioned medium that acts upon myeloid cells. This single protein stimulates colony formation by human granulocyte-macrophage precursors (GM-CSF) (2), inhibits the motility of peripheral blood neutrophils (NIF-T) and activates the microbicidal pathways in granulocytes (3). cDNA clones encoding this GM-CSF have been isolated using expression screening of cDNA inserts prepared from Mo mRNA (4).

The physiological roles of EPA and GM-CSF in hematopoiesis are currently being studied. The potential therapeutic uses of biological response modifiers to increase the number and functional activity of hematopoietic cells are substantial.

1. Westbrook CA et al., J Biol Chem 259:9992, 1984
2. Gasson JC et al., Science, in press, 1984
3. Weisbart RH et al., submitted
4. Clark SC et al., submitted

0237 INDUCER-MEDIATED MODULATION OF GENE EXPRESSION DURING TERMINAL CELL DIFFERENTIATION, Paul A. Marks, Michael Sheffery, Jeffrey Ravetch, Tsuguhiro Kaneda, Takashi Murate, and Richard A. Rifkind, Memorial Sloan-Kettering Cancer Center, New York, NY 10021.

Studies in several systems indicate that the transition from precursor cells capable of self-replication to cells expressing characteristics of terminally differentiated phenotype, including terminal cell division, involves modulation of expression of a number of genes. We have examined the modulation of gene expression during terminal cell differentiation using as a model hexamethylene bisacetamide (HMBA) induced murine erythroleukemia (MEL) terminal cell differentiation. Present studies include: 1) a detailed examination of alterations in chromatin structure and transcriptional activity of α and β globin genes during inducer-mediated terminal differentiation, and 2) the study of expression of genes involved in cell cycle progression. In MEL, virus-transformed cells blocked at the CFU-e stage of erythroid cell development, we found that uninduced cells show certain chromatin structural changes which are associated with actively transcribed genes, e.g., increased sensitivity in DNase 1 and hypomethylation of α and β globin domains. These changes are propagated in cell division and do not change with uninduction. Other changes, namely, the appearance of sites hypersensitive to DNase 1 and S1 nuclease, appear 5' upstream to both α and β globin genes with induction. These inducer-mediated changes precede the onset of increased transcription of α globin genes. Studies on modulation of genes whose products may be involved in cell cycle progression show that the accumulation of p53 protein, which appears to play a role in the progression from G_1 to S, is suppressed by HMBA. This suppression in p53 synthesis correlates with cessation of cell division, with arrest in G_1. We have identified two other genes whose increased expression is related to cessation of cell division. These genes are cloned and being characterized. Thus, HMBA-induced MEL terminal differentiation involves the modulation of expression of several genes, both down and up regulation of genes involved in cell cycle progression and differentiated phenotype.

0238 MOLECULAR CONTROL OF GRANULOCYTE AND MACROPHAGE PRODUCTION, Donald Metcalf, Nicos A. Nicola, Nicholas M. Gough, Ashley R. Dunn, Francesca Walker, Antony W. Burgess, Ed Stanley and Anne Kelso, Walter and Eliza Hall Institute and Ludwig Institute for Cancer Research, Post Office, Royal Melbourne Hospital, 3050, Victoria, Australia.

The formation and function of murine granulocytes and macrophages is controlled by the interaction of four glycoprotein colony stimulating factors - GM-CSF, M-CSF (CSF-1), G-CSF and Multi-CSF (IL-3). Each has been purified to homogeneity and is active in stimulating the proliferation of granulocyte-macrophage (GM) precursors at concentrations in the 10^{-11} - 10^{-13} molar range.

Binding studies using ^{125}I G-CSF have shown the existence on normal and leukemic GM cells of specific membrane receptors (mean number 100 - 500 for normal GM cells, 300 - 700 for WEHI-3B D^+ leukemic cells). Half-maximal proliferative or differentiation-inducing activity is achieved with 5-10% receptor occupancy. Receptor binding is rapid (less than 10 min) but dissociation relatively slow (half-life 6 hr). Comparable binding of murine ^{125}I G-CSF has been observed to normal and leukemic human GM cells, binding being blocked by human CSFβ but not CSFα. Parallel studies using ^{125}I GM-CSF have indicated mean receptor numbers on normal marrow cells of 350. For both CSF's, receptor numbers and turnover are substantially lower than reported for the functionally similar M-CSF. At 20°C the binding of ^{125}I G-CSF to normal mouse BM cells but not leukemic cells can be partially blocked by GM-CSF or Multi-CSF. Similar competition for the binding of ^{125}I GM-CSF to BM cells was observed with G-CSF and Multi-CSF.

cDNA's for GM-CSF and Multi-CSF have been cloned from cDNA libraries constructed using mRNA from con A-induced LB3 cells, a cloned T-lymphocyte cell line with the inducible capacity to synthesize both GM-CSF and Multi-CSF. Genomic clones of GM-CSF and Multi-CSF have also been isolated. The gene for GM-CSF exists in single copy form and has been located tentatively on Chromosome 11. In each case, direct expression of the cloned DNA's has been achieved following transfection of COS cells with synthesis of up to 30 ng CSF/ml of conditioned medium. These CSF's exhibit all the known biological properties of purified GM-CSF and Multi-CSF.

Despite the ability of GM-CSF and Multi-CSF to stimulate the proliferation of the same GM clones, sequencing of the cDNA for GM-CSF has indicated no sequence homology of GM-CSF with Multi-CSF and a very different predicted secondary structure and these two functionally similar GM regulators appear to have developed independently.

0239 ONCOGENESIS BY RECEPTOR GENE TRUNCATION, A. Ullrich, L. Coussens, J.S. Hayflick, T.J. Dull, A. Gray, A.W. Tam, J. Lee, H. Riedel, Y. Yarden*, T.A. Libermann*, J. Schlessinger*, J. Downward+, E.L.V. Mayes+, N. Whittle+, M.D. Waterfield+, and P.H. Seeburg, Department of Molecular Biology, Genentech, Inc., South San Francisco, California 94080, *Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot, Israel, +Protein Chemistry Laboratory, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, UK.

Partial protein sequence analysis and the subsequent isolation and characterization of cDNA clones reveal close similarity between the entire predicted amino acid sequence of the avian erythroblastosis virus (AEV) transforming gene v-erb-B and the human epidermal growth factor receptor transmembrane and cytoplasmic domains. The single 23 amino acid transmembrane region separates a cysteine-rich 621 residue extracellular EGF binding domain from the 542 amino acid long, cytoplasmic, tyrosine-specific protein kinase domain. The receptor gene is amplified and rearranged in A431 epidermoid carcinoma cells and primary brain tumors (glioblastoma) resulting in receptor overproduction and generation of truncated gene products. Two approaches were taken to further investigate the involvement of EGF receptor gene products in oncogenesis. First, a variety of primary human tumors and leukemic cells were analyzed for possible aberrant expression of the receptor gene. Second, in vitro modification of the isolated receptor cDNA sequences and subsequent introduction and expression in tissue culture cells was carried out to identify molecular characteristics responsible for oncogene generation. Insights obtained from these experiments will be presented.

Regulation and Differentiation

0240 GENE EXPRESSION BY VIRAL IMMEDIATE EARLY PROTEINS, R.B. Gaynor, S. Yoshiwaga, N. Dean, L. Feldman, and A.J. Berk, Departments of Medicine, Microbiology, and Micro and Immunology, UCLA School of Medicine, Los Angeles, CA 90024

The adenovirus E1A and the pseudorabies virus immediate early (PRV-IE) proteins induce transcription from adenovirus promoters and are also capable of activating transcription of nonviral genes introduced into cells by infection or transfection.

We investigated whether this activation of viral or nonviral genes by E1A and PRV-IE proteins was limited to genes transcribed by RNA polymerase II (class II genes) or could also function for genes transcribed by RNA polymerase III (class III genes). Results of transfection experiments indicated that both viral and nonviral class III genes were induced 10- to 20-fold by the E1A proteins. This activation is due to the assembly of class III genes into stable transcription complexes. The simian virus 40 enhancer, which is a strong inducer of class II genes, did not induce transcription of a class III gene. Thus the mechanism of transcription activation by viral enhancers and the viral early proteins appears to be different.

We next investigated whether this difference could be observed in in vitro transcription experiments. Tenfold higher rates of in vitro transcription were observed with extracts of E1A-containing cells compared to uninfected cell extracts. This activation is not dependent on the presence of E1A in the extract. Thus the action of the E1A protein appears to be an indirect one.

0241 1,25 Dihydroxyvitamin D_3 and the Hematopoietic System. H.P. Koeffler, H. Reichel, A. Norman, Department of Medicine, University of California, Los Angeles, CA, and Department of Biochemistry, University of California, Riverside, CA.

Recent studies by ourselves as well as others suggest that the seco-steroid known as 1,25 dihydroxyvitamin D (1,25$(OH)_2D_3$) may be intimately involved in proliferation and differentiation of hematopoietic cells. Monocytes, macrophages, activated B and T lymphocytes, and cells from a variety of myeloid cell lines have receptors for 1,25$(OH)_2D_3$. The 1,25$(OH)_2D_3$ (10^{-8}-10^{-9}M) can induce macrophage differentiation of both normal human myeloid stem cells and blast cells of several myeloid leukemic lines (U937, HL-60). Indirect evidence strongly suggests that this induction of differentiation occurs through the cellular 1,25$(OH)_2D_3$ receptors. The presence of receptors does not, however, assure that leukemic blast cells can be induced to undergo differentiation by the seco-steroid. In a related study, we performed a clinical trial of giving 2 ug/dy of 1,25$(OH)_2D_3$ to 18 myelodysplastic patients and observed no improvement in hematopoiesis. In another series of experiments, we showed for the first time that normal human macrophages synthesize 1,25$(OH)_2D_3$ and recombinant gamma interferon (γ-IFN) markedly enhances production of the compound. Pulmonary alveolar macrophages (PAM) or blood monocytes from normal individuals were cultured with γ-IFN; the substrate [^{3}H]-25-hydroxyvitamin D_3 was added to the cultures and conversion of substrate to [^{3}H]-1,25$(OH)_2D_3$ was determined by high pressure liquid chromatography (HPLC). Human PAM from 4 normal individuals synthesized small quantities of 1,25$(OH)_2D_3$ and 24,25$(OH)_2D_3$. In contrast, the PAM cultured with γ-IFN (200 u/ml for 4 days) markedly increased their synthesis of 1,25$(OH)_2D_3$, with approximately 10-16% of the substrate converted to [^{3}H]-1,25$(OH)_2D_3$. The putative [^{3}H]-1,25$(OH)_2D_3$ was collected from HPLC and rechromatographed using three other solvent systems and was found to migrate in exact identity with authentic, chemically synthesized 1,25$(OH)_2D_3$ on each chromatogram. The PAM cultured with inactivated γ-IFN did not have an enhanced synthesis of 1,25$(OH)_2D_3$ as compared to unexposed PAM. Dose-response experiments showed that γ-IFN stimulated macrophages to synthesize 1,25$(OH)_2D_3$ in a linear fashion, with 200 u/ml γ-IFN stimulating 7% and 2000 u/ml γ-IFN stimulating 17% of the substrate to be converted to 1,25$(OH)_2D_3$. Time response experiments showed that by 1 day of exposure of macrophages to γ-IFN (500 u/ml), a maximal stimulation of synthesis of 1,25$(OH)_2D_3$ occurred. Maximal (plateau) synthesis of 1,25$(OH)_2D_3$ by macrophages continued for at least 6 days of exposure to γ-IFN; by day 8, synthesis of 1,25$(OH)_2D_3$ returned to normal. Also, peripheral blood monocytes were capable of synthesizing 1,25$(OH)_2D_3$ and γ-IFN enhanced production of the seco steroid at least 13 fold. The ability of human macrophages to synthesize 1,25$(OH)_2D_3$ may have important consequences for health and disease.

0242 DIFFERENTIATION AND PROLIFERATION OF HEMOPOIETIC STEM CELLS IN CULTURE, Makio Ogawa, VA Medical Center and Department of Medicine, Medical University of South Carolina, Charleston, SC 29403.

Determination of the mechanisms of stem cell self-renewal and differentiation requires the study of individual hemopoietic stem cells. This was enabled by our discovery of a unique type of murine colony consisting of primitive hemopoietic progenitors. Small blast cell colonies observable on day-16 of culture possess self-renewal capacity and the ability to replate with high efficiencies. Injection of a high-dose of 5-fluorouracil (5-FU) significantly enriched for progenitors of blast cell colonies in the spleen. Sequential observations of multilineage colony formation from blast cell colonies suggested that stem cell differentiation processes may be characterized by relatively constant and short generation times. The renewal process of stem cells appears to be accompanied by longer generation times. We subsequently obtained data suggesting that the decision of a stem cell to renew or differentiate is a stochastic process. Studies of lineage potentials of the progenitors with micromanipulation techniques suggested that the restriction of lineage potentials of the multipotential progenitors is also a stochastic process. This stochastic model of stem cell proliferation and differentiation assures the presence of a constant number of committed progenitors whose proliferation may be modified later. The proliferation of early hemopoietic progenitors requires the presence of IL-3 in culture, but IL-3 supports development in all lineages and does not appear to direct their differentiation to particular pathways. Studies of human progenitors in culture also suggest that the restriction of proliferative potentials of the committed progenitors is a random process. The distributional parameters for choices of actions in early processes of stem cell differentiation may not be under the influences of external factors. This notion is compatible with the very stable and long-lasting nature of cell renewal in the hemopoietic system. We believe that "fine tuning" of blood cell production is at the very late stages of differentiation such as erythroid colony-forming or cluster-forming units. It is reasonable to assume that these populations are the targets of the humoral control since these populations are already expanded and are available for immediate use when higher than normal production is necessitated.

Growth and Differentiation

0243 MORPHOLOGY AND CYTOCHEMISTRY OF FISCHER RAT LEUKEMIC CELLS - AN NK CELL LEUKEMIA. Sue A. Bauldry and G. Adolph Ackerman, Ohio State University, Columbus, OH 43210. Fischer rat leukemia, a spontaneous mononuclear cell neoplasm occurring in aged Fischer rats, is proposed to be a natural killer cell leukemia. We have studied leukemic blood and spleen cells and have found that they resemble large granular lymphocytes; cells characterized by a slightly indented nucleus and abundant cytoplasm that contained varying numbers of azurophilic granules and vacuoles; ingested erythrocytes were occasionally observed. Cytochemically, cells were positive for acid phosphatase and non-specific esterase but lacked PAS or peroxidase activity. Ultrastructural examination revealed well developed Golgi, abundant polysomes, large mitochondria and an extensive microtubular system. Granules varied in size, number and density with more "mature" granules occurring in cells from the blood. Numerous autophagic vacuoles containing myelin figures, cellular debri, etc., were evident. Ultrastructural localization of acid phosphatase activity was primarily in the RER and Golgi with infrequent staining of granules. Cells were aryl sulfatase positive with RER and the membrane surrounding the granules showing a strong reaction. Peroxidase activity was absent except after erythrophagocytosis when a few granules were noted to be peroxidase positive; ferritin was not observed in cells which had phagocytized erythrocytes and little evidence was seen of red cell breakdown. The majority of cells were negative for glycogen although a small subset did exist that contained glycogen rosettes. Thus, the leukemic cells proved quite heterogeneous with cells from the spleen appearing more immature than those of the blood. Our research supports the concept that this neoplasm is an NK cell leukemia and gives further information which may help to elucidate the cell line of origin of NK cells.

2044 DIMETHYLSULFOXIDE (DMSO) INHIBITS AUTONOMOUS PROLIFERATION OF HL-60 CELLS AND ENHANCES DEPENDENCE ON GRANULOCYTE-MACROPHAGE COLONY STIMULATING FACTOR(S) (GM CSF). James K. Brennan, Howard Higson, Kim Stagliano and Camille N. Abboud, University of Rochester School of Medicine, Rochester, NY 14642.
HL-60 cells in concentration of 1 x 10^3/ml were cultured in McCoys 5A medium with 10% fetal bovine serum in the presence of 0-1.5% DMSO and 0 or 10% medium conditioned by GCT cells (GCT CM) for 8 days. Viable cell numbers were followed with a particle counter and the number of population doublings/192 hours calculated. In 9 out of 9 experiments DMSO produced a dose-dependent inhibition of HL-60 growth rate in cultures without CM. GCT CM significantly enhanced growth at all DMSO concentrations, restoring growth to control or near-control values, except at a 1.5% concentration of DMSO. Qualitatively similar results were obtained with initial cell densities up to 1 x 10^5/ml, and on colony size when cells were cultured in methylcellulose medium. Increase in median cell volumes and ^{3}HTdr incorporation, but not cell maturation, preceded an increase in growth rates in GCT CM-stimulated liquid cultures and occurred within 2 days; divergence in growth usually occurring by 3 days. Similar effects were observed when semi-purified GM CSF (specific activity 1-3 x 10^7 colonies/mg protein) was substituted for GCT CM. These data indicate that DMSO can suppress autonomous growth of this leukemia cell population and restore dependency on CSF. Moreover, they may provide a practical basis for using this cell-line to measure CSF in biological materials.

0245 Differential Expression of G_1 Specific Cell-Cycle Genes in Human Leukemias, B. Calabretta, L. Kaczmarek, D. Venturelli, R. R. Hirschhorn and R. Baserga. Department of Pathology and Fels Research Institute, Temple University School of Medicine, Philadelphia, PA 19140

The phenotypic manifestation of human leukemias is usually reflected by the degree of maturation arrest and proliferative advantage of the neoplastic clone. We have recently begun to analyze the mechanisms of cell proliferation impairment that occurs in human leukemias by studying the expression of specific genes that are precisely regulated during the cell-cycle progression of normal human cells. We have identified three cDNA clones whose expression is specifically increased during the G_1 phase of the cellular cycle. These cDNA clones were selected from a cDNA library prepared from poly A+ mRNA derived from hamster ts13 cells 6 hours after serum stimulation at the permissive temperature of 34°C. We have found that these cell-cycle genes are evolutionary conserved in their sequence and cell-cycle dependence in human cells. We have also found that these cDNA clones are expressed in human leukemias with highest level of expression observed in patients with CML and the lowest in patients with CLL. Intermediate levels of expression were observed in AML and ALL patients. This differential pattern of expression is somewhat correlated with the type of cell proliferation impairment that characterizes different kinds of human leukemias.

0246 TWO NOVEL CYTOSKELETAL PROTEIN GENES ARE DEVELOPMENTALLY REGULATED IN A HUMAN LEUKEMIC CELL LINE. Chuan-Chu Chou, Janet P. Slovin, Richard C. Davis and Winston A. Salser, UCLA, Los Angeles, CA 90024

We have isolated novel human alpha-tubulin and beta-actin genes from a cDNA library for HL60 cells, using chicken tubulin and actin clones as probes. These human clones are distinct from all other reported alpha-tubulin and beta-actin genes in that they have: (i) untranslated sequences which have little homology to those of previously reported genes, (ii) amino acid deletions or drastic substitutions, e.g. Ser 4 to Cys 4 and Asp 69 to Arg 69 in alpha tubulin, and Ser 343 to Phe 343 in beta actin. Using the specific untranslated regions as probes on Northern blots, we demonstrated that both the alpha-tubulin and beta-actin mRNAs are differentially expressed in a variety of human tissues, and that, during the in vitro differentiation of HL60 cells to either granulocytes or macrophages, the expression of these genes is dramatically regulated. The HL60 alpha-tubulin gene described here is the first reported example of a human gene, in which the 5'-noncoding region of the messenger contains multiple AUG codons. All four AUGs are followed by in-frame termination codons, resulting in four short open reading frames all terminating 5' to the putative initiation site of alpha-tubulin translation, suggesting that the expression of alpha-tubulin gene may also be regulated at the level of mRNA translation. This is supported by our observation that during the differentiation of HL60 cells, the rate of alpha-tubulin protein synthesis increases and yet, in contrast, the concentration of alpha-tubulin messenger decreases.

0247 HL60 CELLS REGULATE THE EXPRESSION OF TRANSFECTED GENES DURING IN VITRO CELLULAR DIFFERENTIATION, Patrick Concannon, Michele M. H. Lau, Sharon N. Teraoka, Robert Nelson, and Winston A. Salser, UCLA, Los Angeles, CA. 90024

HL60 is a human promyelocytic cell line derived from a leukemic patient which is able to differentiate into either granulocytes or macrophages in vitro. Our laboratory has isolated and characterized over 25 cDNA clones corresponding to mRNA's whose expression is strongly regulated during the differentiation process. We have used these cDNA clones to isolate genomic clones from human chromosomal lambda libraries using the pi recombination system. These genomic clones and subfragments of them have been reintroduced into HL60 cells by cotransfection with pSV2neo and selection for G418 resistance. Approximately 25% of the clonal cell lines isolated from these cotransfections transcribe the reintroduced gene, as measured by the detection of the inserted pi plasmid sequences in the cytoplasmic mRNA of these cells. In all of the cell lines examined to date, the level of this mRNA is modulated during cellular differentiation in a manner consistent with that expected for the particular gene whose genomic clone was transfected. This is true for genes whose expression is either increased or decreased during cellular differentiation. In one case, this regulated expression has been achieved from a subfragment of a genomic clone that allowed us to localize the site at which presumed regulatory sequences reside to an area of approximately 2.8 kilobases. Such a result indicates that we may expect to find DNA sequences involved in developmental regulation within close proximity to the genes they control.

0248 MURINE ERYTHROLEUKEMIA CELL VARIANTS:ISOLATION OF CELLS THAT HAVE AMPLIFIED THE DIHYDROFOLATE REDUCTASE GENE AND RETAIN THE ABILITY TO DIFFERENTIATE, Robert E. Corin and Paul Szabo, Sloan-Kettering Institute, New York, NY 10021

A group of murine erythroleukemia cell (MELC) variants was generated by selection for the ability to grow in increasing concentrations of methotrexate (MTX). Growth of the parental MELC strain DS-19 was completely inhibited by 0.1 uM MTX. We isolated cells able to grow in up to 800 uM MTX, and for all variants growth was essentially the same in the presence or absence of the selective dose of MTX. MTX resistance was not the result of a transport defect. Variants had increased dihydrofolate reductase (DHFR) activities. The specific activity of DHFR was proportional to the selective concentration of MTX used to isolate a given variant. Hybridization studies performed in situ established the presence of amplified genes in MTX-resistant but not MTX-sensitive (parental) cells. Thus, MTX resistance was probably by virtue of increased levels of DHFR due to amplification of the DHFR gene. The variants retained the ability to be induced to differentiate as determined by hemoglobin production in response to dimethyl sulfoxide (DMSO), butyrate, and hexamethylene bisacetamide (HMBA). Variants were also inducible to terminal cell division by DMSO and HMBA. A DHFR gene amplified MELC was also isolated that was resistant to the action of inducers with respect to terminal cell division and hemoglobin production. Preliminary studies indicated that DHFR was regulated during induced differentiation of MTX-resistant MELC.

0249 ADHERENT-LAYER DEPENDENT MAINTENANCE OF A NEOPLASTIC PHENOTYPE IN LONG-TERM MARROW CULTURES FROM A PRELEUKEMIC CHILD, L. Coulombel, M. Derycke, M. Rault and G. Tchernia, Université Paris XI, 94270 Bicêtre, France

Short-term progenitor assays applied to AML or preleukemic marrow cells usually show reduced numbers of normal progenitors and variable numbers of progenitors of abnormal colonies and clusters. Studies of AML marrow in long-term cultures (LTC) have revealed heterogeneity in the behavior of neoplastic cells in this system also. An unusual result has now been obtained from studies of a 2 year old girl who presented with all the criteria required for a diagnosis of preleukemia. Methylcellulose assays revealed normal numbers of erythroid and granulopoietic progenitors. To test the possibility that conditions prevailing in LTC might allow expression of progenitors not able to differentiate in short-term assays, we established and maintained LTC for 12 weeks. The adherent layer was normal in morphology and total cellularity at 4, 6 and 8 weeks. However, progenitors (giving rise to either normal or abnormal colonies) were never detected in assays of the non-adherent and the adherent layer, although input values of BFU-E and CFU-C were normal, and a large population of granulocytic cells was maintained in the non-adherent fraction of the LTC. Four wk old adherent cells from these LTC did not inhibit the growth in methylcellulose of normal marrow cells or 4 wk old normal LTC adherent cells. A similar growth pattern has recently been observed in AML and preleukemic adult patients, although assays of the initial marrow in these cases usually do not detect normal progenitors only. This suggests that in some neoplastic disorders, the growth of neoplastic cells (maintenance of clonal dominance) in vitro might depend on interactions with non-hematopoietic elements in the adherent layer.

0250 "PRODUCTION OF COLONY STIMULATING FACTOR(S) FROM HUMAN SOMATIC TxT CELL HYBRIDS AND A CARCINOMA CELL LINE ACTIVE IN THE GROWTH OF MULTI-POTENTIAL HEMOPOIETIC PROGENITOR CELLS" Philip Crosier, Peter Macdonald and Peter Bissell, Department of Medicine, Christchurch Clinical School, P.O. Box 4345, Christchurch, NEW ZEALAND.

Human hemopoietic stem cell proliferation and differentiation is regulated by a family of glyco-protein growth factors. We have been investigating a number of sources of human hemopoietic growth factors which support the growth of CFUmix colonies in culture. The usual source of these growth factors is phytohaemagglutinin-stimulated leucocyte conditioned medium (PHA-LyCM). We here discuss the generation of a human T x T cell somatic cell hybrid line whose conditioned medium supports the generation of CFUmix in semi-solid culture. Details of producing this cell line will be presented. In brief, cells from a 6-thioguanine resistant Jurkat line were fused with pokeweed mitogen-stimulated peripheral blood lymphocytes.

In addition, we have been characterising the conditioned medium obtained from a fast-growing variant of a bladder carcinoma cell line. The medium from this line used with erythropoietin can replace the need for PHA-LyCM in the formation of macroscopic hemoglobinised erythroid bursts and multipotential colonies containing megakaryocytes, neutrophilic and eosinophilic granulocytes and monocyte-macrophages (CFUmix). Details will be presented on how the T cell hybridoma and bladder line conditioned medium support short-term cell proliferation in liquid culture of T cell-depleted cord blood cells. Results on preliminary biochemical analysis of these factors will be discussed.

0251 REGULATION OF ALPHA TUBULIN SYNTHESIS DURING DIFFERENTIATION OF THE HL60 HUMAN LEUKEMIC CELL LINE, Richard C. Davis, Chuan Chu Chou, and Winston A. Salser, UCLA, Los Angeles, CA. 90024

HL60 cells can be induced to differentiate from promyelocytes to either macrophage-like cells in response to phorbol esters (TPA) or vitamin D3, or to granulocyte-like cells in response to DMSO and amiloride. During both differentiation pathways, the cells dramatically change the rates of synthesis of more than 100 different proteins. We have used 2D gels to measure the relative rate of alpha tubulin synthesis in treated and untreated HL60 cells and have used northern blots to compare these rates of synthesis with the concentration of alpha tubulin message in similarly differentiated HL60 cells. Strikingly, the fraction of cytoplasmic poly A+ RNA with homology to an alpha tubulin cDNA probe decreases by more than 10 fold in cells treated with TPA, vitamin D3, or DMSO and amiloride, while the rate of alpha tubulin protein synthesis increases by at least 10 to 100 fold during monocytic or granulocytic differentiation. This unexpected discordance between alpha tubulin messenger concentration and protein synthesis has been consistently seen in cells of the original HL60 line maintained in our lab. The alpha tubulin cDNA clone we have isolated from HL60 has four short (15 to 66 bases) overlapping open reading frames (ORF's) in the region 140 to 230 nucleotides upstream of the translation start site. This is the first reported example of such ORF's in a human mRNA but it has been proposed that, in yeast, short ORF's in the 5'-untranslated mRNA have a role in translational regulation of protein synthesis. Such regulation could explain the high concentration of untranslated alpha tubulin mRNA in undifferentiated HL60.

0252 IN VITRO DEVELOPMENT OF B LYMPHOCYTES FROM LONG TERM CULTURED PRECURSOR CELLS, Kathleen A. Denis and Owen N. Witte, UCLA, Los Angeles, CA 90024

B lymphocytes stem cells and early B cell precursors have been difficult to isolate and characterize in vitro. Pluripotential hematopoietic stem cells and more mature elements of the myeloid and erythroid lineages can be maintained under culture conditions developed by Dexter (J. Cell. Physiol. 91:335). Innovations in long term culture also make it possible to specifically maintain B lymphocytes under a different set of conditions (Whitlock and Witte, PNAS 79:3608). By shifting cells cultured under Dexter conditions into B cell culture conditions, we have achieved the in vitro differentiation of B lymphocytes. In this manner, stem or early precursor elements may be channeled along the B cell pathway using culture manipulations.

Bone marrow cultures are established with corticosteroids at 33°C. Under these conditions, pluripotent stem cells and myeloid and erythroid cells were maintained for several months. No T or B lymphocytes were present. However, when these cells were shifted to culture conditions known to support long term B cell growth, production of myeloid and erythroid cells ceased and B lineage cells could be identified within 3-4 weeks. These lymphoid cells were 5-20% cytoplasmic mu positive and had a heterogeneous array of immunoglobulin gene rearrangements. Thus early precursor cells, perhaps pluripotent stem cells, can undergo committment and differentiation to the B cell lineage in vitro. This will aid in the definition of the cells and events important in early B cell development. (K.D. is a Lievre Fellow of the ACS, CA Div.)

0253 MORPHOLOGICAL AND BIOCHEMICAL COMPARISONS BETWEEN SPONTANEOUS AND TRANSPLANTED MONONUCLEAR CELL LEUKEMIA IN FISCHER 344 RATS. Michael P. Dieter, John E. French, and Robert R. Maronpot, NIEHS, P.O. Box 12233, Research Triangle Park, NC 27709 Mononuclear cell (MNC) leukemia was identified in 26 mo. old F344 rats by splenomegaly, reduced red cell and elevated white cell counts. Atypical MNC were predominant in spleen and blood with acentric nuclei and red cytoplasmic granules. Rate-limiting enzymes representative of major pathways of carbohydrate metabolism were elevated 100-900% in the enriched MNC fractions (Ficoll-Paque density gradients, 1.077 g/ml) isolated from spleen and blood. A leukemic MNC line was maintained by subcutaneous transfer of 2×10^7 spleen cells into 7-8 wk. old syngeneic recipients. In replicate experiments leukemia that was clinically and morphologically indistinguishable from spontaneous leukemia in aged rats was induced at 103 and 112 days after inoculation. The pattern of biochemical response in spleen MNC from transplanted cases was inversely related to the enzyme elevations previously noted in spontaneously leukemic rats, with 50-70% decreases in the specific activities of carbohydrate metabolizing enzymes. In addition, acetylcholinesterase activity decreased 34-85% in MNC of spleen and blood. Reversal of the expression of carbohydrate metabolizing enzymes in MNC may be related to a difference in the growth rate of the tumors or to selective destruction of the transplanted leukemic cells, and could be important in the study of chemotherapeutic intervention. Mononuclear cell acetylcholinesterase enzyme activity may serve as a non-destructive index of the severity of leukemia for this animal model, and may also prove to be a predictive indicator of the disease.

0254 AVIAN LEUKEMIC CELLS INDUCE FIBROBLASTS TO RELEASE CSF, William H. Dodge and Shanta Sharma, Wake Forest University Medical Center, Winston-Salem, NC 27103.

Confluent, quiescent chick embryo fibroblasts maintained in protein-free medium (M-199) released CSF(s) active on granulocyte and monocyte progenitors and on AMV-induced leukemic cells when exposed to formaldehyde-fixed AMV leukemic cells (held in protein-free medium). The CSF was assayed under serum-free conditions. All recent data indicates that avian "myeloblastosis" virus induces an acute monocytic leukemia (Boettiger & Durban, 1979, Cold Spring Harbor 44:1249; Jurdic et al, 1982, J Cell Physiol 2:85). Of several cell types tested, including populations enriched for normal blast cells, induction was limited to the leukemic cell and to the macrophage. Thus, the induction probably occurs via the monocyte/macrophage-derived recruitment activity (MRA) which induces fibroblasts to release CSF (Bagby et al, 1983, J Clin Invest 71:340). The fixed-cell data suggest that the factor is active even when membrane-bound. Production of such factors as well as an inhibitor which preferentially acts on normal progenitors (Bryant et al, 1981, Exp Hematol 9:457) probably insures a growth advantage for the leukemic cell. Production of MRA in an unregulated fashion could lead conceivably to a self-perpetuating cascade (leukemic cell→MRA→fibroblast→CSF→leukemic cell). This process would be exacerbated by the fact that these leukemic cells release CSF(s) and as with normal monocytes/macrophages (Glenn and Ross, 1981, Cell 25:603), release fibroblast growth factor [Dodge et al, 1983, Exp Hematol 11 (Suppl 14):48].

0255 EVIDENCE THAT MLA-144 DERIVED BURST PROMOTING ACTIVITY ACTS DIRECTLY ON PURIFIED PROGENITORS, R.E. Donahue, D.C. Linch and D.G. Nathan, Department of Pediatric Oncology, Dana Farber Cancer Institute, Boston, MA 02115 and Department of Haematology, School of Medicine, University College, London, England.

Burst promoting activity (BPA) has been demonstrated to be important in the growth and differentiation of erythroid progenitors in vitro. Several leukemic T cell lines are capable of producing BPA, but most of these cell lines are either TCGF dependent or are infected with the human T cell leukemia virus (HTLV). Conditioned medium (CM) from six T cell lines that were TCGF independent and were known not to produce HTLV were tested for BPA. These cell lines were a gibbon T cell line MLA-144, and the human T cell lines Jurkat, HSB-2, Molt-4, T-8402, and Hut-78. When added to methylcellulose cultures containing adherent cell depleted (Ad^-), non-E-rosetting (E^-) peripheral blood mononuclear cells, CM from the Jurkat and MLA-144 cell lines were capable of enhancing erythroid burst formation. This enhancement was not due to IL-2 production by these cell lines. Conditioned medium from the Jurkat and MLA-144 were then tested for BPA using highly purified progenitors as the target population. These highly purified progenitors were isolated from the Ad^-, E^- peripheral blood mononuclear cells by depleting granulocytes, monocytes, T cells, B cells, and NK cells using a panel of monoclonal antibodies and selecting for HLA-DR positive cells. Using these highly purified progenitors, MLA-144 conditioned HB101^{tm} serum-free medium enhanced burst formation, while the Jurkat CM failed to. This suggests that several factors may be involved in the formation of BFU-E, one of which may act on an accessory cell and another which can act directly on the erythroid progenitor.

0256 EVIDENCE OF ADHERENT LAYER DEPENDENT MAINTENANCE OF LEUKEMIC CELLS IN THE ABSENCE OF BLAST COLONY-FORMATION IN METHYLCELLULOSE, A.C. Eaves, L. Coulombel, D.K. Kalousek and C.J. Eaves, Terry Fox Laboratory, B.C. Cancer Research Centre, 601 West 10th Avenue, Vancouver, B.C. V5Z 1L3, Canada, Universite Paris XI, 94270 Bicetre, France

We have shown that marrow from untreated AML patients can be used to establish long-term cultures in which clonogenic hemopoietic cells are usually maintained for several weeks. In most cases progenitors of abnormal colonies/clusters initially present decline rapidly, and are not detectable after 4 weeks. In contrast, progenitors of morphologically normal hemopoietic colonies either continue to be detected at normal or near normal levels, or in some instances actually become detectable only after the first 4-5 wks in culture. However, in a few cases, progenitors of abnormal colonies/clusters have been found to persist (in both non-adherent and adherent fractions). We now describe a third pattern. The patient was a 79 yr old woman who presented with M2-AML (WBC count = 10,000/mm^3, 10% blasts, and 47% blasts in the marrow). No cells capable of forming any type of colony or cluster in methylcellulose cultures supplemented with 5% PHA-LCM and erythropoietin were detected either initially, or subsequently in assays of either the non-adherent or the adherent fraction of long-term cultures maintained in this case for 6 weeks. Nevertheless, in spite of the weekly removal of half of the non-adherent cells, blasts and differentiating myeloid cells were maintained in this fraction at levels above 2 x 10^6 per culture and the proportion of blasts remained relatively constant. These studies raise the possibility of leukemic cell responsiveness to as yet undefined stimulatory factors not found in PHA-LCM but present on the surface of, or released by, adherent marrow cell types.

0257 ISOLATION OF HUMAN FETAL LIVER PROGENITORS: A MODEL SYSTEM FOR THE STUDY OF LEUKEMIC PROGENITORS. Stephen G. Emerson and David G. Nathan, Harvard Medical School Boston, Ma. The pathogenetic events in the leukemias are believed to occur at the level of the leukemic progenitor cell, which populates the marrow pool with its partially differentiated progeny. We have utilized human fetal liver as a model system to develop a simple and efficient method for the purification of large numbers of committed progenitors from a larger population of variably differentiated cells. Low density, nonadherent fetal liver mononuclear cells were coated with a panel of eight mouse monoclonal antibodies specific for determinants of lymphoid, myeloid and erythroid precursor cells, and the antibody coated cells were removed by panning over plastic dishes coated with rabbit anti-mouse immunoglobulin. The nonadherent, panned cells are highly enriched for progenitors when assayed in methylcellulose cultures, containing 18-55% BFU-E, 3.2-15% CFU-GM and 0.5-2.8% CFU-GEMM. These cells are immature blasts with prominent Golgi, are DR^+, DC^-, HLE^+ and require less exogenous growth factors for in vitro differentiation than do adult marrow progenitors--CFU-GM require 2-5 fold less GM-CSA, and BFU-E growth is augmented only 0-25% by the addition exogenous BPA. This technique yields 2-30 x 10^6 progenitors per fetal liver sample, and thus provides enough progenitors to allow direct biochemical and immunologic manipulation. This panning technique is extremely flexible, and inclusion of the proper anti-myeloid precursor antibodies in the incubation mixture should permit direct application of this technique to the purification of leukemic progenitors from clinical samples, thus facilitating the direct study of leukemic progenitors and their differentiation.

0258 EVIDENCE FOR THE CLONAL DEVELOPMENT AND STEM CELL ORIGIN OF PHILADELPHIA POSITIVE T-CELLS IN CHRONIC MYELOGENOUS LEUKEMIA, A.A.Fauser, L.Kanz, G.W.Löhr, B.A.Cooper, Division of Hematology, Royal Victoria Hospital, McGill University, Montreal, and Department of Hematology, Med.Univ.-Klinik, Albert-Ludwigs-Universität, Freiburg,W.-Germany.

Studies on glucose-6-phosphate-dehydrogenase isoenzymes in female heterozygous with chronic myelogenous leukemia demonstrated that erythroblasts, granulocytes, platelets, and probably lymphocytes arise from a malignant multipotential progenitor cell, because the involved cells displayed only one single glucose-6-phosphate-dehydrogenase allele. Cytogenetic studies have provided further evidence that the various cell lineages involved in this disease contain the characteristic Ph' Chromosome. The origin and stage of differentiation of T-lymphocytes and B-cells in chronic phase of CML however is uncertain. A culture assay for pluripotent stem cells that form mixed colonies containing granulocytes, erythroblasts, megakaryocytes, macrophages and lympocytes (CFU-GEMMT) was used to determine whether T-lymphocytes and B-cells can be identified in such colonies in CML. Multilineage hematopoietic colonies from one patient with Ph' positive CML in chronic phase were examined for lymphoid markers. Subclones derived from prescreened primary mulilineage colonies were analyzed for lympopoietic cells and were subjected to cytogenetic studies.The result indicates that T-cells arise from the malignant CML-clone as they were identified in multilineage and subcloned colonies by their reaction with monoclonal Anti-T-cell antibodies. Cells from the same subcloned colony contained the Ph' chromosome. B-cell associated antigen positive cells were also identified in Ph' positive mixed colonies; whether subclones of these cells contain the Ph' chromosome remains to be determined.

0259 DIFFERENTIATION OF CLL CELLS

M.Ferrarini, S.Guazzi, R.Sitia, S.Roncella and A.Rubartelli - I.S.T. Genova, Italy

Cells from 15 patients with B CLL were stimulated in vitro with supernatants of mitogen activated T cells (TF) and studied for their capacity of differentiating into secreting cells. In seven patients only was a response detected. Their cells appeared to have undergone a maturation process already in vivo since they comprised a number of cells with ultrastructural features of more mature cells and were capable of secreting amounts of fully assembled Ig molecules in endogenous labelling experiments. The cells from the remaining patients had more immature morphological feature and did not secrete but degraded secretory molecules. Cells that did not respond to TF were not blocked as for their maturation capacities since TPA treatment induced an apparently normal secretion. Studies on the mRNA of the Ig heavy chain, showed that, following stimulation with TF, there was an increased synthesis of mRNA of the secretory chain accompanied by a decreased synthesis of that of membrane chain, a change that paralleled that seen for the production of the corresponding polypeptide chain. The pattern of mRNA production following TPA stimulation was more complex and varied in the different cases. Analysis of the factors contained in TF showed that BCDF was responsible for the differentiation process. IL2 had also some effects on the malignant cells since a number of them expressed the relevant receptor.

0260 **SWITCH IN ULTIMATE MATURE LINEAGE OF HL-60 PROMYELOCYTIC LEUKEMIA CELLS IN RESPONSE TO ALKALINE CONDITIONS** Steven A. Fischkoff and Margaret E. Condon, University of Maryland Cancer Center, Baltimore, MD 21201

The HL-60 promyelocytic leukemia cell line is capable of selectively differentiating to neutrophils, macrophages or eosinophils in response to different chemical stimuli. In the latter case, mildly alkaline growth conditions have been shown to promote eosinophilic differentiation. This effect is most marked when the culture medium is allowed to become exhausted; few eosinophils are seen in cells growing vigorously at pH 7.6. We now report that among the diverse list of compounds known to induce neutrophilic differentiation of HL-60 cells, there is a subgroup that selectively induces eosinophilic differentiation of up to 80% of cells previously grown at pH 7.6, but induces neutrophilic differentiation in cells previously cultured at pH 7.2 or below. The alkaline conditions need not be maintained during the maturation period for efficient inductions. These observations suggest two conclusions. First, lineage commitment and maturational commitment can be separately manipulated in this multipotential cell line so may be distinct processes. Second, various maturation inducers likely operate by different biochemical mechanisms in the cell. Furthermore, this two-stage approach permits more uniform, extensive and complete inductions of eosinophilic differentiation than the prior one-step technique.

0261 PRODUCTION OF B CELL GROWTH FACTOR BY NEOPLASTIC HUMAN B LYMPHOID CELLS, R.J. Ford, D. Kwok, M Cramer, S. Sharma, C.G. Sahasrabuddhe, M.D. Anderson Hospital; Houston, Texas 77030

Recent studies have shown that normal B lymphocyte proliferation is dependent on a lymphokine growth factor, BCGF, that is produced by activated T lymphocytes. The growth factor has been shown to exist in both low (14-16 kD) and high (>50kD) molecular weight (mw) forms, which are able to sustain growth in activated normal human B cells. We have also found that various types of human neoplastic B cells, obtained directly from the patient, including non-Hodgkin's lymphomas (NHL) and the chronic B cell leukemias, hairy cell leukemia (HCL) and chronic lymphocytic leukemia (CLL) can proliferate in vitro in response to BCGF, and that the neoplastic cells can be grown in vitro for extended periods of time if BCGF is provided. Our most recent studies indicate that a variety of neoplastic human B cells such as HCL, also contain a high mw BCGF in their cytoplasm and can secrete both high and low mw forms of the growth factor into cell culture supernatants. Both the cytoplasmic and secreted form of the growth factor from neoplastic B cells can stimulate growth in long term BCGF dependent normal human B cell lines, as well as in the autochthanous neoplastic B cells. These findings suggest that at least some types of human B cell neoplasms can proliferate by autocrine stimulation, and that the growth factor for these tumor cells is similar if not identical to the normal BCGF molecule is involved in neoplastic B cell growth. Characterization of the tumor cell derived factors also suggests their homology to the normal T cell-derived BCGF molecules.

0262 BIOLOGICAL CHARACTERIZATION OF A LEUKEMIC CELL DIFFERENTIATING FACTOR FROM CONDITIONED MEDIUM OF A HUMAN BLADDER CARCINOMA CELL LINE, 5637.

Janice Gabrilove, Karl Welte, Paul Harris, Erich Platzer, Roland Mertelsmann and Malcolm A.S. Moore. Sloan-Kettering Institute, NY,NY 10021.

We have identified a differentiation factor for the human promyelocytic leukemic cell line HL-60 and the human monoblast/monocytic cell line U937, constituitively produced and released by the human bladder carcinoma cell line 5637. The purification utilized sequential ammonium sulfate precipitation, anion exchange chromatography, AcA 54 gel filtration, reverse phase high pressure liquid chromatography (RPHPLC) and hydroxylapatite column chromatography. This factor has the capacity to induce the differentiation of HL-60 cells in a clonal agar assay and to induce the expression of chemotactic peptide receptors (fMPLF) in HL-60 and U937 cells, in a suspension culture system. In addition, this factor has minimal differentiation activity on WEHI-3BD+ leukemic cells, when assayed in a clonal agar culture. This factor also exhibits weak proliferative capacity for normal human bone marrow granulocyte-macrophage progenitors and exhibits no capacity to promote the growth of human mixed colony progenitors (CFU-GEMM), or human early erythroid progenitors (BFU-e). This factor appears to be biochemically and biologically distinct from the human pluripotent CSF, which we have previously purified to apparent homogeneity, from this same source of conditioned medium.

0263 PURIFIED rIL-2 CAUSES THE DIFFERENTIATION OF "NULL" CELLS INTO KILLER LYMPHOCYTES Elizabeth A. Grimm, Jack A. Roth, SNB, NINCDS, NIH, Bethesda, MD 20205

Evidence from our laboratory demonstrates that purified recombinant interleukin-2 (rIL-2) is not solely a growth factor, but also that it can induce differentiation of null lymphocytes (non T, non B, non NK). Within 48 hours of exposure of null lymphoid populations to IL-2 in vitro, the expression of T cell markers can be detected (OKT3, OKT8, HLA-Dr). These activated killer cells also express a novel killing activity that is preferentially directed toward NK resistant tumors and modified self (TNP-PBL). The tumor target spectrum includes leukemia and lymphoma cells from both human and murine sources, as well as murine 3T3 cell lines transfected with human oncogenes (Ha-ras or N-ras). Lymphokine activated killer (LAK), have shown therapeutic efficacy in murine adoptive therapy experiments, significantly increasing the survival of tumor bearing animals. Therefore, rIL-2 may be a useful therapeutic modality, both for differentiation of T lymphocytes and for activation of an antitumor cell killing mechanism.

0264 INDUCTION OF DIFFERENTIATION AND INHIBITION OF PROLIFERATION IN MURINE LYMPHOCYTES BY DUALTROPIC MINK CELL FOCUS-INDUCING MURINE LEUKEMIA VIRUS, Mark D. Howell and Nathan O. Kaplan, Dept. of Chemistry and Cancer Center, UCSD, La Jolla, CA 92093.
Differentiation of murine lymphocytes is often accompanied by the production of infectious murine leukemia virus (MuLV) or MuLV gene products. Despite many such correlations suggestive of a role for MuLV in lymphocyte differentiation, a virus with this activity has yet to be described. We now report the isolation, by mink lung cell (MLC) co-cultivation, of two such viruses. These dualtropic, mink cell focus-inducing MuLVs are unique in that they: 1) are of athymic mouse origin; 2) grow to considerably high titers in MLCs; and 3) induce differentiation and inhibit proliferation in murine splenocytes. When splenocytes from Balb/C mice heterozygous for the athymic allele are cultured with viral supernatants, lymphoblast formation is observed within 48 hours and is followed by the appearance of plasma cells in 3-4 days. This activation is accompanied by an increase in protein synthesis, but surprisingly not by a proportional increase in DNA synthesis. Following treatment with Con A, splenocyte DNA synthesis is markedly inhibited by viral supernatants, but lymphoblast formation and protein synthesis are synergistically enhanced. Neither differentiation nor the inhibition of proliferation is induced by uninfected MLC supernatants. The properties of these viruses suggest that they may possess a unique class of genes intimately involved in the normal processes of growth and differentiation in murine lymphocytes.
Supported by NIH grant #CA11683 and NCI Postdoctoral Training Grant #CA09290-06.

0265 LEUKEMIC CELLS CONSTITUTIVELY RELEASE FACTORS ACTIVATING IN VITRO MALIGNANT AND NORMAL T-CELLS. V. Georgoulias, M. Allouche, G. Kosmatopoulos, C. Jasmin.
Peripheral blood T colony-forming cells (T-CFC) from patients with T-cell malignancies can generate colonies in methylcellulose in the absence of added growth factors and/or mitogenic stimulation. In 50% of the patients, the spontaneous plating efficiency was low (less than 100 colonies/$5x10^5$ seeded cells) (1,2). In 6 patients with T-cell Acute Lymphoblastic Leukemias (T-ALL) presenting a low spontaneous plating efficiency, the activation state on the proliferation capacity of leukemic cells was studied. In 5 of them semi-purified or/and cloned bearing TCGF enhanced the colony growth. Although 2 of 6 patients displayed fresh leukemic cells bearing TCGF receptors (14% and 34% Tac^+ cells, respectively), in all patients a 48-96 h incubation of leukemic cells in the absence of growth factors and mitogenic stimulation allowed the expression of TCGF receptors. The increase of Tac^+ cells was concomitantly associated with an increased proportion of $HLA\text{-}DR^+$ (55-73%) and $OKT9^+$ (39-63%) cells. Semi-purified or/and cloned TCGF induced secondary colony formation from these cells which could be inhibited by anti-Tac mAB(57-100%). Incubation of normal T^+ lymphocytes for 48h with media conditioned by leukemic T-cells, induced the expression of TCGF receptors and HLA-DR antigen (4-19% and 9-26% respectively). Semi-purified TCGF induced cell proliferation and colony formation from these cells in the absence of mitogenic stimulation. These results seem to indicate that leukemic T cells release spontaneously factors which can activate in vitro both leukemic cells (autostimulation) and normal T lymphocytes as do mitogens and antigens.
1. Georgoulias et al. Int. J. Cancer, Oct. 1984 (in press).
2. Georgoulias et al.Leuk. Res., Oct. 1984 (in press).

0266 ISOLATION OF A MYELOMONOCYTIC TUMOR CELL LINE FROM THE SPLEEN OF MYELOPROLIFERATIVE SARCOMA VIRUS INFECTED MICE : SECRETION OF A MIXED-COLONIES PROMOTING ACTIVITY (MPA) A.M. BEVILACQUA-BERTOLI, F. SMADJA-JOFFE, M.C. LE BOUSSE-KERDILES, V. DEGIORGIS, M.A. BUENDIA, M. LONGUET and C. JASMIN.
Clonogenic tumor cells were sought in the spleen of DBA/2 mice with the myeloproliferative syndrome induced by the Myeloproliferative Sarcoma Virus (MPSV). These cells could be isolated because of their ability to proliferate on the greater omentum of sublethally irradiated isogenic recipient mice. The tumor masses obtained could be transplanted subcutaneously into non-irradiated isogenic recipients. Using a subcutaneous tumor obtained in this way, we have established a permanent suspension myelomonocyte cell line in vitro (TE_8). The line is tumoral, clonogenic in semi-solid medium containing agarose and produces MPSV virus particles. It also secretes a Colony Stimulating Activity (CSA), an activity termed Mixed Colony Promoting Activity (MPA) which enables pluripotent hematopoietic stem cells (HSC) to proliferate and differentiate, and an activity (perhaps Interleukin 3) enabling Factor Dependent Cell Line Paterson (FDCP) to proliferate. TE_8 cells proliferate in the absence of exogenous growth factors, requiring relatively little serum (2%) in the culture medium. In addition, they can differentiate through the granulomacrophage cell line independently of exogenous factors other than those which may be present in the serum. This differentiation, both granulocytic and macrophagic, was also observed in the clones obtained from this line, suggesting that MPSV transforms granulomacrophage progenitor cells.
Finally, the secretion of an MPA like activity by the tumor cells would be sufficient to explain the proliferation of pluripotent and committed stem cells which may provoke the Myeloproliferative syndrome characterizing the MPSV-induced neoplasia.

0267 T CELL COLONY-FORMING CELLS FROM CHRONIC MYELOID LEUKEMIA PATIENTS IN LYMPHOID BLAST CRISIS, M. Allouche, A. Bourinbaiar, V. Georgoulias, R. Consolini, H. Auclair and C. Jasmin - U 268, Hôpital Paul Brousse, 94800 Villejuif (France).

Cytochemical and immunological analysis of cells obtained from two patients with chronic myeloid leukemia during lymphoid blast crisis reveals markers suggestive of an immature T cell phenotype. Peripheral blood mononuclear cells (PBMC) from both patients generated spontaneous lymphoblastoid colonies in methylcellulose, a phenomenon observed in T-acute lymphoblastic leukemia (ALL) and T-non-Hodgkin's lymohoma (NHL) but not in any other type of leukemia (1,2). Colonies derived from one patient expressed predominantly OKT_3 (89% of the cells) whereas those from the second patient expressed OKT_3 and OKT_6 in equal proportions (42%, 43%). In the second patient's colonies, 5 out of 5 mitoses contained the Philadelphia chromosome (Ph') and 2 out of 5 displayed the same additional karyotypic abnormalities as the blast crisis cells. Cells obtained from the two patients during remission still gave rise to T cell colonies (85% OKT_3^+) and Ph^1 was detected in 33% and 60% of the metaphases respectively. However, when colony growth was induced by an interleukin 2-containing conditioned medium, less than 5% of mitoses were Ph'-positive. These date suggest that : 1) the T cell lineage might be involved in CML; 2) a subset of T cells may remain unaffected by the leukemic process, as demonstrated by the virtual absence of Ph^1 in induced T cell colonies, and 3) the spontaneous colony assay seems to select for the growth of malignant T cells.

0268 MATURATION ARREST FACTOR OF CANINE MEGAKARYOCYTIC LEUKEMIA, Thomas G. Kawakami, University of California, Davis, CA 95616

Dog and man who are acutely or chronically exposed to radiation have been reported to have a higher incidence of myeloproliferative diseases. Recently, a dog that was chronically exposed to gamma radiation in the 60-cobalt field developed megakaryocytic leukemia with a high peripheral blood count consisting of 90% undifferentiated megakaryoblasts. Separating leukemic cells by sedimentation rate method, it was found that 20-40% of the leukemic cells were in an active S-phase by ^{3}H-thymidine incorporation while 60-80% of the cells were at maturation arrest. The leukemic cells with an active S-phase were found to undergo maturation arrest with time, while leukemic cells at maturation arrest were capable only of undergoing differentiation to a more mature cell stage. Both serum and cell products of the leukemic dog were effective in inducing maturation arrest of proliferative leukemic cells. In an attempt to determine the source of maturation arrest factor (MAF), leukemic cells that were metabolically labelled with 35-methionine were found to synthesize the MAF. Preliminary characterization study of the MAF indicates that it consists of 2 peptides that can regulate DNA synthesis and differentiation of the megakaryoblasts. Future studies will be directed toward establishing the mechanism by which these peptides induce maturation arrest and clinical expression of leukemia.

0269 A NOVEL HEMATOPOIETIC INHIBITORY PROTEIN FROM CULTURED AIDS BONE MARROW Ira Z. Leiderman, Michael L. Greenberg, Bernard R. Adelsberg, Frederick P. Siegal, Mount Sinai School of Medicine (CUNY), New York, NY 10029

Inhibitors of granulopoiesis have been well described in numerous acute diseases and syndromes. Many of these inhibitors are T-lymphocyte mediated while others are factors released by specific cell populations. We have previously reported (Clin Res 32:2,351A) that bone marrow (BM) cells from patients with the Acquired Immune Deficiency Syndrome (AIDS) suppress normal proliferation of the granulocyte-macrophage progenitor cell (CFU-GM), whether the AIDS BM cells are in direct contact with or in feeder layers beneath the normal cells. A cell free conditioned media (CM) prepared by the liquid culture of BM cells from patients with AIDS or the AIDS related complex (ARC) inhibit CFU-GM growth in a manner similar to the BM cells alone. CM prepared from normal BM cells had no such effect. Polyacrylamide gel electropheresis (PAGE) demonstrated a unique band in lanes containing AIDS and ARC CM but not in the control lanes. Periodic acid-Schiff staining revealed it to be a glycoprotein (gp). A molecular weight for this gp was determined to be ≈84 Kd by SDS-PAGE. Eluates of this band from preparative PAGE inhibited hematopoietic proliferation similar to the complete CM. This hematopoietic inhibitory protein (HIP) did not inhibit mitogenic stimulation of normal T-lymphocytes. CM from AIDS BM cells depleted of adherent cells did not have the unique band though non-depleted CM from the same patients contained the band. We are presently determining the effect of HIP on the growth and differentiation of other BM progenitors while concurrently investigating its origin.

0270 AUTOSTIMULATORY GROWTH OF A PRE-B ALL CELL LINE. Glenn Miller, Karl Welte, Karen Holloway, Malcolm Moore and Roland Mertelsmann. Memorial Sloan-Kettering Cancer Center, New York, NY 10021.

The study of growth factors important to the proliferation of leukemic cells has relevance in the understanding of growth patterns, differentiation and avenues of treatment of this disease. The pre-B ALL cell line Nalm-12 was adapted to serum-free growth by culturing cells in a medium(SFM) consisting of RPMI 1640, 0.04% fatty acid free BSA, 2.5 µg/ml transferrin and 1% glutamine. Serum-free medium conditioned by Nalm-12 cells(SF-CM) was removed at five day intervals. SF-CM was tested for autostimulatory activity by culturing Nalm-12 cells in SFM at a non-self-supporting concentration in the presence of serial doubling dilutions of SF-CM. Proliferation of cells in the presence of SF-CM was measured at day three by uptake of ^{3}H-TdR. Crude SF-CM induced proliferation of Nalm-12 cells as well as of all hematopoietic cell lines tested. Normal peripheral blood mononuclear cells, however, were not affected by this factor. Growth curve analysis of serum-free Nalm-12 cultures revealed increased growth of cell in the presence of SF-CM. Preliminary characterization of this growth factor has resulted in an approximate molecular weight of 2000-4000. The factor was stable at pH 2.0 but was substantially inactivated after heating to 96^{o}C for 2 min.. Further biological and biochemical characterization of this constituitively produced growth factor is currently underway.

0271 THE EFFECTS OF 1:25 DIHYDROXY VITAMIN D_3 ON WEHI-3 GROWTH AND DIFFERENTIATION, Penelope A. Morel*, Dale R. Wegmann*, D.M. Provvedini+, S.C. Manolagas and Jacques M. Chiller*, Lilly Research Laboratories*, and UCSD, V.A. Medical Center+, La Jolla, CA 92037

$1:25(OH)_2$ vitamin D_3, the active metabolite of vitamin D can promote the differentiation of monocytes towards macrophages and osteoclasts. Specific receptors for $1:25(OH)_2D_3$ have been demonstrated in several immune cells, suggesting a role for this hormone in the immune system. We have studied the murine myelomonocytic cell line WEHI-3 for the presence of $1:25(OH)_2D_3$ receptors and determined whether this hormone modulates some of the known activities of this cell. Large numbers of specific receptors for $1:25(OH)_2D_3$ were detected in WEHI-3 cells (KD 3.3 x 10^{-10}M) and were found to be functional DNA binding receptors. $1:25(OH)_2D_3$ added to WEHI-3 in vitro had the following effects: 1) Inhibited proliferation; the recovered cells appeared to be blocked in the GO/Gl stage. 2) Enhanced adherence to plastic. 3) In combination with IFN-γ, the expression of Ia normally induced by IFN-γ was enhanced 2-3-fold. Such cells were also enhanced for their capacity to stimulate antigen specific Ia-restricted activation. 4) $24:25(OH)_2D_3$, an inactive metabolite, did not influence any of these effects. These data suggest that $1:25(OH)_2D_3$ may play a role in modulating the IFN-γ induction of Ia antigens and thus permit the cell to function more efficiently as an antigen presenting cell.

0272 PRODUCTION OF A-MULV "TRANSFORMED" PRE-PRE B CELLS WHICH ARE GROWTH FACTOR DEPENDENT Gregory J. Palumbo, Brad Ozanne, and John R. Kettman, University of Texas Health Science Center at Dallas, Dallas, Texas 75235

We have found that a combination of Witte-type bone marrow culture (MWCSN) and ANN-1 (ANN-1SN) conditioned medium lead to a 300-400% increase in efficiency of A-MuLV induced transformation of bone marrow cells in vitro. Upon removal of cells from agarose and culture in liquid (in conditioned medium), these early transformants exhibit poor growth for several months before entering a crisis period. Through formal limiting dilution analysis we can show growth dependence of these early transformants on a factor(s) present in our conditioned medium (MWCSN and ANN-1SN). Further analysis indicates that these cells are only dependent, in a dose dependent manner, on a factor(s) present in MWCSN. As the concentration of MWCSN is increased there is a concommitant increase in cells entering into cell cycle and their burst size. However, with continued passage factor-independent variants arise within the clone (crisis) with these cells now having excellent growth characteristics in liquid. The factor independent cells (late transformants) are malignant in vivo, B220^{+}, 2XJ? of the immunoglobulin heavy chain region rearranged, surface and cytoplasmic-µ negative pre-pre B cells.

0273 MODULATION OF NORMAL AND LEUKEMIC HUMAN MARROW CFU-GM HLA-DR (Ia) EXPRESSION. Louis M. Pelus, Karl Welte, Sloan Kettering Institute, N.Y.,N.Y. 10021.

Expression of Ia antigen on CFU-GM, BFU-E and CFU-GEMM and their response to in vitro inhibition by prostaglandin E (PGE) and acid ferritins (AIF) are related to the proportion of CFC in S-phase of the cell cycle and can be modulated by preexposure to PGE (10μ-10pM) in vitro. In patients with CML, CFU-GM Ia-antigen expression is absent or quantitatively deficient and corresponds with absent or reduced sensitivity to inhibition by PGE and AIF. However, culture of CML CFU-GM with PGE for 24 hrs in suspension culture restores normal CFU-GM Ia expression and sensitivity to PGE and AIF, to PH+ CML CFU-GM. The ability of PGE to modulate CFU-GM Ia antigen and growth sensitivity requires HLA-DR$^-$ T-lymphocytes having the cytotoxic/suppressor phenotype (OKT8+, Leu2+, Lyt3+ (E+)) as determined using mAbs+C'. Positive selection of marrow Lyt3+ cells using mAb (panning, purity >98%) and readdition to autologous T-depleted marrow (T$^-$BM) confirmed the T-cell requirement. Exposure of Lyt3+ cells to OKT8 or Leu2 mAb prior to addition to T$^-$BM blocked the restoring effect of these cells. Media conditioned (CM) by positively selected (panning, FACS sorting) OKT8+ but not OKT4+ cells replaced the effect of T-cells. Biochemical analysis of CM by ACA filtration, DEAE-, blue agarose- and procion red agarose- chromatography followed by reverse phase HPLC indicates a glycoprotein of MW 15,000-35,000, distinct from IL2, interferon, IL3 and GM-CSF. These results indicate a role for T-cells and PGE in regulating CFU-GM cell cycle, Ia-antigen expression and as a consequence, sensitivity to PGE and AIF.

0274 MAJOR PLASMA MEMBRANE CHANGES ACCOMPANY THE TRANSITION OF MYELOBLAST TO PROMYELOCYTE, J. Peyman and A.K. Sullivan, McGill Cancer Centre and Division of Hematology, Royal Vistoria Hospital, McGill University, Montréal, Québec, Canada, H3G 1Y6.

A major step in myeloid maturation is the transition from myeloblast to promyelocyte and, it is between these limits that the phenotype of most myeloid leukemia cells appears to be expressed. As a model to study this process we have compared the cell surface products of the human promyelocytic line HL60 and its blastic derivative HL60-D (Fitz-Gibbon *et al*, Br. J. Hematol. 55:311,1983).

When adequate precautions were taken to inhibit proteolysis caused by primary granule enzymes the following was observed:

(1) Incorporation of ^{35}S-methionine into total membrane protein produced very similar profiles on SDS-PAGE analysis.
(2) Labelling of more peripheral surface structures by methods of periodate oxidation-NaB^{3}H4 tritiation and lactoperoxidase-catalysed iodination revealed very major differences.
(3) Analysis of the major high mannose glycoproteins by affinity chromatography on Con A-Sepharose also confirmed major changes.
(4) Examination of the specificity of iodination catalysed by endogenous myeloperoxidase indicated a non-random labelling pattern.

In conclusion, it appears that, as reflected in these cultured cells, there may be major changes in the cell surface that accompany the acquisition of primary granules as the myeloblast matures to a promyelocyte.

0275 A STOCHASTIC MODEL FOR MAST CELL PROLIFERATION, Pamela N. Pharr, Jerry Needleman*, Heather P. Downs, Alan J. Gross and Makio Ogawa, Medical University of South Carolina and VA Medical Center, Charleston, SC 29403 and *Clemson University, Clemson, SC 29631.

Our previous study showed that pure mast cell colonies could be serially replated. The replating efficiency of cells in the primary colonies varied over a wide range suggesting a stochastic process. We posed the question as to whether the birth-death model developed by Till et al (1) for the self-renwal of stem cells could be used to describe the proliferation of mast cells. A modification of this model was developed which fitted our data on secondary mast cell colony formation reasonably well. According to the model, each secondary mast cell colony starts one proliferative cell. At each generation, each cell chooses among three possibilities: 1) division into two proliferative cells; 2) division into two non-proliferative cells; or 3) disappearance. At each step, a non-proliferative cell either does nothing or disappears. A computer simulation of this model was tested against data for the number and size of secondary mast cell colonies recorded after different culture periods. The distribution of colony size showed a large number of small colonies and a long spread in the distribution emcompassing large colonies. The model could be fitted to the data by appropriate choice of parameters.

1. Till JE, McCulloch EA, Siminovitch L: Proc Natl Acad Sci USA 51: 29-36, 1964.

0276 PHYSIOLOGY OF ADHERENT STROMAL LAYER FORMATION IN SERUM FREE HUMAN LONG-TERM BONE MARROW CULTURE, August J. Salvado, Richard C. Meagher, Daniel G. Wright, WRAIR,Wash. DC. Investigation of critical nutrient requirements has resulted in development of completely defined serum free(SF) methods for assays of committed hematopoietic precursors. No analogous data for Dexter type human long-term bone marrow cultures(LTMC) is available. Since hematopoiesis in LTMC is dependent upon the initial formation of an adherent layer of stromal cells any defined SF system must first support establishment of such an adherent layer. We previously reported that defined medium supplemented with horse serum lipoprotein (Lp) will not support establishment of an adherent layer in human LTMC. However, addition of the proteoglycan heparin to this medium allows development of a morphologically distinct adherent monolayer. Heparin indirectly prevents the downregulation of HMG-Co reductase, the critical enzyme in de novo cholosterol systhesis. 25 OH-cholesterol is a potent direct inhibitor of HMG-CoA reductase. Addition of 10ug/ml of 25 OH-cholesterol completely inhibited adherent layer development in both serum and SF LTMC at 2-3 wks. Further, during the first 3-4 wks of culture returning half the nonadherent cells to each SF LTMC results in at least 5-10% greater coverage of culture surface area compared to controls. The effect of O_2 tension was also investigated. Low O_2 tension resulted in $<10\%$ of the culture surface coverage in serum fed cultures that was observed with high O_2 controls. SF LTMC demonstrated that low O_2 resulted in approximately 60% of the culture surface coverage observed with high O_2 controls. In conclusion we have found that a)development of an adherent monolayer in serum fed or SF human LTMC appears dependent upon de novo cholesterol systhesis and b) thisprocess is aided in SF LTMC by return of half the nonadherent cells and by high vs low O_2 tension.

0277 TRANSFERRIN RECEPTORS IN CHICKEN ERYTHROID DIFFERENTIATION AND RETROVIRUS TRANSFORMATION. Bob G. Sanders and Kimberly Kline. University of Texas, Austin, Texas. Antiserum prepared against gradient purified reticuloendotheliosis virus (REV) identifies the avian transferrin receptor. Immunochemical analyses show the avian transferrin receptor to exhibit many properties identical to mammalian transferrin receptors. The avian transferrin receptor is a 190,000 dalton membrane molecule consisting of two similar 95,000 dalton disulfide-bonded subunits, and is expressed on lymphoid and erythroid cells in both monomeric and dimeric forms. The transferrin receptor is expressed on all differentiation stages of primitive cells and definitive type I erythroid cells undergoing erythropoiesis in the chicken embryo. The transferrin receptor is expressed on immature erythroid cells, but not mature erythrocytes, in adult chickens. Transferrin receptor expression on retrovirus transformed lymphoid and erythroid cells is different from normal cells. REV-transformed lymphoid cells express two distinct transferrin receptors composed of 98,000 and 110,000 dalton disulfide-bonded subunits, respectively. Comparisons of transferrin receptor peptides between erythroid and REV-transformed lymphoid cells reveal a high degree of homology; however, the transferrin receptor isolated from REV-transformed lymphoid cells exhibits a 50,000 dalton peptide that is not found on the erythroid transferrin receptor. Avian erythroblastosis virus (AEV)-transformed erythroleukemia cells express low levels of transferrin receptors composed of 100,000 dalton disulfide-bonded subunits. Cloned AEV-transformed erythroleukemia cells grown in the presence of 1mM butyric acid express elevated transferrin receptor levels, and immunochemical analyses show the induction of a new form of transferrin receptor composed of 110,000 disulfide-bonded subunits.

0278 A new model system to study normal human lymphocyte growth. K.J. Scanlon, M. Kashani-Sabet, the Chemotherapy Foundation Laboratory, Department of Neoplastic Diseases and Biochemistry, Mount Sinai School of Medicine, New York, N.Y. 10029

Interleukin (IL)-stimulated normal human lymphocytes were used as a model system to study cell growth and drug cytotoxicity in culture. Normal peripheral blood mononuclear cells were stimulated with optimal combinations of purified IL-1 and IL-2. Only "T" lymphocytes (helper and suppressor, 1:1) grew in culture, as characterized by monoclonal antibodies. These lymphocytes were maintained in culture with IL-2 for several months. Mitogen (PHA)-stimulated lymphocytes were also maintained in culture with IL-2. The PHA-stimulated and IL-stimulated lymphocyte cell lines had a similar generation time (30 h). Yet the PHA stimulated cell line showed similar properties to some tumor cells since both required large amounts of exogenous methionine and had unusual amino acid transport systems (Cancer Treat. Rep. 67, 631, 1983). The uptake, efflux and metabolism of chemotherapeutic agents were compared in the IL-stimulated and PHA-stimulated cell lines. These pharmacological properties of cisplatin and methotrexate were similar in both cell lines, yet the PHA stimulated cells were 3-5 fold more sensitive to the cytotoxic effects of these drugs. This greater drug sensitivity of the PHA-stimulated cell line was associated with an enhanced sodium-dependent methionine transport and altered amino acid transport systems when compared to the IL-stimulated cell line. Differences in the nutrient transport systems may be one explanation for different drug sensitivities in growing human lymphocytes.

0279 ROLE OF AUTOGENOUS PRODUCTION OF HEMOPOIETIC GROWTH FACTORS IN MYELOID LEUKEMIA, John W. Schrader, Sabariah Schrader, Kevin Leslie and Ashley Dunn, The Walter and Eliza Hall Institute of Medical Research, P.O. Royal Melbourne Hospital, Victoria 3050, Australia.

Previously we have reported the derivation of autonomous variants of an immortalized line of mast-cell-megakaryocytes that depended for its growth on a T cell derived hemopoietic growth factor, P cell stimulating factor (PSF). The autonomous variants were producing a factor with PSF activity and unlike the parental line were leukemogenic. Results of biochemical and biological analysis and of Northern blot hybridization with a cDNA probe, indicate that the factor released by the autonomous variants results from aberrant activation of the PSF gene. Analysis of another murine myeloid leukemia that this time arose in vivo, indicates that it too responds to an autogenously produced hemopoietic growth factor. At low but not high cell-densities, colony-growth in agar depended on the addition of medium conditioned by the leukemic cells or of purified growth factors. Preliminary data indicate that autostimulatory mechanisms may be relevant to certain malignancies of cells derived from pluripotential hemopoietic stem cells in the human. Because available evidence indicates that autostimulation depends on the interaction of autogenous growth factor with cell surface receptors, these results point to a number of possible therapeutic strategies based on antibodies or competitive antagonists.

0280 INCREASED ECTONUCLEOTIDE TRIPHOSPHATASE ACTIVITY IS A FEATURE OF NORMAL B- AND CLL-LYMPHOCYTES. George B. Segel and Marshall A. Lichtman, University of Rochester School of Medicine, Rochester, NY 14642.

The ectoadenosine triphosphatase activity of CLL B-lymphocytes has been reported to be quantitatively greater and qualitatively different from blood B-lymphocytes (Blood 62:1041, 1984). We have studied the kinetic parameters of the ecto-ATPase from CLL B-lymphocytes and compared them to blood and tonsillar B- and T-cells. The V_{max} for ATP activation of ecto-ATPase activity in CLL B-lymphocytes was 65 ± 9 fmol Pi/cell/30 min compared to 39 ± 2 in blood B-lymphocytes and 8.5 ± 1.5 in blood T-lymphocytes. Ecto-ATPase of membranes prepared from CLL, tonsillar B and T and blood T-lymphocytes showed a similar relationship to intact cells. However, no qualitative differences could be demonstrated in the ecto-ATPase in that the K_m for ATP, .15 mM, and the K_m for magnesium, .17 mM, in both intact cells and in membranes of CLL-lymphocytes did not differ from blood or tonsillar B-cells. The ectoenzyme hydrolyzed GTP, ITP, CTP and UTP as well as ATP. Further, ATP added to an enzyme assay containing an alternative nucleotide did not result in increased phosphate release. Nucleotide acceptance by CLL-cells and membranes was very similar to blood B and T lymphocytes. ATP inhibited phosphate release when present in excess of magnesium in both CLL and blood B-lymphocytes. These data indicate that there is greater ectonucleotide triphosphatase activity in tonsillar and blood B-lymphocytes, including CLL, as compared to T-lymphocytes. However, CLL cells showed no qualitative difference from blood or tonsillar B-cells in ectonucleotidase activity. Thus, the higher activity in CLL cells is B-cell "like" and may reflect also their maturation stage or monoclonal origin.

0281 TRANSPLANTATION OF FUNCTIONAL STROMA FROM LONG TERM MARROW CULTURES, J.G. Sharp, G.C. Udeaja, S.L. Mann, and D.A. Crouse, Univ. Neb. Med. Ctr., Omaha, NE 68105.

The stromal cells (SC) of hematopoietic tissues, both *in vivo* and *in vitro*, may play an important role in protecting primitive hematopoietic stem cells from differentiation by providing protective niches. In this study, the SC of the adherent layer of murine long term marrow cultures (LTMC), together with their associated hematopoietic cells, were transplanted in lethally irradiated syngeneic recipients. The SC recovered from LTMC by gentle scraping were organ cultured briefly prior to grafting beneath the kidney capsule. The recipients were autoposied after either 8 or 12 d, the grafts recovered for morphological evaluation and the spleens examined for spleen colonies (CFUs). Histological analysis of the recovered organ cultures showed prominent hematopoietic foci which displaced many of the fat cells seen in unirradiated grafted controls. The grafted organ cultures from LTMC of various culture ages (5-12 weeks) showed small amounts of bone which were usually located at the junction of the graft with the kidney parenchyma. When LTMC adherent cells were grafted as organ cultures in irradiated recipients only a few CFUs were seen at day 8 but a large number were observed at day 12. In contrast, when the adherent layers were completely disaggregated and administered intraveneously, both 8 and 12 d CFUs were observed in high frequency. These data indicate that the adherent SC of LTMC can establish ectopic hematopoiesis on transplantation, particularly in lethally irradiated recipients and further suggest that SC *in vitro* may play an important role in maintaining the hematopoietic stem cell hierarchy by regulating the response of more primative stem cells to differentiation pressures. (Supported by AM26636 and UNMC Seed Grant Funds)

0282 ALTERED GROWTH FACTOR DEPENDENCE OF CFU-C CORRELATES WITH PRELEUKEMIC CAPACITY IN RADIATION LEUKEMOGENESIS. Allen E. Silverstone, Cell Biology and Genetics, Memorial Sloan Kettering Cancer Center, New York, NY 10021.

Long term liquid bone marrow cultures were established from mice treated with fractionated irradiation. Cultured cells were assayed for several parameters including production of ecotropic and xenotropic virus, terminal deoxynucleotidyl transferase, capacity to form agar colonies in the presence of optimal and limiting growth factors, and the ability to transfer leukemia to sublethally irradiated animals. While little correlation was found with the induction of ecotropic virus production , a high correlation between a capacity to transfer leukemia in sublethally irradiated recipients and the generation of agar colonies in the presence of suboptimal growth factors was observed. The phenotype of cells in these colonies will be discussed.

0283 AN AUTOSTIMULATORY FACTOR SECRETED BY CULTURED HUMAN PRE-B LYMPHOBLASTS, R. Graham Smith, Jerome Zack and Brad Ozanne, University of Texas Health Science Center, Dallas, Texas 75235.

The reasons why only a few human acute leukemias grow permanently in vitro are not understood. Established T-cell acute lymphoblastic leukemia (ALL) and Burkitt lymphoma cell lines produce autostimulatory growth factors in vitro. This mechanism may provide one explanation for successful in vitro growth adaptation. We noticed that in vitro growth of the human pre-B ALL cell line SMS-SB is density dependent; cells grow well when seeded at 10^6/ml but die when seeded at $\leq 10^4$/ml. This observation suggested that SMS-SB cells might produce autostimulatory growth factor(s) whose concentration may become limiting in sparsely seeded cultures. To explore this possibility, SMS-SB cells were adapted to grow in serum free medium (SFM) and supernatants from cultures at high cell density were tested for stimulation of growth at critical (growth-limiting) density ($\leq 10^4$/ml). This conditioned medium (CM) clearly stimulated growth of cells down to 10 cells/ml. In thymidine uptake assays, the half-maximal response was obtained with 15% (v/v) CM; the maximum stimulation index was 80 in the presence of 40% CM. In SFM without CM, no clones were obtained from cells plated at limiting dilution, while 8% of the expected maximum number of clones grew in the presence of 40% CM. CM from two other ALL cell lines (REH and NALM-6) also stimulated growth of SMS-SB cells. CM from SMS-SB cells promoted anchorage-independent growth of rat fibroblasts in an assay for transforming growth factor activity (J. Zack, R.G. Smith, B. Ozanne, submitted to this meeting). These data extend the phenomenon of autocrine growth factors to cultured human pre-B ALL cells.

0284 INHIBITION OF DNA REPLICATION AND THE EXPRESSION OF THE C-MYC GENE IN HL60 CELLS EXPOSED TO VITAMIN-D_3 ANALOGS. George P. Studzinski, Zamir S. Brelvi and Amarjit Bhandal, Department of Pathology, UMDNJ-New Jersey Medical School, Newark, NJ 07103.

1,25 dihydroxy-vitamin D_3 and other cholecalciferol derivatives induce monocytic differentiation in HL60 cells, a line of promyelocytic leukemia cells. These cells characteristically overexpress the c-myc oncogene, but the expression of this gene is markedly reduced in cells treated with vitamin D_3 analogs. Since the expression of c-myc gene has been reported to be cell cycle dependent, we have studied the kinetics of inhibition of DNA synthesis and c-myc expression in HL60 cells treated with 1,25 dihydroxy-vitamin D_3. We have observed an increased sensitivity to the differentiation-inducing effects of this compound at the G1-S boundary region of the cell cycle. The inhibition of DNA synthesis and c-myc expression are evident within 4 hrs of exposure to 1,25 dihydroxy-vitamin D_3, and precede the onset of phenotypic differentiation by approximately 20 hrs. The results suggest a relationship between the inhibition of DNA replication, a decline in c-myc expression, and monocytic differentiation of HL60 cells.

0285 INDUCTION OF ERYTHROID MATURATION AND SYNTHESIS OF UNTRANSLATED β-LIKE GLOBIN GENE TRANSCRIPTS IN DIFFERENTIATING K-562 CELLS. Asterios S. Tsiftsoglou, Willie Wong, Jack Hensold and Stephen H. Robinson. Charles A. Dana Res. Inst., Department of Medicine, Beth Israel Hospital and Harvard Medical School, Boston, MA 02215.

Treatment of K-562 cells with hemin (HE) stimulates synthesis of embryonic, fetal hemoglobins but does not induce terminal erythroid maturation and synthesis of adult HbA or A_2. We have simultaneously exposed cells to both aclacinomycin (ACL), an anthracycline, and HE (30uM) for 6 days and observed a >60% increase in accumulation of globin mRNAs and hemoglobins. Approximately 35-40% of cells thus treated developed the characteristics of orthochromatic normoblasts. PAGE analysis of cytoplasmic proteins and Triton/Urea fractionation thereof revealed accumulation of ε, ζ, α and γ but not β or δ-globin chains. Dot RNA hybridization analysis with ^{32}P-labeled cloned fragments of globin DNA sequences coding for the 3'end region of α, ζ, ε, α and γ-globin mRNA demonstrated accumulation of the corresponding gene transcripts. No δ-globin gene transcripts were detected with the same techniques. Hybridization, however, was observed with the use of ^{32}P-labeled genomic fragments of β-globin sequences (PstI-Bgl II 3.6kb; Bgl II-Eco R_1, 2.8kb; Bam H_1-Bam H_1, 2.0kb). S_1 nuclease mapping analysis of cytoplasmic RNA with a 5'end ^{32}P-labeled probe (Bam H_1-Bam H_1 2.0kb) revealed 64 base protection in RNA prepared from control, HE, ACL and ACL+HE-treated cells and 212 base protection of RNA extracted from COS cells transfected with a vector carrying β-globin sequences. The level of cytoplasmic RNA protected was higher in ACL/HE-treated cells. These results indicate that induction of erythroid maturation and enhancement of globin mRNA synthesis can be achieved in culture, and differentiating K-562 cells appear to synthesize immature, untranslatable β-like globin gene transcripts.

0286 ESTABLISHMENT OF EBV GENOME-POSITIVE, EBNA-NEGATIVE, RETROVIRUS-NEGATIVE HUMAN T CELL LINES, D.J. Volsky, B. Volsky, M. Hedeskog, T. Gross, C. Kuszynski and F. Sinangil, Dept. of Pathology, U.N.M.C., Omaha, NE 68105.

Epstein-Barr virus (EBV) is a human Herpesvirus which causes infectious mononucleosis and is involved in Burkitt's lymphoma and nasopharyngeal carcinoma. A major distinguishing features of EBV has been its unique restriction to human B lymphocytes. Only mature B cells can be infected by the virus in vitro. The infection results in cell immortalization into lymphoblastoid cell lines carrying latent viral genomes and expressing EB virus-determined nuclear antigen (EBNA).

However, we report here on the establishment of EBV DNA-positive but EBNA--negative human T lymphoblastoid cell lines. The transformed lines, termed HBD, were obtained after transfecting cord blood lymphocytes with purified B95-8 viral DNA enclosed in fusogenic Sendai virus envelopes, followed by exposure to EBV from a P3HR-1 cell subclone. The T cell surface phenotype of HBD cells was determined by flow cytometry using monoclonal antibodies. The presence of EBV genome and viral particles was determined by nucleic acid hybridization and electron microscopy, respectively. In addition, HBD cells are negative for retrovirus, have normal karyotypes and express several antigens associated with the EBV replicative cycle. The lack of EBNA expression suggest that this antigen might not be an essential indicator of EBV infection. This is the first time that EBV genome is found associated with permanently growing hematopoitic cells of non-B lineage origin.

0287 PURIFICATION AND BIOCHEMICAL CHARACTERIZATION OF TWO DIFFERENTIATION INDUCING PROTEINS FOR LEUKEMIC CELLS PRODUCED BY A BLADDER CARCINOMA CELL LINE. Karl Welte, Janice L. Gabrilove, Erich Platzer, Paul Harris, Roland Mertelsmann and Malcolm A.S. Moore. Memorial Sloan-Kettering Cancer Center, NY,NY 10021.

Leukemia-differentiation factors and pluripotent CSF (supporting growth of CFU-GEMM) activities are constituitively produced by the human bladder carcinoma cell line 5637. The purification of these factors involved sequential $(NH_4)_2SO_4$-precipitation, anion exchange chromatography (DEAE cellulose, DE 52), gel filtration (AcA 54), reverse phase HPLC (C18) and Hydroxylapatite chromatography on HPLC. All activities co-eluted at about 0.1M NaCl from a DE 52 ion-exchange column and at 32,000 MW from AcA 54 column. Using reverse phase HPLC (C18) we could separate two distinct activity peaks: Peak 1) containing differentiation activity predominantly for HL-60 promyelocytic leukemic cells (eluted at 30% 1-propanol) and Peak 2) containing differentiation activity predominantly for murine WEHI-3 B(D+) myelomonocytic leukemic cells and pluripotent CSF activity (eluted at 42% 1-propanol). The protein eluted in the second peak revealed a MW of 18,000 in SDS-PAGE, both by silver staining technique and by elution of biological activity from the corresponding gel slice. This protein had an IEP of 5.5. The proteins eluted in the first peak were less pure and were therefore subjected to an hydroxylapatite chromatography HPLC column. The activity eluted as a single peak from this column and had a MW of 22,000 as judged by elution of activity from a SDS-PAGE gel.

0288 CELL SURFACE RECEPTORS FOR GRANULOCYTE-MACROPHAGE COLONY STIMULATING FACTOR, Andrew F. Wilks, Francesca Walker and Antony W. Burgess, Ludwig Institute for Cancer Research, Australia, 3050

Granulocyte-macrophage colony stimulating factor (GM-CSF), supports the differentiation and expansion of neutrophilic granulocyte and macrophage colonies from murine bone marrow precursor cells. The capacity of hemopoietic precursor cells to respond to GM-CSF is presumed to reside in high affinity cell surface receptors. We have developed a binding assay using ^{125}I-labeled GM-CSF and have demonstrated the presence of specific and saturable GM-CSF binding sites on mouse bone marrow cells, the murine leukemic cell line WEHI-3B(D^+) and a number of other myeloid cell lines.

There are only small numbers of GM-CSF receptors on all of the cell lines and cell populations studied, including those which are known to respond biologically. The myelomonocytic leukemic cell line WEHI-3B(D^+), for example, displays two classes of high affinity receptor (Kd 20pM, number of receptors 20-50) and low affinity (Kd 1200 pM, number of receptors approximately 500). We have determined the approximate molecular weight of the ^{125}I-labeled GM-CSF receptor complex by cross-linking. Analysis of this complex using SDS-PAGE gave an apparent molecular weight of 72,000 daltons. Using the data derived from the receptor binding assay and the binding assay itself, we have initiated a programme directed at molecularly cloning the murine GM-CSF receptor by a cDNA subtraction method.

0289 TRANSFORMING GROWTH FACTOR ACTIVITY FROM A HUMAN PRE-B LEUKEMIA CELL LINE, Jerome Zack, R. Graham Smith, and Brad Ozanne, Univ. of Texas Health Science Center, Dallas, TX 75235

Fibroblasts transformed by various means have been shown to produce soluble factors that enable anchorage-dependent recipient cells to grow in semi-solid media. These transforming growth factors (TGFs) act in an autocrine fashion. The human pre-B acute lymphoblastic leukemia line SMS-SB, has been adapted to grow at high cell density (10^6 cells/ml) in serum-free medium supplemented with transferrin, insulin, linoleic acid-albumin, and ethanolamine. Cells seeded in this medium at or below 10^4 cells/ml die. Growth at low density is restored by adding serum-free conditioned medium from high-density cultures of these cells, as judged by an increase in thymidine incorporation. This indicates that an autocrine growth factor is being produced by these cells. Concentrated serum-free conditioned medium from high-density SMS-SB cells allows normally anchorage-dependent rat fibroblasts (NRK 49F) to form colonies in soft agar within 5 days. Thus, this human pre-B leukemia cell line produces TGF activity. This activity may play a role in lymphoid cell growth and/or transformation.

In Vitro Growth of Leukemia Cells

0290 GROWTH OF LYMPHOMA COLONIES: A PREDICTIVE PARAMETER FOR CLINICAL OUTCOME, M. Tweeddale, B. Lim, N. Jamal, M. Minden, H.A. Messner, Princess Margaret Hospital, Toronto, Ontario, Canada

Bone marrow or lymphnode samples of 33 patients with malignant lymphoma of a variety of clinnical and pathological stages were cultured in methylcellulose with 30% human plasma, IDMEM, $5x10^{-5}$M mecaptoethanol and 10% PHA-LCM. Bone marrow cultures also contained 1 unit of erythropoietin. Samples of 9 patients formed tightly cohesive colonies of mononuclear cells. The colony size varied from 50-500 cells. Cells within these colonies reacted positively with antibodies directed against B-cell determinants. The marker pattern was consistent for colonies of the same patient but varied from patient to patient. Some patients grew colonies positive for early B-cell markers (B1, cytoplasmic μ), others stained positive for surface immunoglobulins. Primary colonies of all patients contained cells that gave rise to secondary colonies upon replating under identical conditions. All specimens are now propogated as cell lines under semisolid and liquid conditions.

The growth pattern was highly predictive of the clinical outcome. 8 of the growers died within 6 months of study with a median survival of two months. The median survival for nongrowers from the culture date was 12 months. The mean survival of growers and nongrowers from the date of diagnosis differed significantly (Mantel-Cox, $p = 0.0049$ and $p = 0.003$ respectively).

In conclusion, a culture assay is available that promotes growth of lymphoma colonies in a subset of patients with malignant lymphoma. Colony growth is associated with a poor diagnosis.

Therapy—Acute Leukemias

0291 THERAPY OF ACUTE MYELOGENOUS LEUKEMIA, Robert Peter Gale, Department of Medicine, Division of Hematology and Oncology, UCLA School of Medicine, Los Angeles, 90024.

Since 1970 there has been substantial progress in the therapy of acute myelogenous leukemia (AML). Intensive induction chemotherapy with cytarabine and daunorubicin, with or without 6-thioguanine, produces remissions in > 70% of patients. High doses of cytarabine and/or amsacrine may also be useful for remission induction. Preventing leukemic relapse has proven difficult. Central nervous system prophylaxis with radiation or drugs has no effect on systemic remission duration. Maintenance chemotherapy and immunotherapy, both specific and non-specific, have also proven useless in prolonged remissions. Consolidation (early intensification) therapy has been claimed to be useful but controlled trials to date fail to indicate a substantial benefit. Similarly results of late intensification are contradictory and it is not possible to determine if this is a useful modality. Regardless of these limitations and unanswered questions 3-5 year leukemic-free survival can currently be achieved in ∿ 30% of patients with AML who achieve remission.

Bone marrow transplantation from an HLA-identical sibling has also been used in AML. Transplants in first remission are associated with a 30-40% leukemic relapse rate with 40-50% 3-5 year leukemic-free survival. Direct comparisons of chemotherapy and transplantation in patients with AML have produced contradictory results. Most data suggest an advantage for transplantation in patients < 16-20 years but not in older individuals.

Patients who fail to achieve a remission or who relapse can be temporarily salvaged with investigational chemotherapy or transplantation. Useful drugs include high-dose cytarabine, daunorubicin and amsacrine used either alone or in combination. Mitoxantrone and homoharringtanin may also be useful. Transplants in advanced AML can cure a small but definite proportion of patients (10%).

Current research has focused on developing more effective agents and in more precisely identifying risk factors. For example different morphological subtyping of AML may have different prognoses. Likewise, differeing cytogenetic abnormalities imply discrete risks of leukemic recurrance. These data may be useful in the planning and analysis of clinical trials and possibly in individualizing therapy. New pharmacologies and therapeutic approaches are also being developed such as attempts to "nature" leukemic cells with low-doses of cytarabine or with retinoic acid and the in vivo infusion of monoclonal antibodies.

0292 **ACUTE LYMPHOBLASTIC LEUKEMIA IN CHILDREN,** Mark E. Nesbit, Department of Pediatrics, University of Minnesota, Minneapolis, MN 55455

Acute leukemia represents 32% of all cases of malignancy in children, with approximately 83% classified as acute lymphoblastic leukemia (ALL). Five year survival rates as high as 60-75% are common for patient cohorts diagnosed since 1970 (1), compared to a five year survival of less than 10% in 1960. Using multivariate analysis, outcome of patients with ALL can now be predicted by specific patient and disease characteristics at diagnosis, including initial white blood count (WBC), age, FAB morphology, sex, platelet count, hemoglobin, immunoglobulin levels, and presence of mediastinal mass. Patients can now be stratified at diagnosis into treatment groups based on these categories, with six year survivals of 80% (good risk), 64% (intermediate risk), and 45% (poor risk) (2). Treatment consists of three phases. A) Induction is successful 95% of the time utilizing three to four drugs. B) Central nervous system (CNS) prophylaxis is successful greater than 95% of the time with cranial radiation and intrathecal therapy based on CNS volume rather than total body size (3). C) Remission maintenance is where most new investigative efforts are centered. For example, phases of intensification and delayed reinduction reconsolidation given several months after initiation of therapy are being investigated (4). Two groups of patients with ultra-poor prognosis are presently treated separately; these include infants less than 12 months of age and patients with characteristics of "lymphoma syndrome" presenting with combinations of 1) massive splenomegaly, large spleen, or mediastinal mass; and 2) high WBC, high hemoglobin, or T-cell phenotype. Length of treatment is sex-dependent: recent data indicate that two years of maintenance therapy is sufficient for female patients and that at least three years of therapy is required for males. Increased attention is now also given to extramedullary disease, especially gonadal; new treatment modalities are presently being investigated to both decrease the incidence of extramedullary relapse concomitant with other relapses or as an isolated event. Finally, by curing patients, it becomes increasingly important to determine the potential long-term effects of the disease and the effects associated with its therapy. Based on the incidence of ALL and present survival, it can be estimated that by the year 2000 A.D., more than 24,000 persons, or approximately 1 of every 4500 individuals under 35 years of age, will have been diagnosed and treated for ALL. Abnormal neuropsychological and endocrine functions have already been identified in long-term ALL survival. An increased incidence of second malignant neoplasms has also been noted.

1) Nesbit M, et al: Cancer Research 42:674-680, 1982. 2) Coccia P, et al: IN Murphy SB, Gilbert JR, editors: Leukemia Research: Advances in Cell Biology and Treatment, New York, 1983, 241-250. 3) Bleyer A: Journal of Clin Oncology 1:317-325, 1983. 4) Riehm H: Amer J Pediatr Hematol Oncol 2:299-306, 1980.

Immunology, Pharmacology, and Therapy of Human Leukemias

0293 CLONTYPIC ANTIGENS OF T LYMPHOMA: THE T CELL ANTIGEN RECEPTOR AS TARGET FOR SPECIFIC IMMUNOTHERAPY, James P. Allison, Lewis Lanier, G. B. Kitto, and James Irvin, Univ. of Texas Science Park, Smithville, TX 78957, Becton Dickinson Monoclonal Center, Inc., Mountainview, CA 94043, Univ. of Texas, Austin, TX 78712 and Southwest Texas Univ., San Marcos, TX.

We have raised monoclonal antibodies to individually specific antigens of murine T lymphomas and demonstrated the target structure to be the T cell antigen-specific receptor heterodimer. We have examined the effect of administration of anti-receptor antibodies on the survival of lymphoma-bearing mice. MAb 124-40, an IgG1 antibody directed to the receptor of C6VL lymphoma cells, was found to result in a slight increase in mean survival time (MST) relative to untreated mice or mice receiving an irrelevant IgG1 antibody at doses of 5 and 15 ug. Conjugates of MAb 124-40 and pokeweed antiviral protein (PAP) were found to have a more pronounced effect, and at a dose of 15 ug X 3 resulted in long term survival (>90d) in 4 of 10 mice. Neither 124-40 nor the PAP conjugate increased the MST of mice bearing lymphoma ERLD, which does not express the MAb 124-40 reactive epitope. These results demonstrate that receptor-associated epitopes may serve as targets for lymphoma-specific immunotherapy. (Supported by NCI grant CA26321).

0294 "The affect of dehydroepiandrosterone on murine myeloid leukemic cells" H.G. Bedigian, The Jackson Laboratory, Bar Harbor, ME 04609

Animal models for the study of spontaneously occurring myeloid leukemias are rare. Recently, we have identified a recombinant inbred mouse strain (BXH-2), which has a high incidence of spontaneous leukemia (89% by 8 months of age) associated with the expression of a type C retrovirus. Results obtained from pathological observations as well as histochemical and immunological assays support the notion that the leukemias of BXH-2 mice are of the myeloid lineage. Myeloid cell lines derived from bone marrow and spleen of leukemic BXH-2 mice induce tumors when inoculated into other strains, whereas the oncogenic potential of these lines are lost when induced to differentiate with specific hematopoietic growth factors. We have observed that BXH-2 mice do not spontaneously release detectable levels of endogenous growth factors suggesting that the leukemia originates by a change that uncouples the normal requirement of growth factors for proliferation and differentiation. We have also observed that when these cell lines are exposed to a major adrenal secretory product (dehydroepiandrosterone) the cells have been stimulated to differentiate. The majority of mice inoculated with these differentiated lines (8 of 10 mice) did not show evidence of tumors, whereas the undifferentiated cell lines induced tumors within three weeks. The effect of this steroid on virus and oncogene expression is currently being investigated.

0295 **EFFECTS OF INTERFERON ON LEUKEMIC HAIRY CELLS,** **Brian L. Samuels, Bernard H. Brownstein, Everett E. Vokes and Harvey M. Golomb, University of Chicago, Department of Medicine, Chicago, IL 60637**

Studies on the effects of alpha-interferon (IFN) on leukemic hairy cells suspended in tissue culture medium have shown the induction of several proteins. The most prominent protein (p80) has an approximate molecular weight of 80,000 daltons estimated by SDS-polyacrylamide gel electrophoresis, and appears within 4 hours of exposure to IFN. This p80 protein may be analogous to one induced by IFN in normal lymphocytes. Hairy cells from a patient on IFN therapy for 1 month were found to have been induced for this p80 protein without requiring any in vitro exposure. Prior to treatment his hairy cells could be induced for the p80 protein by in vitro exposure to IFN. We have also looked for effects of IFN on other leukemic cells. A prominent band of 65,000 daltons was induced in chronic lymphocytic leukemia cells suspended in culture medium. In distinction to these observations, certain protein antigens disappear from IFN treated cells as seen by western blot analysis. We are characterizing the cellular location of the IFN induced proteins, and trying to determine their relationship to the effects of IFN on overall cellular mRNA and protein synthesis.

0296 HYDROXYUREA RESISTANT HUMAN CELL LINES WITH INCREASED RIBONUCLEOTIDE DIPHOSPHATE REDUCTASE ACTIVITY, Mark D. Carman and Joseph R. Bertino, Dept. Pharmacology, Yale University, New Haven, CT 06510.

We have developed a series of hydroxyurea (HU) resistant cell lines derived from the human myelogenous leukemia cell line K562 by stepwise selection in increasing concentrations of the drug. The ribonucleotide diphosphate reductase (RdR) enzyme from the parent line and 2 resistant lines, KH150 and KH300 (selected at 2mM and 4mM HU respectively) were examined. The KH150 had a 3 fold increase and KH300 had a 6.5 fold increase in RdR activity compared to the RdR activity of 30 pmol/min/mg for the enzyme from the parent line at a CDP concentration of 0.04mM. The ID_{50} for HU was 0.34mM, 1.64mM, and 2.84mM for the K562, KH150 and KH300 RdR enzymes. Also differing were the Km values for these 3 enzymes, from 0.89mM for K562 to 0.29mM for KH150 and 0.39mM for KH300. The variance in these parameters may be due to the relative concentrations of free M1, M2, and the holoenzyme ($M1_2M2_2$). RdR activity is not linear with protein concentration, and is due to dissociation of the subunits at lower protein concentrations. Resistance which may be by virtue of increased levels of M2, the site of HU action, may shift the relative levels of M1, M2, and the holoenzyme to account for the observed changes. Alternatively, we have selected for a series of mutant lines with altered RdR enzymes. Mixing experiment are being used to determine the rlative amounts of free M1 in these cell lines.

0297 REARRANGEMENT OF THE T-CELL RECEPTOR (Tcr) IN HEMOPOIETIC MALIGNANCIES, G. Cheng, C. L. Richardson, B. Toyonaga, T. Mak, E. A. McCulloch and M. D. Minden, Ontario Cancer Institute, Toronto, Ontario M4X 1K9, Canada.

Recently we have isolated clones specific to the beta chain of the T-cell antigen receptor. Previously, using these probes it was possible to show that there is genomic rearrangement of the Tcr in normal T-cell clones and in cell lines derived from patients with leukemia. In this abstract, we present the results of studies carried out on leukemic cells derived from patients. DNA was extracted from the leukemic cells of patients with AML, ALL, CML in chronic phase, CML in blast phase, and CLL. The DNA's were cut with an appropriate restriction enzyme and studied by Southern blot analysis with various probes to the beta chain of the T-cell antigen receptor. In general, we found that rearrangement occurred only in cases of a T-cell malignancy. However, two exceptions to this were found; one was a patient whose cells have the characteristics of a B cell and the other was a patient whose cells had the characteristics of AML. We conclude from these studies that rearrangement of the T-cell antigen receptor is a good marker of a T-cell malignancy, however, there are some exceptions to this. These exceptions may be examples of lineage infidelity.

0298 ANALYSIS OF THE SYNERGISM BETWEEN THYMIDINE AND ARABINOSYLCYTOSINE IN T-CELL LYMPHOMA MUTANTS, Amos Cohen* and Buddy Ullman†, Division of Immunology, Research Institute, The Hospital for Sick Children, Toronto, Canada M5G 1X8* and Department of Biochemistry, University of Kentucky Medical Center, Lexington, KY 40535†

Thymidine enhances the cytotoxic effect of arabinosylcytosine (araC) on leukemic cells. However, the nature of the synergistic effect of thymidine is unclear. It is well documented that deoxyCTP inhibits the incorporation of araC through feedback inhibition of deoxycytidine kinase the enzyme responsible for araC phosphorylation. On the other hand, deoxyTTP can releave this feedback inhibition of deoxycytidine kinase by deoxyCTP. DeoxyTTP can also deplete the intracellular deoxyCTP pools through feedback inhibition of ribonucleotide reductase.

In order to distinguish between these possible mechanisms of thymidine action we have used T lymphoma mutants with altered deoxyTTP and deoxyCTP levels due to either a feedback resistant ribonucleotide reductase activity or to impaired deoxyCMP deaminase activity. No synergism between thymidine and araC was observed in T cell lymphoma with altered ribonucleotide reductase activity, whereas marked synergism was observed in cells with normal ribonucleotide reductase activity. The synergistic effect of thymidine was in good correlation with deoxyCTP levels and not with deoxyTTP levels. These findings suggest that thymidine increases both araC incorporation and its toxicity by depleting deoxyCTP pools resulting from inhibition of ribonucleotide reductase by elevated deoxyTTP levels (Cohen, A. and Ullman, B. Cancer Chemother. Pharm. 1984, in press).

0299 COMPARATIVE CELLULAR PHARMACOLOGY OF ARABINOFURANOSYL-2-FLUOROADENINE MONOPHOSPHATE (F-ara AMP) AND ARABINOFURANOSYLCYTOSINE (araC) IN PATIENTS (PT) WITH RELAPSED LEUKEMIA. Lynn Danhauser, Stephen Iacoboni, and William Plunkett, The University of Texas at Houston, M. D. Anderson Hospital and Tumor Institute, Houston, TX 77030

F-araAMP is the soluble monophosphate of F-ara A which, unlike araC, is relatively resistant to detoxifying deamination. Like araC, F-araA is phosphorylated initially by deoxycytidine kinase and subsequently, as the triphosphate F-araATP, inhibits ribonucleotide reductase and DNA polymerase. We have compared the pharmacology of F-araATP after a bolus dose of F-ara-AMP (100 mg/m^2 over 30 min) with that of araCTP in the circulating leukemic blasts (CB) of two Pts who, at an earlier time, had received an infusion of araC (3 g/m^2 over 2 hr). Leukemic cells were separated from more mature cells by ficoll-hypaque density centrifugation before cellular nucleotides were extracted and analyzed by HPLC. Pt A (acute myelogenous leukemia) had shown a peak araCTP level, half-life of elimination ($t_{\frac{1}{2}}$), and area under the concentration x time curve (AUC) of 72 μM, 4.4 hr, and 560 μM-hr, respectively. Pt B (chronic myelogenous leukemia in blast crisis) exhibited relatively similar peak (131 μM), $t_{\frac{1}{2}}$ (1.2 hr), and AUC (500 μM-hr) values. The F-araATP cellular pharmacology, however, was markedly different in each Pt. Pt A had a peak F-araATP level of 114 μM, a $t_{\frac{1}{2}}$ of 57 hr, and an AUC of 9500 μM hr, whereas Pt B displayed a peak of 1.2 μM F-araATP, a $t_{\frac{1}{2}}$ of 6 hr, and an AUC of 15 μM-hr. Thus, Pts who exhibit similar ara CTP pharmacology in CB have strikingly different F-ara ATP pharmacology at a later stage of their diseases. These results demonstrate that the cellular pharmacology profiles for araCTP may not predict for F-araATP pharmacologic characteristics despite similarities in metabolic activation pathways of the 2 drugs.

0300 HETEROGENEITY OF DIHYDROFOLATE REDUCTASE SPECIFIC ACTIVITY AND METHOTREXATE SENSITIVITY IN LEUKEMIC BLAST CELLS OF PATIENTS WITH ACUTE MYELOGENOUS LEUKEMIA, Shoukat Dedhar, Daria Hartley, Deirdre Fitz-Gibbons and James H. Goldie, Cancer Control Agency of British Columbia, 600 West 10th Avenue, Vancouver, B.C., Canada, V5Z 4E6

Dihydrofolate reductase (DHFR) activity was determined in the blast cells of 11 acute myelogenous leukemia (AML) patients and in the white blood cells (WBCs) of 3 normal individuals. Whereas the DHFR activity in the normal subjects was low and varied very little (range 0.43 to 0.48 nmoles/20 min/mg protein), the activity in the blast cells of AML patients was highly variable and was generally higher than that in normal WBCs (range: 0.42 to 20 nmoles/20 min/mg protein). The DHFR specific activity in 3 of the patients was significantly higher than the rest. None of the patients had been treated previously with methotrexate (MTX). Furthermore, MTX titration of DHFR activity showed that in 4 (distinct from the 3 with increased activity) of 11 patients, $I.C._{50}$ values of MTX inhibition of DHFR activity were 10 to 20 fold higher as compared to those in the rest of the patients and in the normal subjects. In addition, approximatley 30% of the initial activity remained in the presence of 10 μM MTX in these 4 patients, whereas the activity was totally inhibited by MTX concentrations of 1 μM or less in the rest of the patients and normal subjects. These results show that intrinsic resistance of AML to MTX may be due, at least in some cases, to increased DHFR activity or the expression of DHFRs with low affinities for MTX, and demonstrate the genetic instability of these malignant cells at this locus.

0301 ACTIVATION OF AUTOLOGOUS T-LYMPHOCYTES IN HUMAN ACUTE LEUKEMIA, Markus Fopp and Christine Werner, Med Klinik C Kantonsspital, CH 9007 St Gallen, Switzerland

In vitro proliferation of human lymphocytes in the presence of autologous leukemic blast cells indicates antigenic disparity between stimulating leukemic and responding immune cells. The lymphocyte activating determinants (LAD) of these leukemia-associated antigens are not characterized. Several possibilities exist including acquired new or altered antigens during leukemogenesis, quantitative variation in Ia antigen expression, and normal differentiation antigens expressed on both leukemic blast cells and their normal counterparts in the haematopoietic precursor series.

In order to dissect the polyclonal proliferative T-cell responses against LAD on leukemic blasts we investigated the prerequisites for clonal analysis of autologous mixed lymphocyte tumor cell reactivities (AMLTR) in vitro in acute leukemia during remission. Remission mononuclear leukocytes from 10/16 patients (pts) were reactive in primary AMLTR. Reactive pts included pts with acute lymphatic leukemia (3/7), acute myelogenous leukemia (7/9) but not T-cell ALL. Restimulation of primed lymphocytes (PL) did show crossreactivities with allogeneic leukemic blast cells (7/22), regenerating bone marrow of leukemia pts entering complete remission (3/4) but not with allogeneic monocytes from healthy controls (0/15) or autologous haematopoietic cells including bone marrow cells during remission. Cyclosporin A and monoclonal antibodies specific for human MHC class II molecules inhibit secondary stimulation by autologous leukemic blast cells. These experiments suggest IL-2 dependent T-cell proliferation in the presence of leukemic blasts in the majority of AMLTR-positive patients.

0302 METABOLISM AND CYTOTOXICITY OF 9-β-D-ARABINOFURANOSYLGUANINE (ARA-G) IN CULTURED HUMAN T AND B LYMPHOBLASTOID CELLS, Arnold Fridland and Vernon Verhoef, St. Jude Children's Research Hospital, Memphis, TN 38101

Ara-G, a deoxyguanosine (dGuo) analog resistant to cleavage by purine nucleoside phosphorylase is 100-200-fold more cytotoxic to T than B lymphoblasts and the most selective agent tested toward T-cell leukemias. The present study has examined the metabolism of ara-G in established human lymphoid lines. A deficiency in deoxycytidine kinase (dCK) in human T leukemic blasts (CCRF-CEM) decreased 150-fold the toxicity of ara-G and reduced the capacity to accumulate ara-G nucleotides approximately 7 fold. By contrast, dCK-deficiency in B lymphoblasts (WI-L2) decreased toxicity and ara-G nucleotide accumulation only 2-fold. Levels of ara-G and other nucleoside kinase activities were compared in extracts of CEM and WI-L2 and their kinase-deficient mutants. We found that the activity for ara-G and dGuo phosphorylation was reduced by 70% in extracts of the mutants, while that of dCK activity was decreased by 95%. In addition, ara-G phosphorylation activity in WI-L2 extracts was 20 times greater than that in CEM dCK-deficient cells, despite the fact that these two cell lines accumulated ara-GTP at identical rate. T1/2 of exogenously derived ara-GTP accumulated to 10 pmoles/10^6 cells was 30 min in WI-L2 while in CEM cells ara-GTP was unchanged after 4 h. The data indicate that two enzymes in T and B cells phosphorylate ara-G: one cytoplasmic dCK and another kinase which is localized in mitochondria. The selectivity of ara-G toward T and B cells can be correlated with their differential ability to catabolize ara-GTP.

0303 COMPARATIVE METABOLISM OF ARA-C AND F-ARA-A IN HUMAN LEUKEMIA CELLS. Varsha Gandhi and William Plunkett, The University of Texas at Houston, M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77030

Cellular pharmacology studies have demonstrated a strong correlation between the levels of ara-CTP in leukemia cells and clinical response to high-dose ara-C therapy. F-ara-A is a new anticancer drug entering clinical trial that accumulates as the triphosphate (F-ara-ATP) and is incorporated into DNA as is ara-C. Each nucleoside analog is phosphorylated to the respective monophosphate by deoxycytidine kinase. Because the cellular concentrations of these active triphosphates are determined by the balance between phosphorylation and dephosphorylation, we have investigated these activities in human leukemia cells in vitro. Accumulation of ara-CTP by K562 cells was linear for 3 hr at ara-C concentrations up to 10 μM. After 3 hr of incubation, a plateau cellular concentration of ara-CTP of 90 μM was maintained. When the cells were washed into drug-free medium, the ara-CTP was eliminated with a half-life of 1.3 hr. In contrast, accumulation of F-ara-ATP was not saturated at exogenous F-ara-A concentrations up to 300 μM. Cellular F-ara-ATP accumulated to greater than 250 μM by 2.5 hr. F-ara-ATP was eliminated with a $t_{\frac{1}{2}}$ of 3.3 hr after removal of F-ara-A. These results suggest that, relative to the metabolism of F-ara-A, accumulation of ara-CTP in K562 cells is limited by both the phosphorylative and degradative pathways. These results are consistent with the finding that deoxycytidine kinase is inhibited by high concentrations of ara-CTP, whereas F-ara-ATP is unlikely to have such inhibitory activity. This possibility and the enzymatic basis for the differential elimination of ara-CTP and F-ara-ATP are under investigation.

0304 EFFECT OF DEOXYCYTIDINE ON THE GROWTH AND RESPONSE TO PYRIMIDINE ANTAGONISTS OF NORMAL AND LEUKEMIC HUMAN MYELOID PROGENITOR CELLS, S. Grant and K. Bhalla, Columbia University College of Physicians and Surgeons, New York, NY, 10032

We have examined the effect of the naturally occurring nucleoside deoxycytidine (dCyd) on the in vitro soft agar growth of normal and leukemic myeloid progenitor cells. Bone marrow cells obtained from 30 normal donors and T-lymphocyte depleted leukemic cells obtained from 22 patients with ANLL were plated in agar in the continuous presence of dCyd (10^{-7}-5 x 10^{-3}M) alone or in conjunction with the pyrimidine antagonists thymidine or 3-deazauridine. At the end of 10 days incubation, normal granulocyte-macrophage colonies (CFU-GM), consisting of 50 or more cells, and leukemic colonies (L-CFU), consisting of 20 or more cells, were scored. Normal CFU-GM exhibited an average of 250 ± 80% (range 130-380%) of control colony formation when exposed to 10^{-4}M dCyd. This dCyd concentration was not associated with a significant increase in L-CFU formation in 18 evaluable specimens (mean 110 ± 10% of control values). Co-administration of dCyd (3-5 x 10^{-3}M) restored 40-90% of control CFU-GM formation in 10 normal specimens exposed to 3 x 10^{-3}M thymidine or 2 x 10^{-5}M 3-deazauridine. In only 1 of 18 leukemic specimens was dCyd able to restore greater than 25% of control L-CFU formation following exposure to the same thymidine and 3-deazauridine concentrations. Lack of dCyd mediated protection was observed for cells obtained from untreated leukemic patients and those failing high-dose Ara-C regimens. These studies suggest that dCyd might preferentially stimulate the in vitro growth of normal versus leukemic progenitor cells and may improve the therapeutic index of several clinically available pyrimidine antagonists.

0305 COMPARATIVE EVALUATION OF FLOW CYTOMETRY AND CHROMOSOME ANALYSIS IN CHILDHOOD ACUTE LEUKEMIA. Oskar A. Haas, Wolfgang Hiddemann, Christine Pollak, Peter Bettelheim, Waltraud Schmidmeier, Helmut Gadner, St. Anna Cildren's Hospital, First Med. Dept. Univ. Vienna, Austria, Dept. Int. Med. Univ. Münster, FRG

DNA flow cytometry (FCM) and chromosome analysis were performed on the same BM and/or PB cell samples from 35 cases of acute childhood leukemias and compared with the blast cell count and the immunological phenotype of the leukemic cells. By FCM as an easy method to estimate cell cycle distribution and to measure gross quantitative changes of cellular DNA content DNA aneuploidies were identified in 40 % of cases. FCM failed to reveal near-diploid deviations as well as qualitative changes (translocations) of the genetic material. Chromosomes analysis on the other hand was hampered by preparation difficulties but was successful in 80 % of cases. The presence of only normal metaphases did however not exclude the presence of chromosomally abnormal cells sine FCM revealed DNA aneuploidies in several of theses cases. Hence, both methods complement each other and can therefore provide a more precise and complete information on genetic changes in neoplastic cell populations when used in combination.

0306 SYNGENEIC BONE MARROW TRANSPLANT IN A PATIENT WITH HAIRY CELL LEUKEMIA (HCL), Teodosio Izzi, Giuseppe Rossi and Genesio Balestrieri, Pesaro Hospital and Brescia Hospital, Italy

A 40 year old male affected with HCL was transplanted about two years ago due to a progressive course of the disease despite splenectomy, in November 1982. At the diagnosis the patient presented splenomegaly and severe pancytopenia. Peripheral blood showed 64% mononuclear cells diagnosed as HC B type in 7,200 WBC. Marrow biopsy and a histologic section of the spleen revealed infiltration of the same mononuclear cells. The patient was prepared with cyclophosphamide 120 mg/Kg and total body irradiation (1000 rads) in a single dose and received 2.7 x 10^8 /Kg bone marrow cells from his HLA identical twin. The post transplant period was favourable and he was dismissed on the 33rd day. In the months following bone marrow transplantation the HC disappeared, first in the peripheral blood, then in the bone marrow section in a period of 6-9 months. Bone marrow reticular fibrosis of grade I which was diffuse before BMT, became grade III after 3 months, persisting until 6 months post transplant. At 9 months post BMT the bone marrow was almost completely reconstructed with the possibility of aspirating hemopoietic cells. The patient is presently alive and well with complete hemopoietic recovery both in peripheral blood and bone marrow. The gradual reconstruction of the bone marrow and the presence for months of HC in the histologic bone marrow section may suggest the hypothesis of the particular biology of HC leukemia both for the eradicability of the disease with CY and TBI and the possibility that the buffy coat of the donor after BMT, as in this case, may influence the outcome of the treatment.

0307 MODULATION OF METASTATIC POTENTIAL AND CELL SURFACE PROPERTIES OF A MURINE LYMPHOSARCOMA. S.S. Joshi, N.B. Mathews, J.D. Jackson, F. Sinangil, D.J. Volsky, K.W. Brunson* and J.G. Sharp. Univ. Neb. Med. Ctr., Omaha, NE, *Ind. Univ. Sch. Med. Gary, IN.

The Abelson virus induced murine lymphoma/lymphosarcoma cell line RAW117-P and its *in vivo* selected highly metastatic variant RAW117-H10 have been used to study chemically induced and membrane related changes in metastatic potential. Flow microfluorimetry revealed that the parental P cells contain a higher percentage of lectin positive cells and bind more lectin per positive cell than the metastatic H10 cells. The P cells also express more asialo GM1 than the H10 cells supporting a relationship between glycosylation and malignant potential. When these cells were grown in the presence of differentiation inducing chemicals (butyrate and dimethyl sulfoxide) their *in vitro* growth rates were reduced considerably and the *in vivo* tumorigenicity of H10 cells was decreased. Flow microfluorimetry of the cell surface of these chemically differentiated cell lines revealed an increased expression of asialo GM1 and receptors for various lectins. These results also support the relationship between increased membrane glycosylation and decreased malignancy/metastatic potential. In addition, using the Sendai virus fusion technique, we transplanted the cell membranes from less malignant P cells to H10 cells and vice versa. As a control, normal Balb/c thymocyte membrane was transplanted to both cell types. These experiments showed decreased *in vivo* malignancy of the H10 cells transplanted with either richly glycosylated membranes from less malignant P cells or normal Balb/c thymocytes. Thus, our data clearly support the potential regulatory role played by cell surface glycosylated molecules in the process of metastasis. (Supported by Neb. Dept. of Health)

0308 CELL CYCLE-SPECIFIC FLUCTUATIONS OF dCTP POOLS REGULATE THE ACCUMULATION OF ARA-CTP BY LEUKEMIC CELLS. Jan O. Liliemark and William Plunkett, The University of Texas at Houston, M. D. Anderson Hospital & Tumor Institute, Houston, Texas 77030

Fractionation of exponentially-growing cultures of CCRF-CEM T-lymphoblasts by centrifugal elutriation resulted in enrichment of G_1 (80%) and S-phase (60%) populations. Assays of deoxycytidine kinase in crude sonicates prepared from these cells demonstrated the specific activity of this enzyme to be 2.3 times greater in S-phase cells. Consistent with the findings of others, this activity was inhibited by added dCTP (2-10 μM) in a concentration-dependent manner. HPLC analyses of $HClO_4$ extracts of each cell population demonstrated that the cellular concentration of dCTP rose from 6.2 μM in G_1-cells to 14.2 μM in S-phase cells, i.e. 2.4 times. Correction was made for the larger cell volume in S-phase. However, when cell cycle phase-enriched cells were incubated with ara-C (25 μM and 100 μM), S-phase-enriched cell accumulation of ara-CTP was only 1.7 times greater than that in G_1-enriched cells; less than might be expected from the relative specific activities of deoxycytidine kinase. The accumulation of ara-CTP was linear up to 3 hrs. The same cellular araCTP concentration was achieved at 1 hr independent of the ara-C concentration used. These results suggest that the increased potential of S-phase cells to phosphorylate ara-C may be off-set by the inhibitory action of dCTP on deoxycytidine kinase. If so, cellular dCTP pools may be an important site to affect the modulation of ara-CTP metabolism.

0309 PROTEIN PHOSPHORYLATION AND PROTEIN KINASES IN REVERSE TRANSFORMATION BY cAMP, Arthur H. Lockwood and Maryanne Pendergast, Albert Einstein Medical Center, Philadelphia, PA. 19141

We are investigating protein phosphorylation and protein kinase function in cancer cell lines whose growth and morphology are altered by cyclic AMP. In vivo labelling of chinese hamster ovary (CHO) cells with ^{32}p-orthophosphate reveals that cAMP stimulates the phosphorylation of several cellular proteins. Some of these phosphoproteins are associated with the detergent resistant cytoskeleton. The most prominent changes are the rapid phosphorylation of a 55,000 MW cytoplasmic protein and the dephosphorylation of a 20,000 MW cytoskeletal protein. We have found that the 20,000 MW protein is a regulatory myosin light chain. Since cAMP induces assembly of actyomyosin bundles in several tumor cell lines, changes in the phosphorylation of myosin may, in part, mediate morphological reversion by cAMP. Analysis of total phosphoaminoacids in CHO cells reveals that cAMP suppresses tyrosine phosphorylation. This suggests that the cAMP dependent protein kinase system can attenuate the activity of an oncogene encoded tyrosine kinase in CHO cells. Characterization of this tyrosine kinase and its in vivo substrates is in progress. Interaction cAMP dependent-and tyrosine protein kinases may be one mechanism by which oncogenes and cyclic nucleotides exert their reciprocal effects on the morphologic and growth phenotype of cells.

0310 Expression of T cell or T associated antigens on cells involved in the myeloid differentiation pathway. P. Mannoni+, M. Park+, S. Vassiliadis+, F. Dubreuil*, C. Mawas*. + University of Alberta and * Centre d'Immunologie, Marseille, Luminy.

Monoclonal antibodies (MoAb) defining T cell or T associated antigens define antigens related to the T cell activation, differentiation or proliferation. Some of these MoAb, like anti-TL-like, transferin receptor, CD 1, CD 2, CD 7 or CD 4 have been found expressed on myeloid leukemia cells or cell lines. In order to study the possible relationship of such expression with the myeloid differentiation pathway we studied the reactivity of a series of T-cell MoAb on cells obtained from patients with chronic or acute myeloid leukemia and on leukemic cell lines related to the erythroid differentiation (K 562, HEL) or to the myelo-monocytic differentiation (KG 1, KG 1A, ML 1, ML 2, ML 3, HL 60, U 937). Some MoAb defining the TL-like antigen (CD 1 or T 6) transferin receptors, CD 1, CD 4, CD 7, activated T cells, were found to bind leukemic myeloid cells or cell lines. In order to demonstrate the possible relationship of T-cell antigens and myeloid differentiation pathway we studied the modulation of such antigens on cell lines induced to differentiate by incubating them with lymphokines (recombinant gamma interferon, interleukin II, conditioned medium) or chemicals. The capability of these cell lines to form colonies spontaneously or in response to conditioned medium was studied in relation to membrane antigen expression before and after induction of differentiation. Thus it appears that T-cell markers can be used to identify some stages of leukemic myeloid differentiation, and that the expression of these markers can be related to the capability of the cell line to differentiate or to proliferate.

0311 EXPERIMENTAL REGULATION OF CELL REPLICATION AND DIFFERENTIATION THROUGH THE IN VITRO INDUCTION OF IMMUNOLOGIC UNRESPONSIVENESS. D. Elliot Parks, Judith D. Levich, and William O. Weigle, Scripps Clinic and Research Foundation, La Jolla, CA. 92037.

An experimental model for the evaluation of the molecular events involved in the down regulation of lymphocyte replication and differentiation has been developed. This model employs helper T lymphocyte lines and clones derived from murine and human subpopulations immunologically specific for the protein antigens human immunoglobulin G and tetanus toxoid, respectively. These lymphocytes, maintained with T cell growth factors, will divide in response to exposure to specific antigen. Furthermore, the addition of specific antigen, but not unrelated protein, to cells will induce the expression of helper function through the synthesis and secretion of interleukin-2 and other factors.

Down regulation of these inducible activities can be accomplished by pre-incubating T lymphocytes with doses of soluble antigen above stimulatory concentrations. Whereas down regulation can be demonstrated in as few as 6 hours, unresponsiveness to subsequent induction may last 5 days after removal of the inhibiting ligand. Ongoing investigations into the events accompanying this specific regulation will elucidate the molecular mechanisms effecting the control of lymphocyte division and differentiation. Furthermore, it is hoped that this approach will allow the identification of membrane events responsible for the generation and transmission of both positive and negative cytoplasmic signals originating from ligand binding to the same or similar membrane receptors.

0312 HTLV-I ASSOCIATED T-CELL LYMPHOCYTOSIS NOT PROGRESSING INTO ADULT T-CELL LEUKEMIA, Bernard Poiesz, Bruce Lehr, Tin Han, Fred Davey, Garth Ehrlich, Antonio Planas, Janet Moore, Jeffrey Kirshner, Russ Tomar and John Vournakis, SUNY Upstate Medical Center, VAMC, and Syracuse University, Syracuse, New York 13210 and Roswell Park Memorial Institute Buffalo, New York.

Two patients, one a black female American (MT) and the other a black male immigrant from Liberia (LT), had sustained mature T-cell lymphocytosis for over 3 months. Phenotypic marker studies indicated that the cells were predominantly T4 positive, Ia positive, and in the one patient studied (LT), TAC positive. Both patients had high titer, specific antibody to the gag proteins of HTLV-I as measured in an ELISA assay and to HTLV-I env proteins in HTLV-I membrane antigen assay. Cultured cells from both were positive for HTLV-I p19 using a monoclonal antibody and for HTLV-I homologous DNA sequences using a cloned probe to the gag-pol genes of HTLV-I. A stable TCGF-independent, T4 positive, T-cell line was established from LT patient. It contains one copy per haploid genome of integrated HTLV-I proviral DNA as determined by Southern blot hybridization and is a producer of HTLV-I virions as determined by electron microscopy and reverse transcriptase assays on banded particles. Neither patient has developed a definitive neoplasia. Patient MT now has a normal clinical evaluation, no antibody to HTLV-I, nor detectable HTLV-I positive cells in her peripheral blood. Patient LT has a sustained lymphocytosis, HTLV-I positive, benign lymphadenopathy, and persistent antibodies to HTLV-I proteins. Both patients may represent early phases of HTLV-I infection. Their continued clinical course and further analysis of the integrated viral DNA will be discussed.

0313 AMPLIFICATION OF P GLYCOPROTEIN GENES IN MULTIDRUG RESISTANCE, J. R. Riordan*, N. Kartner+, N. Alon*, K. Deuchars+ and V. Ling+, Hospital for Sick Children*, Ontario Cancer Institute+ and University of Toronto, Toronto, Canada

Multidrug resistance (MDR) is the phenotype exhibited by mutant cells resistant to a certain anti-cancer drug and also cross resistant to a broad spectrum of other drugs having different chemical structures and targets of action. Reduced intracellular levels of drug are maintained as the apparant mechanism of resistance. The P glycoprotein is a large (170 kilodaltons) integral plasma membrane protein which is overexpressed in proportion to the degree of MDR (Kartner et al, Science, 221, 1285,1983). In this study a cDNA library was prepared in the expression vector λgt11 using mRNA from a highly colchicine resistant CHO cell line and screened with a monoclonal antibody to the P glycoprotein. A cDNA corresponding to a portion of the polypeptide sequence of the protein was obtained and employed in molecular hybridization analyses to demonstrate: 1.) an mRNA of approximately 4.7 kb which is overproduced in proportion to the extent of MDR; 2.) multiple genomic DNA bands overrepresented to varying degrees in MDR cells ie. differential amplification of members of a P glycoprotein multigene family; 3.) strong cross species hybridization of the CHO cDNA with mouse and human P glycoprotein genes which are also differentially amplified in MDR. In addition to supporting earlier evidence that the P glycoprotein is a major determinant of MDR these results also provide the tools which can lead to the elucidation of mechanisms at both the DNA and cell membrane levels and to the detection of MDR in patients who become non-responsive to chemotherapy. (Supported by MRC of Canada).

0314 ISOLATION OF A COMMON MYELOGENOUS LEUKEMIA-ASSOCIATED ANTIGEN AND ITS DETECTION IN HUMAN MYELOGENOUS LEUKEMIA USING IMMUNOPEROXIDASE, Robert C. Shipman, Patricia M. Logan, Vincent Lum and Julia G. Levy, University of British Columbia, Vancouver, Canada, V6T 1W5

A 68 Kd protein has been isolated and purified from human myelogenous leukemia cells. This common myelogenous leukemia-associated antigen (CAMAL) has a pl of 7.2, is not phosphorylated and is localized primarily in the cytoplasm of these cells although some membrane association has been observed. Amino acid analysis and peptide sequencing are ongoing. Both rabbit antiserum and monoclonal antiserum (CAMAL-1) have been raised to this 68 Kd protein. By using an indirect immunoperoxidase staining technique the specificity of the monoclonal CAMAL-1 antiserum has been established; most samples from patients with myelogenous leukemia (acute, remission or chronic phase) show significantly higher percentages (≥1%) of CAMAL-expressing cells than those from normals or individuals with lymphoid leukemias. It is possible that expression of the CAMAL protein may represent an underlying pathology in the acute myelogenous leukemia remission state.

- Blood 61:858-866 (1983)
- Diagnostic Immunology 2:86-97 (1984)
- Experimental Hematology 12:539-547 (1984)

0315

T Cell CLL WITH UNUSUAL IMMUNOLOGIC PHENOTYPE AND FUNCTION, H. Simpkins,. D. D. Kiprov and J. L. Davis, Departments of Pathology and Biological Chemistry, University of California, Irvine and The Childrens Hospital of San Francisco, San Francisco, California.

A T cell CLL in an elderly white male presented with lymphocytes expressing a post thymic phenotype with coexpression of T4 and T8 on 80-90% of the cells in the bone marrow. Functional studies showed decreased mitogenic responses but normal helper activity for B cell immunoglobulin secretion and suppressor activity of lectin induced mitogenesis. Morphologic evaluation were consistent with the knobby-type of T cell CLL. Enzyme studies showed low adenosine deaminase and terminal transferase but acetylcholinesterase activity was normal consistent with its T rather than B cell lineage. The patient underwent splenectomy and the lymphocytes showed loss of T4 antigen. HTLV antigens and antibodies were negative. We propose that the cells in this leukemia are at an intermediate stage (not postulated by Rheinherz et al, 1979 Proc Nat Acad, Sci U.S. 76:4061) between the cortical and the medullary thymocyte stage of maturation. It is important to note that a small subset of lymphocytes with this mixed phenotype have been identified in normal individuals. Clinically the patient has continued to do well post-splenectomy (1 1/2 years following diagnosis) which suggests that this is not the aggressive form of T cell CLL and is consistent with no T cell leukemia virus being identified. Recent studies now show that the expression of T4 has decreased from 90% to 30%. It will be interesting to see whether with the emergence of a more "monoclonal disease", the relatively benign clinical course of this patient continues.

0316 MONOCLONAL ANTIBODY (MAB) TREATMENT OF PERIPHERAL BLOOD CELLS (PBC) FROM SOME PATIENTS WITH ACUTE NON-LYMPHOCYTIC LEUKEMIA (ANLL) RESULTS IN GENERATION OF NORMAL CFU-GM IN LONGTERM CULTURE (LTC). J.W. Singer, I.D. Bernstein and P.J. Fialkow, VA Med. Ctr., Univ. Wash. and Fred Hutch. Ca Ctr, Seattle. To determine if MAB treatment of ANLL cells allows expression of normal CFU-GM in longterm culture (LTC), we studied PBC from 4 untreated, glucose-6-phosphate-dehydrogenase (G6PD) heterozygous patients with ANLL. A single G6PD enzyme was found in blast cells and CFU-GM indicating clonal disease. The effect of L4F3 (an MAb that reacts with most normal colony-forming cells) and C' on expression of non-clonal CFU-GM were studied . PBC from the patients were treated with L4F3 or T11D7 (an irrelevant MAB) and C', cultured for CFU-GM growth and placed in LTC on irradiated adherent normal marrow cells in LTC . L4F3 failed to increase expression of normal CFU-GM in 2 samples, and CFU-GM were not detectable after day 7. However, L4F3 and C', but not T11D7, significantly increased nonclonal CFU-GM in the other 2 samples: for patient 1 with a G6PD type B leukemic clone, the ratio of A:B colonies was 7:39 after T11D7 treatment and 27:9 after L4F3 and C' treatment. For patient 2 with an A clone, the values were 76:18 and 31:19, respectively. In these 2 samples, LTC after L4F3 treatment produced 50 CFU-GM/10^5 cells for 8 and 4+ weeks. Without irradiated feeder layers, CFU-GM were not detected. G6PD data indicated that colonies derived from LTC after L4F3 were progeny of normal stem cells. LTC of cells after T11D7 treatment produced small numbers of predominantly leukemic colonies. We conclude: 1) L4F3 treatment of PBC from some patients with ANLL allows normal CFU-GM expression; 2) substantial numbers of normal stem cells not expressing L4F3 are present in ANLL PBC; 3) these stem cells can only be detected if leukemic cells are removed and in LTC; 4) the data suggest a method for detecting a hitherto unmeasurable early stem cell.

0317 THE CONTROL OF Ig BIOSYNTHESIS DURING THE DIFFERENTIATION OF THE I.29 B CELL LYMPHOMA. Roberto Sitia, Sandro DeAmbrosis, Ulrich Hammerling and Janet Stavnezer. Istituto Nazionale per la Ricerca sul Cancro, Genova, Italy- Sloan Kettering Institute New York NY

The I.29 B cell lymphoma consists of cells expressing membrane IgM, IgA or IgE. The three isotypes share identical light chains (λ) and heavy chain variable regions. We have adapted to culture several cell lines expressing μ, α or ε. All of them are inducible by LPS or other B cell mitogens to differentiate into Ig secretion. By day three of culture stimulated cells cease proliferation, increase in size and stop synthesizing membrane heavy chains, while they increase the rate of synthesis of secretory heavy chains. We have further investigated the regulation of Ig biosynthesis during B cell differentiation by Northern blot analyses of the Ig-mRNAs in control and LPS stimulated cells. Our data indicate that the mechanisms that regulate membrane and secreted heavy chain biosynthesis differ in the three isotypes. IgA and IgE biosynthesis is regulated mostly at the pretranslational level, as indicated by the correlation between the rate of synthesis of α_m, α_s, μ_s or ε_s with the amount of the corresponding mRNA. On the contrary, in LPS stimulated IgM cells the synthesis of μm chains stops, while the amount of the 2.7 kb μm specific mRNA increase, indicating that μm and us biosynthesis is regulated also at the translational level.

0318 ALPHA-2 INTERFERON (IFN), MELPHALAN (M) AND PREDNISONE (P) IN THE TREATMENT OF NEWLY DIAGNOSED OF MULTIPLE MYELOMA (MM)

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IFN has shown activity in advanced previously treated MM. Recent in vitro evidence has suggested synergy between cytotoxic agents and IFN. This phase I protocol was initiated to study IFN in combination with M and P. Groups of five patients received S.C. IFN I.I.W for 2 weeks at dose levels of 0.5, 1.0, 2.0 and 5.0 X 10^6 IU/M. During week two M (9 mg/m^2) and P (40 mg/m^2) were administered concurrent with IFN administration followed by a rest period during nadir myelosuppression. Cycles were repeated q 28 days. Twenty-two patients were entered onto study. Median PS was 1 (0-2). There were 15 Stage III, 3 Stage II and 4 Stage I patients. Toxicity information is available on 22 patients. Median nadir WBC/mm at dose levels of 0.5., 1.0, 2.0 and 5.0 X 10^6/M were 3.9 (2.1 - 8.0), 3.6 (1.9 - 7.3), 3.3 (1.9 - 5.6), and 3.1 (2.0 - 4.7), respectively. Platelet counts (days 1-13) were 150 (81 - 381), 124 (48 - 277),179 (72 - 259) and 134 (88 - 197), respectively. Median nadir WBC/platelet/mm (days 14-28) were 2.7 (0.1 - 4.7), 3.2 (2.8 - 8.5),2.9 (1.7 - 5.2) and 2.2 (1.4 - 3.3), respectively. Platelet counts (days 14-28) were 113 (38 - 323), 156 (73 - 582), 184 (105 - 313) and 100 (19 - 238), respectively. Mild transient transaminase elevations occurred in three patients. One patient with hypertensive heart disease expired of a myocardial infarction nine days after the last dose of IFN. A second patient expired of hepatorenal syndrome 29 days after the last dose of IFN. One patient experienced acute renal failure. Constitutional symptoms included reversible flu-like symptoms in nearly all patients at the 1.0 x 10^6 IU level or higher. This Phase I study appears safe and tolerable. Early efficacy information suggests an overall response rate of 70%.

0319 RECOMBINANT HUMAN INTERFERON SENSITIZES RESISTANT MYELOID LEUKEMIC CELLS TO INDUCTION OF TERMINAL DIFFERENTIATION. P.J.R. Spooner[1], S. Grant[2], S. Pestka[3], I.B. Weinstein[2], and P.B. Fisher[1], Departments of Microbiology and Medicine[2], Columbia University, College of Physicians & Surgeons, New York, NY 10032 and Roche Institute of Molecular Biology[3], Nutley, NJ 07110

A major problem in chemotherapy is the development of resistant tumor cell populations. By growing HL-60 cells in increasing concentrations of 12-O-tetradecanoyl-phorbol-13-acetate (TPA), dimethylsulfoxide (DMSO) or trans retinoic acid (RA) a series of resistant variants have been generated which are not inhibited in their growth or induced to differentiate by concentrations of TPA, DMSO or RA which affect the parental HL-60 cells. When applied alone to the appropriate resistant variant, TPA (10^{-7}M), DMSO (1.2%), RA (2.5 x 10^{-5}M) or recombinant human leukocyte interferon (IFN-αA) (1000 units/ml), resulted in a 23, 19, 40 or 18 to 36% reduction in growth, respectively, whereas the combination of IFN-αA and the appropriate inducer resulted in a >90% inhibition in growth and a concomitant induction in differentiation as demonstrated by NBT staining. Analysis of the effect of IFN-αA and DMSO, used alone or in combination, on the levels of c^{myc} RNA indicates a complex biphasic response including a synergistic suppression in c^{myc} expression in cells treated with IFN-αA plus DMSO. The ability of interferon to interact synergistically with agents which promote leukemic cell maturation may represent a novel means of reducing resistant cell populations. (Supported by an award from Hoffmann La Roche Inc.)

0320 IMMUNOTOXINS AS POTENTIAL PURGATIVE AGENTS FOR AUTOLOGOUS BONE MARROW TRANSPLANTATION FOR LEUKEMIA, Daniel A. Vallera, Esmail D. Zanjani, Franz K. Jansen and Robin C. Stong, University of Minnesota, Minneapolis, MN 55455, Clin-Midy, Montpellier, France. Supported by NIH grants CA-31618 and CA-36725.

Immunotoxins represent a new class of pharmacological reagents whereby monoclonal antibodies are chemically linked to potent toxin. Such reagents are currently employed for antibody-directed cell targeting. The two most important characteristics of immunotoxins are their a) selectivity and b) potency. In these studies we have investigated an immunotoxin composed of the monoclonal antibody T101 directed against a P65 glycoprotein on the surface of most human T cell acute lymphoblastic leukemias. This monoclonal was covalently linked to the A chain of the potent phytotoxin ricin. We have studied potency using a clonogenic assay system. We showed that immunotoxin alone eliminated less than 1 log of T-ALL cells (CEM cell line).

It has been reported that certain lysosomotropic potentiators can augment the activity of A chain immunotoxins. We compared as potentiators, ammonium chloride and the carboxylic ionophores X537A and monensin. When added to the preincubation mixture containing immunotoxin, these agents increased potency as much as 1000-fold. Monensin enhanced toxicity to the greatest degree. Toxicity was selective since inhibitory doses were minimally toxic to pluriportent stem cells of granulocytic, erythroid, monocytic and megakaryocytic lineage. We conclude that this purgative approach may be effective in eliminating contaminating leukemia cells from grafts for autologous bone marrow transplantation.

0321 HIGH DOSE CYCLOPHOSPHAMIDE, BCNU AND VP-16 (CBV) AND AUTOLOGOUS BONE MARROW RESCUE AS TREATMENT FOR ADULT ACUTE LEUKEMIA IN RELAPSE. L. Vellekoop, A. R. Zander, G. Spitzer, S. Jagannath, L. Horwitz, M. Keating, K. McCredie and K. Dicke, The University of Texas, M. D. Anderson Hospital and Tumor Institute, Houston, TX 77030.

28 patients with acute leukemia in relapse, median age 25 yrs, range 17-63, received high dose combination chemotherapy consisting of Cyclophosphamide 6 gm/m^2, BCNU 300 mg/m^2 and VP-16 600 mg/m^2 , followed by infusion of autologous bone marrow stored during previous remission. Of 12 patients with acute myelogenous leukemia (AML) 6 achieved a complete remission (CR) and of 16 patients with acute lymphocytic leukemia (ALL) 7 reached a CR, for a total CR rate of 46%. When the patient population was broken down by number of previous reinduction attempts, the CR rate was as follows;

CBV as	1st	2nd	3rd	4th	5th	salvage regimen
Number of patients	1	13	11	2	1	
Number of CR	1	7	5	0	0	

Median remission duration was 4 months, range 1-8 months. Main toxicity was myelosuppression with related bleeding and infection. There was only one early death, from hemorrhagic pneumonia. In the patients who achieved CR median time to recovery to 1000 granulocytes/µl was 35 days (range 14-70) and median time to 100.000 platelets/µl was 37 days (range 17-80). It is concluded that CBV is an active regimen, even in 3rd salvage, where conventional treatment has a CR rate of only 10%, but that remissions are of short duration.

Therapy—Chronic Leukemias

0322 CHRONIC MYELOGENOUS LEUKEMIA: REVIEW OF RECENT ADVANCES. Richard Champlin, M.D., Div. Hematology/Oncology, UCLA Center for Health Sciences, Los Angeles, CA 90024.

Chronic myelogenous leukemia (CML) is a clonal disorder of pluripotent stem cells resulting in massive expansion in pools of committed myeloid progenitors. B lymphocytes and some null cells are also derived from the malignant clone. Circulating T lymphocytes are polyclonal but several cases of T-cell blast crisis have been reported indicating that T-lymphocytes may also be involved. The Philadelphia (Ph^1) chromosome t(9;22) is a cardinal feature of CML. This translocation results in rearrangement of the cellular proto-oncogenes c-sis from chromosome 22 to 9 and c-abl from chromosome 9 to 22. C-sis expression is not increased but a novel 8 Kb RNA transcript of c-abl has been reported in cells with the Ph^1-chromosome. Considerable data suggests that CML results from a multi-step process; clonal populations of Ph^1-negative cells have been demonstrated with the Ph^1 chromosome may be acquired as a second or subsequent step in the oncogenic process.

CML can be divided into 3 phases. 1) Chronic phase in which cellular maturation is normal and the disease can be controlled by chemotherapy; 2) accelerated phase where symptomatic progression occurs and resistant to chemotherapy develops; and 3) acute phase, the terminal stage associated with maturation arrest similar to acute leukemia. Myeloid and lymphoid variants of acute phase occur. Median survival in CML is 3-4 years but survival following development of accelerated or acute phase is generally < 6 mo. A number of prognostic factors have been identified at the time of diagnosis. Patients with high leukocyte counts, marked hepatosplenomegaly and a large proportion of immature cells have the worst prognosis.

The conventional treatment of CML has not substantially prolonged the duration of chronic phase or survival. Chemotherapy with hydroxyurea or busulfan is successful to control the leukocyte count and the symptoms of CML during the chronic phase but cannot eradicate the malignant clone. More aggressive combination chemotherapy may transiently eliminate Ph^1-positive cells from the bone marrow but the disease generally recurs rapidly and survival has not been improved. Recently, interferon has been reported to suppress granulocytosis and also reduce the proportion of Ph^1 cells in the bone marrow. These interesting data must be confirmed.

The most encouraging advance in therapy involves the use of bone marrow transplantation (BMT). High dose cyclophosphamide, total body irradiation and BMT is capable of eradicating Ph^1-chromosome positive cells and reestablishing normal hematpoiesis. The results of BMT have been best for patients in chronic phase where > 60% have achieved over 3 year disease free survival. Results have been worse in patients with accelerated or acute phase where the risk of leukemic relapse is higher and extended survival has been achieved in 10-30% of patients.

0323 CHRONIC LYMPHOCYTIC LEUKEMIA, Kenneth A. Foon* and Robert Peter Gale†
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Chronic lymphocytic leukemia (CLL) is a hematologic neoplasm characterized by proliferation and accumulation of relatively mature-appearing lymphocytes. Almost all of the cases involve a clonal proliferation of B lymphocytes. CLL is the most common leukemia in the United States, accounting for 40% of all cases; patients have a median age of 50 years. The B lymphocytes characteristic of CLL display a relatively small amount of surface immunoglobulin, and in most cases, cells display a single heavy chain class, typically μ, sometimes with δ, and the light chain is either κ or λ. These cells also display receptors for mouse erythrocytes, Fc and complement receptors, and HLA-DR antigens, as well as other B-cell-associated antigens. Patients with B-CLL typically have hypogammaglobulinemia; this is most likely related to decreased B-cell function and possibly to abnormalities of T cells as well. There are numerous metabolic abnormalities associated with B-CLL lymphocytes, including abnormal membrane fluidity and permeability, decreased 5' nucleotidase, increased ratio of thymidine kinase isoenzyme 1 to 2, decreased cholesterol and tocopherol, decreased actin content, and decreased receptors for some lectins. The phenotypic expression of CLL is usually stable over months to years; however, several forms of transformation have been described, including the development of a diffuse lymphoma (Richter's syndrome), prolymphocytic transformation, and progression to an acute (or blast) crisis. Chromosomal abnormalities are being described with increasing frequency, particularly trisomy 12, which, in combination with other chromosomal changes, is associated with more advanced diseases. Other unfavorable prognostic factors include advancing age, increased CLL cell count, anemia, thrombocytopenia, and a diffuse pattern of bone marrow involvement. The three classes of therapeutic agents most commonly used in CLL are alkylating agents, radiation therapy, and corticosteroids. Little progress has been made in the past ten years that improves the prognosis for CLL. More recently, biological response modifiers such as monoclonal antibodies and interferons have been studied in patients with chronic lymphocytic leukemia, but major advances have been difficult to achieve. These newer approaches to therapy will be presented.

0324 HAIRY CELL LEUKEMIA, Harvey M. Golomb, James W. Vardiman, Mark J. Ratain, Karen Daly, Department of Medicine, The University of Chicago, Chicago, IL 60637

Hairy cell leukemia is a chronic lymphoproliferative disease with a variable course. The diagnosis requires a bone core biopsy specimen as the peripheral blood findings can be misleading. Some patients do well for many years without treatment, these are usually older men without palpable spleens. Once an indication for treatment arises, splenectomy should be considered as the first choice. The response to splenectomy can be dramatic and various degrees of response are seen in almost all patients. However, approximately one-third of patients progress between the next several months and up to 10 or more years later with either the leukemic phase or progressive pancytopenia. Although daily chlorambucil chemotherapy reverses the progressive bone marrow involvement in the majority of patients treated, approximately 25% still succumb to a serious infection. For the past one year, we have been treating patients with progressive disease with recombinant alpha-2-interferon. Twenty patients have completed at least 8 weeks of IFN and are evaluable. Patients characteristics were as follows: median age 47, 17M:3F, 19/20 prior splenectomy, 11/20 prior chlorambucil. Reasons for treatment were: Hb <10 (9/20), plts <100,000 (12/20), plts <40,000 (7/20), neutrophils (NP) <1000 (15/20), leukemic phase (7/20). Pts 1-4 received an initial dose (later reduced) of 10 million IU (MU) /m^2 thrice weekly, and all other pts received 2 MU/m^2. Five pts achieved a partial response (normalization of peripheral blood counts and differential), and 11 pts were minor responders (Hb >12 or plts >100,000 or NP >1500 or hairy cells <5%, if initially leukemic). No patient achieved a complete response (absence of hairy cells from bone marrow (BM) but 2 pts had <5% HCL in the BM. Median time to response was: plts 5 weeks, leukemic phase 14 weeks, NP 18 weeks, Hb 19 weeks. Transient myelosuppression occurred during the first month of IFN: ↓Hb 9±7%, ↓plts 31±25%, ↓NP 49±30%. Typical IFN side effects were seen in most pts, as well as alopecia (5 pts), paresthesias (3 pts), and chemical hepatitis (15 pts). A BM was obtained monthly, revealing ↓hairy cells in all pts. However, BM changes did not always correlate with changes in peripheral blood counts. Other BM changes included ↑M:E ratio, ↑eosinophils, ↓ plasma cells, and ↓cellularity. Recombinant IFN at doses of 2-10 MU/m^2 thrice weekly, is safe and effective in the management of pts with HCL. Responses are achieved rapidly; especially correction of life-threatening thrombocytopenia. The optimal dose of IFN and duration of therapy remain to be determined. It is clear, however, that hairy cell patients with progressive disease can be treated with IFN, have an objective improvement in their blood counts, and only minor morbidity.

Pharmacologic Approach to Leukemia

0325 SELECTIVE APPROACHES TO ERADICATE DRUG RESISTANT CELLS, Joseph R. Bertino and Enrico Mini, Department of Pharmacology, Yale University School of Medicine, New Haven, CT 06510

Development of drug resistance to chemotherapeutic agents represents a major obstacle to the cure of acute leukemia, in particular adult leukemia. Since drug resistant cells presumably are genetically different from the cells they are derived from, as well as normal renewal populations, they became unique targets for selective approaches. We have developed sensitive assays for detecting drug resistant leukemia cells to the drug methotrexate. Since 4 different mechanisms are known that give rise to drug resistant populations, we have developed an extended [^{3}H]-deoxyuridine incorporation assay in the presence of MTX that detect resistance to MTX regardless of the cause. If resistance is present, then the exact mechanism is determined by "dot blot" assay (gene amplification), ^{3}H-MTX uptake studies, inhibition of dihydrofolate reductase by MTX (altered enzyme), and ^{3}H-MTX polyglutamate formation (altered or defective folate polyglutamate synthetase).

We are testing (phase I clinical trials) a second generation "non-classical" antifolate, trimetrexate, that is equally effective vs. MTX transport resistant cells, as the parent sensitive line. Combinations of MTX and trimetrexate, or alternating treatment with these drugs may prevent or delay MTX resistance.