HEMATOPOIETIC CELL DIFFERENTIATION

D. W. Golde, M. J. Cline, D. Metcalf, Organizers

Plenary Sessions

Poster Sessions

Erythropoiesis

380 ERYTHROPOIESIS-NORMAL ERYTHROPOIESIS AND INDUCED MURINE ERYTHROLEUKEMIA DIFFERENTIATION, Paul A. Marks and Richard A. Rifkind, Cancer Center and Departments of Medicine and Human Genetics and Development, Columbia University, New York, New York 10032

Normal erythropoiesis is a useful system for defining several aspects of the regulation of eukaryotic cell differentiation (1-4). Regulation of the rate of erythropoiesis may be achieved by control mechanisms which exert their effects on 1) proliferation of the pluripotent hematopoietic stem cells, 2) commitment of stem cells to erythropoiesis, 3) proliferation of committed erythroid precursor cells (which include several recognized sequential stages differing in proliferative capacity and responsiveness to erythropoietin), 4) commitment of erythroid precursor cells to express the program of biosynthetic and morphogenetic activities characteristic of terminal erythroid differentiation. The influence of the hematopoietic environment on the developmental potential of the stem cell is supported by considerable data. Erythropoietin appears to have its principle effect on the proliferation of the target erythroid precursor cell. Differentiation of progeny of these precursors is an inherent property of the cell lineage, following a probabalistic model. Stimulation of RNA synthesis is the earliest detected effect of erythropoietin on macromolecular synthesis. Early RNA includes rRNA and 4 and 5S RNA but not globin RNA. DNA synthesis may be required for the transition to globin mRNA production and hemoglobin synthesis. Investigations with normal erythropoietic cells have been limited by several factors. Friend virus infected murine erythroleukemia cell (MELC) lines have been established in continuous culture. MELC show a low level of spontaneous differentiation (0.5%); when cultured with dimethylsulfoxide (Me_2SO) or a variety of other agents, are induced to erythroid differentiation at a much greater level. This program of differentiation has many similarities to normal erythropoietin regulated differentiation. This system permits analysis at the molecular and cellular levels the relationship of inducer action to commitment to differentiation, defined as the continued expression of the erythroid program in cells removed from culture with inducer. Inducer causes metabolic changes before commitment which are possibly reversible. Events occurring at or after commitment appear to be irreversible.

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381 PATHWAY-INDIFFERENT VERSUS PATHWAY-SPECIFIC HUMORAL REGULATION OF HEMOPOIESIS: A MODEL. Norman N. Iscove, Basel Institute for Immunology, Basel, Switzerland.

Early and late stages in red cell formation prior to hemoglobin synthesis can be delineated independently on the basis of clonogenic properties in culture. When erythropoietin levels are manipulated in vivo and in culture, the responses of these red cell precursors suggest that the earliest committed erythroid progenitors are not sensitive to erythropoietin. Rather, responsiveness appears and accumulates only with maturation beyond the initial stages. During early but not later stages, proliferation in culture is dependent on the presence of other molecules which are distinct from erythropoietin. These can be supplied either by serum or by medium conditioned by mouse spleen cells treated with pokeweed mitogen or concanavalin A. The conditioned media reduce the concentration of erythropoietin required for proliferation and maturation through the intermediate stages of erythropoiesis. They also induce the formation of colonies containing both erythroid and granulocytic cells. It will be argued that the molecules responsible for this activity represent a new class of humoral regulator acting not only on early committed red cell precursors, but also on pluripotential hemopoietic stem cells and early committed cells of each of the derived differentiation pathways.

382 HORMONAL CONTROL OF ERYTHROPOIESIS, J.W. Adamson, W.J. Popovic, and J.E. Brown. Veterans Administration Hospital and University of Washington, Seattle, Washington.

Erythropoietin (ESF) is the primary hormone which regulates erythropoiesis in mammals, although other hormones also influence red cell production. In culture, several hormones interact with ESF to influence erythropoiesis, including steroids, growth hormone, catecholamines, and thyroid hormones. The results of several studies suggest multiple mechanisms of interaction and provide evidence that the effect of these hormones may be linked through common intracellular pathways.

The differentiation and proliferation of many cellular systems are influenced by changes in levels of cyclic nucleotides. Cyclic adenosine nucleotides (cAMP) specifically enhance erythroid colony formation while cyclic guanosine, cytosine, inosine, and non-cyclic nucleotides do not. Several hormones, known to act through surface receptors to raise intracellular levels of cAMP, also enhance erythroid colony formation. Among these are the β-adrenergic agonists, isoproterenol and epinephrine. This activity is blocked by low concentrations of propranolol. With combinations of specific agonists and antagonists, the adrenergic effect was shown to be mediated by receptors with β_2 subspecificity. The antagonists had no effect on ESF-dependent colony formation, indicating that the adrenergic receptor and the putative receptor for ESF are distinct.

Thyroid hormones also enhance erythroid colony growth and the concentrations required resemble physiologic levels in man. The thyroid hormone effects are also blocked by propranolol in culture and the populations of responsive cells for thyroid and adrenergic hormones are identical as assessed by velocity sedimentation. Thyroid hormones also appear to interact with a receptor having β_2 subspecificity when antagonists of known affinity are employed. In the hypothyroid state, a pattern of hormone/target cell interaction is found which reflects clinically described alterations in adrenergic activity. Thus, β-adrenergic agonists are ineffective in the hypothyroid state while α-agonists, such as phenylephrine, become active. This change in the display of receptor activity is reversible upon preincubation of marrow cells with thyroid hormone. These findings provide evidence that the pattern of hormonal interactions defined in vitro reflects the endocrine state in vivo and that such interactions may significantly contribute to erythropoietic regulation in the intact animal.

Accumulating information suggests that a number of hormones may interact with subpopulations of erythroid colony forming units to modulate the response of these target cells to ESF. Of particular interest, studies with catecholamines and thyroid hormones suggest that some of these interactions may occur through a common intracellular pathway which involves cyclic nucleotides. Such interactions provide an example of growth modulation which may have in vivo as well as in vitro correlates and suggest mechanisms by which other hormones and small molecules may affect terminal erythroid differentiation.

383 FRIEND VIRUS AND MALIGNANT ERYTHROPOIESIS, Arthur A. Axelrad, Shigetoh Suzuki, Henk Van der Gaag, Bryan J. Clarke and David L. McLeod, Division of Histology, Department of Anatomy, University of Toronto, Canada M5S 1A8.

Friend Virus (replication-defective spleen focus-forming virus SFFV together with helper LLV) induces a progressive defect in regulation of hemopoiesis whose most prominent expression is along the erythrocytic line of differentiation. Susceptibility to the development of this malignant condition, called erythroleukemia or polycythemia, is under the control of several genes. The Fv-2 gene locus (Chromosome 9) is recognized by its effect on the induction by this virus of macroscopic spleen foci or spleen enlargement. Susceptibility is dominant; resistance is solid. However neither the cellular site nor the mechanism of action of the gene is known.

Kumar et al. have proposed a hypothesis implicating marrow-dependent immunocompetent M cells as the site of Fv-2 gene action. This hypothesis has been reexamined with the help of the congenic strains B6(C57BL/6) and B6.S, which we have shown to differ at Fv-2. Results of experiments on spleen focus induction by Friend virus after repopulation of supralethally irradiated hosts with mixtures of bone marrow cells from sensitive ($Fv\text{-}2^{ss}$) and resistant ($Fv\text{-}2^{rr}$) donors were consistent with the concept that the site of action of the Fv-2 gene is in cells that are potentially capable of undergoing erythroleukemic transformation. The results were not consistent with the M cell hypothesis.

Friend virus infection in vivo with assays for erythropoietic progenitor cells in vitro have shown that the development of erythropoietin-independent CFU-E was strongly affected by the Fv-2 status of the host.

High specific activity ^3H thymidine had little or no effect on the marrow or spleen BFU-E of B6 mice but substantially reduced the number of BFU-E of B6.S mice. This suggests that Fv-2 may control the state of cycle of BFU-E. The implications of this finding for the mechanism of action of Fv-2 on susceptibility or resistance to SFFV will be explored.

384 ERYTHROPOIESIS, Chairman's Opening Remarks, Allan J. Erslev, Cardeza Foundation, Thomas Jefferson University, Phila., PA 19107

About 100 years ago, Dr. Dennis Jourdanet, a little known friend and mentor of Paul Bert, observed that the blood of his surgical patients from the highlands of Mexico was thick and viscous. In a book "The Anemia of Altitude" published in 1863, he proposed that this plethoric blood in some way was caused by the low oxygen content of arterial blood. Thirty years later Fredric Miescher suggested that hypoxia had a direct stimulating action on the bone marrow leading to increased red cell production and polycythemia. However, this hypothesis could not be confirmed experimentally and in the nineteen fifties, an alternate hypothesis, first proposed by Carnot and Deflandre in 1906, was tested and expanded. It was shown that the bone marrow rather than being stimulated directly by hypoxia is stimulated indirectly via a hormone, named erythropoietin, and that the kidney is the main site of production of this hormone. This production apparently involves the interaction of an oxygen sensor with an erythropoietin producer but the exact sites and mechanisms of these two functions are still quite obscure.

It is generally accepted that the main production of erythropoietin is regulated by an oxygen sensor located either in the cortex or the medulla of the kidney. However, the actual mechanism of production of erythropoietin is unsettled. Is it carried out by the oxygen sensing cells, by cells at a distance, inside or outside the kidney, and controlled by short-lived releasing hormones or does it involve the production of an enzyme by the oxygen sensor, an enzyme capable of activating a circulating inactive erythropoietin precursor? Current experimental evidence appears to favor the first concept and oxygen sensing and erythropoietin production seems to be caused by the same cells probably located in the medulla. The one cell concept is supported by the fact that oxygen sensing and erythropoietin production apparently can be carried out by a number of extrarenal cells, including cells of the reticuloendothelial system, normal hepatocytes and malignant cerebellar and hepatic cells. Actually, it seems possible that all cells may have the capacity to produce erythropoietin in response to a hypoxic stress and that the accelerated extrarenal erythropoietin production observed in the rapidly growing fetus, after partial hepatectomy and in hemolytic anemia are all caused by a relative oxygen deficiency in rapidly metabolizing or regenerating cells. Although evolution has placed erythropoietin production in the kidney at a distance from the bone marrow, local extrarenal production of erythropoietin in a hypoxic bone marrow, as dimly envisioned by Fredric Miescher, may have some regulatory function on erythroid stem cell differentiation.

385 MOLECULAR ASPECTS OF THE INITIATION OF ERYTHROPOIESIS, Eugene Goldwasser and George Inana, Department of Biochemistry and Franklin McLean Memorial Research Institute, University of Chicago, Chicago, IL 60637

Erythropoietin (epo) has an early effect on transcription by target cells. In addition there is evidence, of an indirect nature, suggesting that there is a protein that may be a specific epo receptor on the external surface of marrow cells. The question then arises: how does the interaction of epo at the outside surface of a cell affect transcription in the nucleus? The possibility of epo being internalized after interaction with cells is not yet susceptible to experimental test because, to date, all methods of preparing a radioiodine derivative of epo have resulted in inactivation. We have described the existence of a cytoplasmic protein factor derived from epo-treated marrow cells, (termed MCF, for marrow cytoplasmic factor) that was devoid of epo activity toward whole cells and appears to be the sought for mediator. It has been possible to stabilize MCF activity and further study of its mode of action has been undertaken. Marrow, kidney or liver nuclei were permitted to interact with an MCF preparation; then washed, sonically disrupted and the activities of RNA polymerases I and II and of DNA template were measured using the differences between total RNA synthesis and that resistant to α - amantin as an indicator of the relative amounts of the two polymerases. Similarly, the effect of MCF on transcription occuring in the presence of a saturating amount of exogenous rat RNA polymerase was used to determine its action on template availability. An effect of MCF was apparent on all three components. Using the increment in cpm due to MCF as a baseline, we found an increase in transcription due to excess DNA of 124% to 208%, depending on the source of nuclei. With all three types of nuclei, the effect of MCF on transcription was made up of approximately 60% due to polymerase I and 40% due to polymerase II. Adding excess polymerase resulted in an increase in MCF effect, over that with no additions of, 64% for kidney nuclei, 300% for marrow and 800% for liver nuclei. The absolute change in template availability due to MCF was, however, small. The effect of MCF seems to require intact nuclei, i.e. there is no direct effect of MCF on partially purified rat liver polymerase.

386 REGULATION OF THE INDIVIDUAL GLOBIN GENES, Arthur W. Nienhuis, Jane E. Barker, Edward J. Benz, Jr., Richard Croissant, Judy Kantor, Peter Kretschmer, Donald M. Miller, and Neal Young, Clinical Hematology Branch, NHLBI, NIH, Bethesda, MD 20014.

The erythropoietin-induced switch from Hb A ($\alpha_2\beta^A_2$) in the adult sheep of Hb F ($\alpha_2\gamma_2$) in the fetal sheep to the synthesis of Hb C ($\alpha_2\beta^C_2$) provides a model to explore the cellular and molecular basis for regulation of the individual globin genes. Induction of Hb C synthesis in cultures of sheep fetal liver represents an action of erythropoietin on an early progenitor cell.[1] Fractionation by unit gravity sedimentation has permitted partial separation of the BFU-E and CFU-E sub-populations of progenitor cells. At a high ESF concentration, CFU-E derived erythroid colonies synthesize 15-25% Hb C between 72-96 hr in culture whereas the larger colonies derived from the more slowly sedimenting BFU-E population make 45-60% Hb C between 144 and 168 hr in culture. This evidence would suggest that ESF may act on CFU-E and earlier progenitor cells in inducing expression of the β^C globin gene. Using pancreatic DNAse I to explore the structure of the individual globin genes in chromatin,[2] we have exposed nuclei from adult anemic sheep making only Hb C ($\alpha_2\beta^C_2$) to this nuclease resulting in digestion of 5-20% of the total nuclear DNA. Each of the globin genes was digested equally; the concentrations of γ, β^A and β^C sequences were reduced 4-5 fold as shown in a specific molecular hybridization assay. These data appear to imply that modulation of expression of the individual globin genes in maturing erythroblasts occurs by modulation of the transcriptional rate of the individual genes or selective processing of precursor mRNA molecules. To further explore these mechanisms, totally pure probes for the individual globin gene and mRNA sequences are required. To achieve this we have synthesized double stranded globin genes using reverse transcriptase and prepared these for insertion into bacterial plasmids by the A-T tailing method.[3] Recombinant plasmids have been obtained as indicated by preliminary hybridization and restriction endonuclease analysis. These will facilitate further analysis of globin mRNA synthesis.

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Granulopoiesis

387 PURIFICATION AND HETEROGENEITY OF CLONABLE MURINE GRANULOCYTE AND MACROPHAGE-PROGENITOR CELLS (CFU-c), N. Williams and R. R. Eger, Sloan-Kettering Institute for Cancer Research, 145 Boston Post Road, Rye, NY 10580.

Colonies of mouse macrophages and granulocytes (colony-forming units--culture) can be grown in semisolid cultures in vitro from a precursor cell. The growth of these cells is dependent on obligatory stimulators (colony-stimulating factors). These moieties may be specific hormonal regulators of macrophages and granulocytes in the intact animal. CSFs generally fall into two classes based on the morphology of the end cells in the colonies (macrophages and mixed granulocyte-macrophage colonies), and on the dose responsiveness of the progenitor cells to the stimulus. Despite extensive investigation, an activity which exclusively stimulates the production of neutrophil colonies has hitherto not been described. Such an entity has recently been isolated from the conditioned medium of a myelomonocytic leukemic cell line (WEHI-3), which stimulates colonies of which 60-90% contain only granulocytes. The dose-response curve of this fraction differs from other preparations, in that the percentage of granulocytes stimulated increases as the stimulator is diluted. As well as heterogeneity in morphology, murine CFU-c subpopulations separated by equilibrium density centrifugation differ in their responsiveness to the different CSFs and potentiators, in their cell cycle characteristics and in the optimum time required for colony formation. Since the CFU-c are extremely heterogeneous in their biological and physical properties, purification of these progenitor cells has proved difficult. Biological enrichment of CFU-c has been achieved, however, by selective depletion of lymphoid and erythroid cells from bone marrow cells which proliferate in vitro over several months. Further enrichment is obtained by subsequent incubation of these cells with WEHI-3 conditioned medium and selective removal of adherent cells and cells bearing Fc receptors, such that 10-25% of all remaining cells clone in vitro.

388 DIFFERENTIATION PROGRAMMES UNDERLYING CELLULAR HETEROGENEITY IN THE MYELOBLASTIC LEUKEMIAS OF MAN, Ernest A. McCulloch, Ronald N. Buick, Mark D. Minden, Carlos Izaguirre and James E. Till, Institute of Medical Science, University of Toronto and the Ontario Cancer Institute, Toronto, Ontario, Canada M4X 1K9

Continuing myelopoietic differentiation contributes to cellular heterogeneity in Acute Myeloblastic Leukemia (AML). Chromosome markers of leukemic hemopoiesis have been identified in granulopoietic and erythropoietic precursor cells; accordingly AML is considered to originate in pluripotent stem cells (for a review see 1). Myelopoietic differentiation programmes are studied using clonal culture assays for granulopoietic (CFU-C) and erythropoietic (BFU-E, CFU-E) progenitors. With these assays, striking patient-to-patient variation is observed. During clonal re-expansion following chemotherapy, a similar heterogeneous distribution of values is seen, but individual patients reassort themselves in the distribution rather than retaining their original rank. This finding is compatible with random events during clonal expansion rather than a heritable lesion as the basis for patient-to-patient variation (2). Further, numerical correlations between erythropoietic and granulopoietic progenitors are compatible with a normal myelopoietic differentiation pattern at the time of diagnosis; these correlations are maintained during remission induction (3). On the basis of these data, the programmes of myelopoiesis appear unlikely to contain lesions primarily responsible for AML phenotypes.

An alternative may be found in the programme leading to morphologically identified blast cells. A progenitor class in leukemic blood may be identified by its capacity to give rise to colonies of blast-like descendants in culture (4). With this assay plating efficiency is correlated with blast cell concentration (Spearman rank correlation coefficient 0.90 for 18 patients at diagnosis). Chromosomal markers of leukemic clones have been identified in metaphase cells from the colonies. Progenitors of colonies of T-lymphocytes co-exist with blast progenitors in leukemic blood. These form rosettes with sheep erythrocytes and may be separated from blast cell progenitors by centrifugation in ficol-hypaque after rosette formation.

The blast cell assay has been used to measure anthracycline sensitivity (blast cell precursors more sensitive to drug than CFU-C or T cells) and cell cylce state (blast cells in rapid cell cycle, while T cells may be cycling or quiescent). Blast cell progenitors may be replated from single colonies, indicating that they may have self renewal properties.

The suggestion is advanced that primary leukemic lesions may be identified in the differentiation programme leading to blast cells. This programme may have a normal counterpart. Alternatively it may be assembled abnormally from the repetoires of other programmes.

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389 PURIFICATION AND CHARACTERIZATION OF CELL-SPECIFIC COLONY STIMULATING FACTORS, Antony W. Burgess, Donald Metcalf, Nicos A. Nicola and Susan Russell, Cancer Research Unit, Walter and Eliza Hall Institute of Medical Research, Parkville, 3050, Australia.

Conditioned media capable to stimulating the in vitro clonal proliferation of hematopoietic progenitor cells often contain several colony stimulating factors (CSF's) of different cellular specificity, as well as substances which nonspecifically inhibit or potentiate colony growth. Human placental conditioned medium (HPCM) and pokeweed mitogen stimulated spleen conditioned medium (PKM-SCM) will be used to compare the efficacy of different techniques for the purification and characterization of cell-specific CSF's. HPCM contains eosinophil (EO)-CSF, granulocyte-macrophage (GM)-CSF (which stimulate human progenitor cells) and substances which inhibit colony growth. PKM-SCM contains EO-CSF, GM-CSF, megakaryocyte (MEG)-CSF, erythroid (E)-CSF (which stimulate mouse bone marrow or fetal liver progenitor cells) and substances which inhibit colony growth. Although HPCM is easily concentrated ten-fold by ultrafiltration, high concentrations of inhibitors are present which completely prevent colony formation. It is necessary to partially purify the CSF's before a supramaximal stimulus for colony growth can be obtained. A 300-fold purification of the CSF's in HPCM has been obtained by dialysis, calcium phosphate gel absorption and gel filtration on Sephadex G100. These techniques remove the inhibitory substances and much of the contaminating proteins but are not effective in separating EO-CSF from GM-CSF. The distribution of EO-CSF across the peak of colony stimulating activity on gel filtration was not uniform, suggesting that EO-CSF was slightly larger than the GM-CSF. Hydrophobic chromatography has proved useful, in that all of the EO-CSF and 50% of the GM-CSF elute directly from Cibachrome blue-Sepharose, but only GM-CSF is present in the proteins bound and eluted from the column. The MEG-CSF, EO-CSF and E-CSF present in PKM-SCM were lost on dialysis. Partial purification (40-fold) can be obtained, however, by concentration using hollow-fibre ultrafiltration, Sephadex G150 and concanavalin A-Sepharose. The CSF's in PKM-SCM appeared to have identical molecular weights and all bound to and eluted from the lectin column in the same way. Thus these techniques facilitated purification, but not separation of the cell-specific CSF's. After the initial purification has been achieved, phenyl-Sepharose chromatography, thin layer electrophoresis and isoelectric focussing have been used to partially separate EO-CSF and MEG-CSF from GM-CSF.

390 MOLECULAR PROBES OF HEMOPOIESIS, G.B. Price, D. Bazett-Jones, R. Krogsrud and P. Ottensmeyer, Department of Medical Biophysics, University of Toronto, Toronto, Ontario, Canada M4X 1K9.

Proteins and peptides isolated from leukocyte conditioned media which stimulate the growth of hemopoietic colonies in cultures have been characterized. Two types of colonies have been used to assay these stimulators, granulocyte and cytotoxic T-lymphocytes colonies. These potential physiological regulators of hemopoiesis are membrane-associated and bear antigenic similarities to β_2-microglobulin and products of the major histocompatibility complex. One low molecular stimulator of hemopoiesis may bear Ca^{++} ions across the lipid bilayer of cell membranes as a mechanism of activation of hemopoietic progenitor cells; dark-field electron micrographs of these molecules have provided structural estimates compatible with this suggestion. The membrane association of these molecules, a possible close relationship to major histocompatibility antigens, and the possible role for an "ionophore" in regulation of hemopoiesis make these molecules and analogs interesting probes for investigation of the progenitor cells' response characteristics. Research supported by the National Cancer Institute of Canada.

391 INHIBITORS OF GRANULOPOIESIS. M.J. Cline, Dept. of Med., UCLA, Los Angeles, CA 90024

A variety of inhibitors of granulopoiesis have been described in normal and disease states. Two groups of inhibitors thought to have physiologic significance are the neutrophil-derived chalones and immunologic suppressors of myelopoiesis. The existence of normally occurring chalones is controversial. We looked for their presence in the products of purified populations of human neutrophils (PMN) using several different proliferating hematopoietic cells as targets, and various assays including ^{3}H-thymidine incorporation, mitotic indices, CFU-C and CFU-E culture. PMN-conditioned medium inhibited the proliferation of erythropoietic precursors and some leukemia cells without affecting CFU-C or more differentiated myeloid cells. The PMN-derived factor has been partially characterized. Turning to established cell lines, we identified a line from a patient with chronic myelocytic leukemia (CML) whose conditioned medium inhibits CFU-C much more potently than CFU-E or Friend erythroleukemia cells. Screening of 30 other cell lines revealed a lung carcinoma whose product is also potently inhibitory of CFU-C. These inhibitors have now been partially characterized. The carcinoma-derived factor is stable at 56^o and labile at 70^{o}C, >100,000 daltons, and inhibits at least some myeloid leukemia cells. The CML factor is >10,000 daltons. Immune inhibitors of hematopoiesis have recently been described. In studies of 62 patients with congenital (7) and acquired (17) neutropenia, aplastic anemia (12), collagen vascular diseases (11) and other disorders, we have identified serum inhibitors of CFU-C in 5 patients: autoimmune panleukopenia, systemic lupus erythematosus, idiopathic aplastic anemia, aplastic anemia in infectious mononucleosis, and acquired neutropenia. In one, a complement-independent IgG and IgM antibody was found; in another, a complement-dependent IgG inhibitor.

Hematopoietic Stem Cells

392 DIFFERENTIATIVE POTENTIAL OF STEM CELLS, Robert A. Phillips, Division of Biological Research, Ontario Cancer Institute, Toronto, Ontario M4X 1K9.

Lymphocytes like erythrocytes have a limited life span and their continuous production depends on a stem cell-based cell renewal system. Many investigators have shown that a common stem cell produces both erythroid and lymphoid progeny. However, there is little detailed information concerning the early events in the differentiation along these pathways. This paper will describe experiments designed to investigate three aspects of lymphoid differentiation: 1) Existence of restricted stem cells. By using radiation-induced chromosome markers it has been possible to identify two classes of restricted stem cells. One is restricted to myeloid differentiation and does not produce detectable numbers of lymphoid progeny. A second type is restricted to T cell differentiation. Both types of restricted stem cells are present in bone marrow and both have extensive self renewal capacity. 2) Regulation of early events in the differentiation of stem cells. Preliminary data suggest that restricted stem cells can influence the differentiation of pluripotent stem cells. The factors which mediate this interaction between stem cells have not been elucidated but are being studied in vitro with long term cultures of stem cells as described by Dexter. 3) Clonal analysis of early stages of B cell differentiation. The experiments described above did not detect any stem cells restricted to B lymphocyte differentiation. To examine the B cell pathway for differentiation we have established an in vitro assay for the precursors of B lymphocytes. This clonal assay for the precursors for B lymphocytes should be useful in studies on regulation of B lymphocyte differentiation and in studies on the acquisition of specificity by B cells.

393 STEM CELL INVOLVEMENT IN EXPERIMENTAL LEUKEMIA, Dirk W. van Bekkum, Radiobiological Institute TNO, 151 Lange Kleiweg, Rijswijk, The Netherlands.

Our observations are based on a series of investigations with a transplantable promyelocytic leukemia in BN rats (1). This leukemia has many characteristics in common with AML in humans and serves presently as a most realistic experimental model of the human disease. In the course of the progression of the leukemia in rats, the bone marrow becomes depleted of normal pluripotential hemopoietic stem cells (HSC) (2). This has been shown to be due to a physical disappearance and not to inhibition of spleen colony forming properties (3). Simultaneously with the disappearance of HSC from the marrow, there occurs a steep increase of HSC in the peripheral blood and in the spleen, but the extramedullary hemopoiesis is insufficient to meet the hemopoietic needs of the leukemic animal. With regard to the leukemic stem cells (clonogeneic leukemic cells, CLC) these can be determined in a spleen colony assay using non irradiated BN rat recipients. The proportion of CLC among the leukemic cell population is uncertain because estimates based on leukemic colony formation differ from those based on TD_{50}. The kinetics of CLC during remission induction and prolonged induced remissions will be discussed.

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Hematopoietic Microenvironment

394 OVERVIEW OF HEMOPOIETIC INDUCTIVE MICROENVIRONMENTS, John J. Trentin, Division of Experimental Biology, Baylor College of Medicine, Houston, TX 77030.

The pluripotent bone marrow stem cell is currently assayable only by in vivo hemopoietic colony formation (CFUs). Its "decision" for differentiation into any one of several possible hemopoietic or lymphoid cell lines was variously thought to be random (stochastic) or to be determined by humoral substances such as erythropoietin. However, extensive studies of clonal hemopoietic colony formation in the spleens and in the bone marrow (*in situ* or transplanted) of lethally irradiated mice transfused with bone marrow or spleen cell suspensions from isologous, allogeneic or xenogeneic donors, of normal or genetically anemic strains (W W^v or Sl Sl^d), under a variety of conditions, have revealed that "decision" by the pluripotent stem cell for differentiation into any one of four different hemopoietic cell lines (erythroid, neutrophilic granuloid, eosinophilic granuloid or megakaryocytic) is controlled by its interaction with spleen or marrow stromal hemopoietic inductive microenvironments (HIM) of each of the four types. The function of the erythroid HIM has been shown to involve conversion of some of the progeny of the CFUs to erythropoietin responsive stem cells (ERC). Erythropoietin acts on the ERC, causing them to self-replicate, and secrete hemoglobin and differentiate along the erythroid line.

The functional units of the HIM are presumed to be different types of radioresistant stromal cells. Erythroid and granuloid spleen colonies were each found to have a characteristic stromal cell type in close apposition to stem cells in early stages of differentiation. The characteristic stromal cell of the erythroid colonies was comparable to the central reticular cell of the "erythroblastic islands" of Bessis and of Ben-Ishay and Yoffey, but different from the characteristic stromal cell of granuloid spleen colonies.

Differentiation of CFUs into various kinds of T or B lymphocytes is presumably also controlled by interaction with stromal cells, but since grossly visible and dissectable (retransplantable) colonies of lymphoid cells do not occur, comparable studies have not been possible.

395 HYBRID RESISTANCE: RECOGNITION IN VITRO BY F_1 HYBRID CYTOTOXIC LYMPHOCYTES OF PARENTAL OR SYNGENEIC Hh-1 TARGET DETERMINANTS, Gustavo Cudkowicz, Keiichiro Nakano, Ichiro Nakamura, Yee Pang Yung and Paula S. Hochman, Departments of Pathology and Microbiology, State University of New York at Buffalo, Buffalo, N.Y. 14214.

F_1 hybrid resistance to parental bone marrow and leukemia cells (Hh system) and natural killer cell activity (NK system) are two noninduced host reactivities mediated in vivo by cells lacking T-lymphocyte markers. Similarities between the two systems, setting them apart from conventional immunity, are (1) maturation after the age of 3 wk; (2) low sensitivity of effector cells to radiation coupled with radiosensitivity of their precursors; (3) requirement for integrity of bone marrow; (4) unequal organ distribution of effectors; (5) suppression of reactivity by antimacrophage agents, (6) antispecies antisera, and (7) multiple injections of parental spleen cells.

In vitro, F_1 anti-parent cell-mediated lympholysis (CML) is mediated either by natrually occurring or by "induced" cytolytic cells. Classes of effectors are endowed with undistinguishable target specificities but they may differ for being Thy-1 negative (or weakly positive) and cortisone-sensitive in one case (NK system), or Thy-1 positive and cortisone-resistant in the other (induced Hh system). Genes coding for target antigens of induced CML can be mapped by direct assays and cold inhibition of specific lysis, employing mice of appropriate strains. Both methods map the gene(s) in the H-2D region where Hh-1 was previously located by in vivo grafting. Whereas syngeneic F_1 target cells are not detectably lysed in the direct assays, such cells are specific inhibitors whenever they carry appropriate Hh-1 determinants. Autoreactivity and gene expression under heterozygosity are thus detectable in vitro in contrast to the exclusive detection of Hh-1 under homozygosity in vivo.

The induction of F_1 anti-parent CML requires the interaction of a minimum of three cell types and possibly more: prekiller Thy-1 positive responder lymphocytes, macrophages of responder or stimulator genotype, and irradiated Thy-1 positive stimulator cells. Macrophages are required early in the induction of cytolytic effectors from prekiller cells but not for the effector function itself. Selective elimination of macrophages by silica particles, carrageenans, carbonyl iron, and Sephadex G-10 reduce or abolish induction of CML. Irradiated parental T cells furnish a necessary signal, via a specific reaction to F_1 alloantigens, which may activate or derepress F_1 prekiller cells. Involvement of an additional cell type, i.e., suppressors, is evident in two situations: after in vivo administration of the bone-seeking radioisotope ^{89}Sr, and after in vivo or in vitro administration of carrageenans. Both the inductive and effector phases of CML can be suppressed by such cells which are immunogenetically nonspecific.

These developments suggest that hybrid resistance is far more complex than originally thought and that the mechanisms involved regulate under physiologic conditions the proliferation of normal and transformed cells of the lympho-myeloid system.

396 ROLE OF THE THYMUS AND THYMIC MICROENVIRONMENT IN THE DIFFERENTIATION OF T-LYMPHOCYTES. Erwin W. Gelfand, Hans-Michael Dosch, Abraham Shore and Jacob W. W. Lee
Department of Immunology, Research Institute, Hospital for Sick Children, Toronto, Canada.
Since the thymus is directly concerned with the acquisition of cell-mediated immunity, studies of the normal processes of thymic differentiation have been of great interest and importance. The thymus derives from an epitheliomesenchymal rudiment originating from the third and fourth pharyngeal pouches. Blood borne hematopoietic stem cells originating from the fetal yolk sac, fetal liver or bone marrow, migrate to the thymus where they are induced to proliferate and differentiate into thymic-dependent T-lymphocytes. This differentiation is marked by morphologic alterations and the expression of characteristic T-cell surface alloantigens, receptors and functions. From the thymus these T-lymphocytes are eventually exported to peripheral lymphoid tissues where they play a vital role in the expression of an immune response.
The nature of the inductive influences within the thymic stroma which lead to the initiation of T-cell differentiation have been actively investigated. Evidence for cell-free thymic "hormones" or differentiation factors has been based on reconstitution of several immune functions and T-cell associated markers, in vitro and in vivo, using extracts of thymic tissue, especially from bovine thymus. Although the majority of these studies have been carried out in mice, induction of E-rosette formation and reactivity with an anti-human T-cell antiserum following incubation of human cells with similar extracts, has been interpreted as evidence for the induction of T-cell differentiation in man. In an attempt to clarify some of these questions, long term cultures of human thymic epithelium were established. Using the induction of E-rosette formation (a marker of T-lymphocytes in man) and the generation of T-helper cell function in a plaque forming cell assay following incubation with the epithelial monolayer or epithelial derived factors, we have developed a model for the study of the role of thymic epithelium in the induction of T-cell differentiation in man. Our studies suggest that the initial stages of T-cell differentiation within the thymus involve the direct interaction (contact) between bone marrow T-precursor cells and thymic epithelial cells and that subsequent differentiation can be effected by epithelial-derived factors or by agents which lead to elevated levels of intracellular cyclic AMP. Application of this model to the study of infants with congenital abnormalities of T-lymphocyte function and combined immunodeficiency have revealed disorders of thymic epithelial cell maturation and function. The reconstitution of immune function in vivo following implantation of thymus epithelium in selected patients supports the role of thymic epithelium in the induction of T-cell differentiation.
Supported by the Medical Research Council of Canada.

In Vitro Differentiation of Other Hematopoietic Cells

397 MIXED HEMOPOIETIC COLONIES IN VITRO, D. Metcalf and G.R. Johnson, Walter and Eliza Hall Institute, Melbourne 3050, Australia.

In agar cultures of CBA fetal liver cells stimulated by pokeweed mitogen-stimulated C57BL spleen conditioned medium, pure and mixed erythroid colonies develop in addition to colonies containing neutrophils, and/or macrophages. No erythropoietin was detected in this conditioned medium. The frequency of erythroid colony-forming cells (E-CFC) is highest in the 10 day fetal liver and yolk sac ($600/10^5$ cells) and falls with increasing fetal age. Low numbers of E-CFC ($1\text{-}2/10^5$ cells) were found in adult CBA marrow cells.

One third to one half of colonies containing benzidine-positive erythroid cells also contained one or more of neutrophils, macrophages, eosinophils or megakaryocytes. Identification of these cells was confirmed by electronmicroscopy. Single cell transfer studies showed that mixed cells were clones.

Fetal liver E-CFC were non-adherent, radiosensitive (D_o = 110 rads), had a relatively light buoyant density of 1.056 - 1.069 g/cm^3, sedimented at 6-9 mm/h and 65-75% were in the S phase of the cell cycle - properties similar to other progenitor cells in fetal liver.

Recloning of individual mixed colonies in agar showed that 15% contained small numbers of colony-forming cells (7.5 - 26.7 per colony). Daughter colonies usually were small (< 400 cells) and most were composed only of neutrophils. A low frequency of spleen colony-forming cells was observed in pooled mixed colonies.

The active factor in pokeweed mitogen-conditioned medium that stimulates the formation of pure and mixed erythroid colonies is a glycoprotein of approximately 40,000 M.W. and the in vitro synthesis of this molecule is a radiosensitive process depending on DNA, RNA and protein synthesis and the interaction of two cell types - T lymphocytes and adherent cells.

398 IN VITRO CLONOGENIC GROWTH OF MEGAKARYOCYTES, Alexander Nakeff, Section of Cancer Biology, Mallinckrodt Institute of Radiology, Washington University School of Medicine, St. Louis, MO 63110.

Studies in our laboratory have been concerned with analyzing the growth of megakaryocyte colonies (CFU-M) from mouse marrow in the plasma culture system by defining the specific progenitor cell populations responsible for colony growth in terms of their cell size, density, DNA content, cycling characteristics and physiological response to platelet demand. In addition, we are also attempting to define those specific stimulatory factors capable of supporting the proliferation and maturation of these progenitors in vitro. Recent preliminary data has been obtained on megakaryocytes and their progenitors following their separation from mouse marrow cells by elutriation (cell size) and subsequent analysis of DNA content by flow microfluorometry (FMF) using a vital stain (Hoerscht 33342). Megakaryocyte enrichment ratios of 20-30 were obtained which permitted the physical sorting of cells on the basis of ploidy and subsequent testing for clonogenicity. The use of proliferative probes such as hydroxyurea and cytosine arabinoside has revealed that about 30-40% of CFU-M are cycling and in DNA synthesis in normal marrow. In the marrow of genetically-anemic mice (W/W^V and Sl/Sl^D) which exhibits a kinetic defect in megakaryocyte production reflected in a decreased number of CFU-M, the proportion of cycling CFU-M is increased to about 80%. The response of normal CFU-M to immune-induced thrombocytopenia has been further characterized indicating a limited potential for self-regulation. This may be under the control of a circulating "thrombopoietin" since the addition of thrombocytopenic mouse serum to normal mouse bone marrow increased the number of CFU-M. However, CSF activity is also high in this serum as indicated by increased numbers of CFU-GM. Our most recent data will be presented on the systematic testing of stimulators of megakaryocyte proliferation present in culture media conditioned by lectin-stimulated mouse spleen cells and embryonic kidney cells following their isolation by Amicon ultrafiltration and concentration by membrane dialysis. These studies are important in the optimization of culture conditions that will reproducibly permit maximal CFU-M proliferation as well as aiding in the analysis of the initial events involving the proliferative induction of CFU-M.

This investigation was supported by Grant Number 5P01CA13053-05 awarded by the National Cancer Institute, DHEW and Grant Number R01HL20825-01, awarded by the National Heart, Lung and Blood Institute, DHEW.

399 LYMPHOCYTE COLONY GROWTH IN VITRO, L.A. Rozenszajn, A. Zeevi, J. Radnay, I. Goldman and B. Sredni, Hematological Laboratories, Meir Hospital, Kfar Saba, and Department of Life Sciences, Bar-Ilan University, Ramat-Gan, Israel.

Human and murine resting lymphocytes are able to transform into blast-like cells, to divide and develop into colonies in a soft agar culture system without requiring a humoral stimulating factor provided that T and B mitogens are present in the culture system. These mitogens seem to trigger that particular subset of lymphocytes whose colony formation mechanism operates in the presence of the stimulating agent. The two-layer soft agar system was found to be a convenient and expedient tool for studying in vitro lymphocyte proliferation and differentiation, cellular interaction leading to lymphocyte activation and regulation of lymphocyte colony formation. Changes in the number of colonies per culture and in the growth rate of individual colonies reflect the dependence on culture conditions, the number of lymphocyte clone-forming cells seeded, sensitivity of the lymphocyte populations to the mitogen and the presence of human- or mouse-derived biological material in the culture system. A number of experiments attempting to clarify the contribution of various hemopoietic cells to the development of colonies were performed. The influence of extracellular soluble factors secreted by cultured lymphoid cells and monocytes-macrophages on the proliferative potential of mitogen-stimulated lymphocytes was examined. The clonal proliferative potential was subjected to a number of different controlled, experimental conditions and studied. Several properties of the substances stimulating and inhibiting colony growth were elucidated. Monocytes-macrophages secrete substances which behave as biologic regulators of lymphocyte clonal growth. The clonal proliferation of T lymphocytes was suppressed when culture fluid from macrophages was incorporated in the agar culture system. The inhibitory effect disappeared with dialysis and the dialyzed culture fluid exhibited an enhancing effect on lymphocyte colony formation. It appears that monocytes-macrophages release two biologic substances able to modulate the cloning of T lymphocytes - lymphocyte colony inhibitory factor and lymphocyte colony enhancement factor. Unless the two are separated, the mild activity of the enhancement factor is completely masked by the relatively strong action of the inhibitory factor. Enhancement of colony growth was noted when human lymphocytes were cultured in the presence of culture fluid derived from T mitogen-stimulated lymphocytes. It is evident that the sensitized cells release a lymphocyte colony enhancement factor into the culture medium. It is possible that, like the monocytes-macrophages, mitogen-stimulated lymphocytes also release substances having antipodal effects, stimulatory and inhibitory, on lymphocyte colony formation. In the soft agar culture system the effect of the enhancement factor strongly prevails. Cells in colonies that developed from stimulated B lymphocytes exhibited definitive differentiation into immunoglobulin-producing plasma cells. Cytochemical and ultrastructural studies of these cells gave leading indications that a potential secretory capacity was present.

Culture Systems and Disease

400 HEMOPOIESIS IN VITRO AND ITS PHYSIOLOGICAL RELEVANCE, Karel A. Dicke, Department of Developmental Therapeutics, University of Texas System Cancer Center, M.D. Anderson Hospital and Tumor Institute, Houston, Texas 77030.

Changes in hemopoiesis occur generally in diseases especially in those which effect the bone marrow. Such changes were detected predominantly on the basis of morphological examination of peripheral blood and bone marrow. The last decade functional test systems for hemopoietic cells became available of which the progenitor myeloid in vitro colony assay was the first system for progenitor hemopoietic cells being developed. Using this system, regulation mechanisms of the myeloid compartment were studied in mice and man. Also hematological disorders were tested in the CFU-C myeloid (colony forming unit culture) system such as aplastic anemia, and hematologic malignancies. This assay can predict leukemic transformation in preleukemia, as well as leukemia proliferation in oliogoblastic leukemia. In addition the results in the CFU-C system are prognostic for response to chemotherapy in acute leukemia. Cloning techniques of progenitor erythroid cells have been developed and have given us information of regulation of erythropoiesis in polycytemia vera. In the mouse normal erythropoiesis has been studied and in the near future elucidation of regulation mechanisms of the red cell system in man can be expected using the CFU-E and BFU-E assay. Cloning techniques of lymphoid cells (T cells and B cells) have also been developed; the value of these assays is still uncertain. In the systems mentioned, a specific stimulus is present such as CSF (colony stimulating factor). erythropoietin and spleen conditioned medium prepared by Pokeweed stimulation of spleen cells in vitro. Specific cloning assays for multipotential hemopoietic stem cells are not yet available. although in the mouse by our group and recently by Metcalf culture systems have been described in which a small proportion of the colonies may be derived from hemopoietic stem cells. It was Dexter who developed in the mouse a liquid culture system in which the hemopoietic stem cell population can be maintained for weeks and this system when developed in man offers ideal opportunities to study induction of leukemic diseases and viral detection in hematologic malignancies. Moreover, using this sytem the microenvironment of the bone marrow can be studied since stroma cells of the bone marrow are necessary to grow hemopoietic stem cells in this culture system. Other groups (Boyum et al.), developed liquid culture systems in which progenitor cells of various hemopoietic cell lines can be maintained and simultaneously enables studies of in vivo regulation of hemopoiesis. The systems mentioned above facilitate growth of normal hemopoietic cells as well as growth of abnormal hemopoietic cell lines. Most recent culture systems have been developed in which leukemia cells can be cloned specifically. Such systems are of value to detect early proliferation of leukemia cells especially in remission of acute leukemia. Moreover, these systems may open pathways to study growth regulation of leukemic cells on which new therapeutic modalities can be based.

401 LONG TERM GROWTH OF HAEMATOPOIETIC STEM CELLS IN VITRO
T. Michael Dexter, Paterson Laboratories, Manchester, England

A method has been described in which proliferation of stem cells (CFU-S) and production of granulocytic (CFU-C), megakaryocyte (M-CFU) and erythroid (BFU-E) precursor cells can be maintained in vitro for many weeks (1,2,3). The technique has been used to study viral and chemical leukaemogenesis in vitro (4,5) and provides a model system for studying genetic resistance mechanisms to marrow transplantation (6). The maintenance of stem cells in vitro is dependent upon the prior establishment of a suitable population of bone marrow-derived adherent cells, which appear to provide a necessary haemopoietic microenvironment for self-renewal and commitment (7,8). The defective environment of genetically-anaemic mice possessing the steel (Sl) mutation has been successfully reproduced in vitro and attempts to characterise the lesion(s) involved will be reported. Furthermore the effect of chronic and acute X-ray exposure and cytotoxic chemotherapy of the adherent cell population will be discussed in relation to the late aplasias and marrow failure often seen following such regimes.

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402 REGULATION OF HEMOPOIESIS IN DIFFUSION CHAMBERS, Arne Bøyum & Dagfinn Løvhaug, Norwegian Defence Research Establishment, Division for Toxicology, N-2007 Kjeller Norway.

The differentiation and proliferation of bone marrow cells in diffusion chambers (DC)implanted intraperitoneally, are affected by host-mediated factors. Examples are the enhanced formation of granulocytes and macrophages in irradiated hosts, and the reduced cell yield when the hosts are maintained in hypoxia (Bøyum et al. 1972), whereas the combination of irradiation and hypoxia promotes erythropoiesis. Recent studies have indicated that different growth regulators also enter DC in normal mice during the culture period. To better characterize the culture technique, we have implanted DC inoculated with medium, and tested the chamber content for effects on CFU-C in agar. Three different activities were found in the chamber fluid: i) CSA, ii) enhancer, which itself did not promote colony formation, but together with lung cell conditioned medium (L-CSF) it yielded more colonies than an optimum concentration of L-CSF alone, iii) a granulocyte promoting factor, which increased the fraction of granulocyte colonies. Changes in the levels of these factors have also been tested with hemopoietic cells present in the chambers. This kind of information, held together with the growth pattern of CFU-S, CFU-C and their progeny cells in DC, may provide a better understanding of the regulation of hemopoiesis.

Bøyum, A., Carsten, A.L., Lærum, O.D. and Cronkite, E.P. Blood 40, 174, 1972.

403 RECENT RESULTS FROM CELLULAR AND VIROLOGICAL STUDIES ON HUMAN MYELOGENOUS LEUKEMIA CELLS. R. C. Gallo, F. Ruscetti, R. Gallagher, W. C. Saxinger. S. Collins, and P. Jacquemin, National Institutes of Health, Bethesda, Maryland 20014.

This report will focus on reviewing recent results from our laboratory with liquid suspension culture of human myelogenous leukemia cells. We will describe: (1) experience with conditioned media from select human embryonic fibroblasts in promoting growth of these cells in short term (<3 months) culture; (2) evidence that differentiation can frequently be induced; (3) a detailed description of the new HL-60 human promyelocytic leukemia cell line, including evidence that most or all of these cells can be induced to mature in vitro, despite karyotypic abnormalities, and the effect of certain primate RNA tumor viruses on this process; (4) new results on the association of RNA tumor virus-related markers (antibody and nucleic acid sequences) in association either with myelogenous cells in suspension culture or with fresh myelogenous leukemic blood or bone marrow leukocytes; and (5) attempts to develop testable models which consider these disorders in terms of the abnormalities of differentiation seen in vivo but sometimes terminal differentiation observed in vitro, the presence of karyotypic abnormalities, and the presence or absence of certain viral markers.

Clinical Disorders of Hematopoiesis

404 MYELO- AND LYMPHOPROLIFERATIVE DISORDERS, D. Golde, UCLA SCH. OF MED., LOS ANGELES, CA 90024

In vitro culture techniques have been useful in elucidating the pathophysiological mechanisms involved in certain of the myelo- and lymphoproliferative disorders. Clonal and liquid culture methodologies have provided data confirming a maturational block as the fundamental lesion in acute myelogenous leukemia. Chronic myelogenous leukemia is largely a disorder of cell proliferation. Methods for culturing erythroid progenitors in vitro have led to information causing a restructuring of our concepts of the pathophysiology of polycythemia vera. Patients with polycythemia vera have greatly increased numbers of erythroid progenitors in the bone marrow which respond to erythropoietin in vitro in a near normal manner. The major abnormality in polycythemia vera appears to be an increased feed-in of committed erythroid progenitor cells from the pluripotent stem cell compartment. Lymphoid malignancies have been somewhat more difficult to analyze by in vitro techniques. Hairy-cell leukemia is an example of a recently defined disorder characterized by the proliferation of neoplastic mononuclear cells in the bone marrow and spleen. Our in vitro studies show that these cells behave as lymphocytes in liquid culture and studies on permanent cell lines obtained from these patients have confirmed the B-lymphoid nature of this disorder. Recently, we observed a patient with typical disease in whom the proliferating cells had properties of T lymphocytes. A permanent cell line grown from spleen cells of this patient has T-cell properties and carries the marker enzyme isozyme 5 of acid phosphatase. This cell line responds to PHA and rosettes with sheep erythrocytes. Thus, hairy-cell leukemia may be a B- or T-lymphoid neoplasm. There is good evidence that these myelo- and lymphoproliferative disorders are clonal in origin.

405 BONE-MARROW FAILURE A. Morley, Flinders University & Medical Centre, Adelaide, Australia.

Bone-marrow failure is a syndrome and may be classified on many bases - on an aetiological basis, on whether marrow cells are primarily or secondarily disturbed, on whether precursor compartments or maturation compartments are primarily involved and on the clinical course. The prototypal form of marrow failure is aplastic anaemia. Experimental studies and the effect of immunosuppressive therapy suggest that some cases of aplastic anaemia may be immunologically mediated. Our own studies suggest that another group of cases may be due to permanent damage by alkylating agents to marrow stem cells and their descendants and that the damage results in proliferative failure.

Erythropoiesis

406 BLOOD ISLAND FORMATION OF AVIAN EMBRYO YOLK SAC CELLS IN VITRO. Yasusada Miura, Takashi Terasawa and Reiko Masuda, Division of Hemopoiesis, Institute of Hematology, Jichi Medical School, Tochigi-ken 329-04, Japan.

Mechanism of blood island formation and hemoglobin(Hb) synthesis in the avian embryo yolk sac cells has been investigated. Yolk sac cell suspension from embryos at definitive primitive streak stage were reaggregated using a gyratory shaker in liquid medium for 24 hrs. The single reaggregate thus formed was cultured on a whole egg agar medium for 48 hrs. By then, groups of erythroblasts or blood islands were observed. Hb synthesis was estimated by incorporation of ^{59}Fe into the heme. This system provides a quantitative method for measuring the formation of blood islands or Hb synthesis still keeping cell-to-cell interactions. More than 10^6 cells were required to form a single aggregate containing blood islands. Addition of 0.5-0.75% dimethyl sulfoxide(DMSO), an inducer of Hb in cultured Friend leukemia cells(FLC), stimulated Hb synthesis in the yolk sac cells upto two to three fold. Other dipolar compounds like NN dimethylacetamide, triethylen glycol etc also stimulated Hb synthesis. DMSO was effective during the early period of erythroid differentiation. By DMSO there was no change in pattern of Hb, DNA synthesis during the early period of incubation, nor numbers of blood islands. Whether DMSO works through the modification of cell membrance permeability or conformational changes in DNA as suggested in the previous papers on FLC is still obscure. It is interesting that the stimulatory effect of Hb synthesis was also observed in butyric acid and bleomycin.

407 DENSITY-GRADIENT SEPARATION OF RBC's OF DIFFERENT HEMOGLOBIN TYPES, Allan R. Dorn and Robert H. Broyles, Univ. of Okla. Health Sciences Center, Okla. City, OK 73190

In bullfrogs (Rana catesbeiana), there are a total of eight different major hemoglobins (Hb's): 4 larval Hb's and 4 adult Hb's. The adult Hb's have no globin chains in common with the larval Hb's and completely replace the larval Hb's during metamorphosis. Density gradient cell separation of artificial mixtures of larval and adult erythrocytes by self-forming density gradients of Ludox HS colloidal silica (Dupont de Nemours) and PVP-40 (polyvinylpyrrolidone, m.w. 40,000) produced fractions containing only larval Hb and fractions with only adult Hb- as detected by polyacrylamide gel electrophoresis. Results show larval and adult erythrocytes to have mean densities of 1.085 g/cm^3 and 1.105 g/cm^3 respectively. The bulk of the generated gradient covered a density range of 1.09 g/cm^3 to 1.10 g/cm^3. Larval Hb containing erythrocytes banded near the top of the centrifuge tube just below a well separated band of WBC, while the adult Hb containing erythrocytes banded near the bottom of the tube with a large area in between which contained very few cells. This approach will be used to determine whether the Hb transition during metamorphosis occurs within a population of erythrocytes synthesizing both larval and adult Hb, or with a population synthesizing only adult Hb as other results suggest. Results also indicate that larvae with different Hb patterns, as detected by gel electrophoresis, exhibit different erythrocyte densities upon density gradient separation. Parameters which have been found to affect density gradient formation include the concentrations of Ludox HS, PVP-40, and NaCl as well as pH, centrifugation time, and centrifugal force, and the order of addition of the various components. (Supported in part by NIH grant #5 RO1 AM21386 to RHB).

408 ERYTHROPOIETIC MICROENVIRONMENT—SPECIFIC HEMOGLOBIN SYNTHESIS IN LARVAL BULLFROGS, Robert H. Broyles and Allan R. Dorn, Univ. of Okla. Health Sciences Center, Okla. City, OK 73190

Bullfrog tadpoles have 4 larval hemoglobins (Hb's) separable by disc gel electrophoresis. By amino acid sequencing, Riggs et al. have shown that these Hb's share no globin chains, with the possible exception that two may have a common α chain. (The larval Hb's have no globin chains in common with 4 adult Hb's which appear and replace the larval Hb's during metamorphosis). Previous results with organ cultures and drug-induced anemia by Broyles et al. (Nature New Biol. 241, 207, 1973; Science 190, 471, 1975) have shown that tadpole liver and kidney tissues are both erythropoietic but support the snythesis of different larval Hb's, indicating that different RBC lines containing different larval Hb's differentiate in the two organs. Recent results show that RBC's of at least two distinct morphologies are found in circulating blood of tadpoles, that enriched populations of two different RBC types can be obtained from the two organs, and that the predominant cell type in each organ correlates with organ-specific Hb synthesis. Histological sections reveal characteristic but different types of erythropoietic centers in the two organs. The approaches of organ culture, hemopoietic cell separations in shallow density gradients of colloidal silica (method will be presented), and cell culture are being used to investigate alternative hypotheses: (1) that these two RBC lines share a common precursor cell and that the two different microenvironments determine which Hb genes are expressed, or (2) that the two RBC lines differentiate from different precursor cells that become determined in these sites at an earlier developmental stage. (Supported in part by NIH grant #5 RO1 AM21386 to RHB).

409 CONTROL OF PROTEIN SYNTHESIS BY HEMIN. Martin Gross, University of Chicago, Dept. of Pathology, Chicago, Ill. 60637

Studies in rabbit reticulocytes have indicated that the maintenance of a close balance between the synthesis of the heme and globin components of hemoglobin is regulated by a high molecular weight protein inhibitor of polypeptide chain initiation, termed the hemin-controlled translational repressor (HCR). HCR is formed in the absence of hemin from a precursor, the prorepressor, and then acts by phosphorylating the initiation factor responsible for promoting binding of methionyl-tRNA$_f$ to 40 s subunits. We have purified the prorepressor approximately 600-fold. When it is converted to HCR by prolonged warming in the absence of hemin or by incubation with N-ethylmaleimide for 5 minutes and then incubated briefly with ATP-γ-[^{32}P] and Mg+2, a protein that migrates as a 100,000 mol. wt. (100 K) component on SDS-polyacrylamide gels becomes phosphorylated. This component is not labeled when phosphorylation is attempted with the prorepressor or prorespressor warmed in the presence of hemin, indicating that the protein kinase responsible is probably HCR itself. Since the 100K protein appears to copurify with the prorepressor and with HCR, the data suggest that HCR, once activated, may autophosphorylate itself. The fact that this phosphorylation is very rapid (50% complete within 30 seconds at 34°) and insensitive to dilution also suggests an autophosphorylation. We estimate that about 6 moles of phosphate become bound per mole of 100K protein. Neither the rate of phosphorylation of the 100K component nor the slow conversion of prorepressor to HCR by warming in the absence of hemin is enhanced by cAMP or reduced by incubation with cyclic nucleotide diesterase.

410 ALTERATIONS IN tRNA DURING ERYTHROID DIFFERENTIATION OF THE FRIEND CELL, Lawrence Kleiman and Jo Ann Woodward-Jack, Lady Davis Inst for Med Res, Montreal, Quebec

The Friend cell, a transformed murine cell infected with Friend erythroleukemia virus complex, undergoes erythroid differentiation when exposed to various chemical inducers, and loses the capacity to proliferate. During erythroid differentiation, changes in the tRNA population can be detected using reverse phase chromatography. Lysine tRNA can be resolved in 5 isoacceptor species in mammalian cells, and one of these, lysine tRNA$_4$, has been shown to vary in amount according to the proliferative capacity of the cell. It is absent in non-dividing tissues such as brain or muscle, but represents a major portion of lysine tRNA in rapidly-dividing cells such as are found in tumor or embryonic tissue (Ortwerth, B.J., G.R. Yonuschot and J.V. Carlson, Biochemistry 12 (1973) 3985). Lysine tRNA$_4$ comprises 30% of the total lysine tRNA in rapidly dividing, uninduced Friend cells, but less than 10% of the total lysine tRNA in uninduced cells which have reached a density-dependent stationary growth phase. Friend cells undergoing erythroid differentiation divide more slowly than uninduced cells, and finally cease proliferation, but lysine tRNA$_4$ becomes the major lysine tRNA species (greater than 50%). This does not appear to reflect erythroid properties of the cell, since the lysine tRNA of the mouse reticulocyte contains very little lysine tRNA$_4$. The non-dividing erythroid Friend cell, therefore, represents an exception to the finding that non-dividing cells usually have little or no lysine tRNA$_4$ present.

411 GENETIC STUDIES ON THE CONTROL OF ERYTHROID DIFFERENTIATION, Marcy E. MacDonald, Michelle Letarte and Alan Bernstein, the Ontario Cancer Institute, Toronto, Canada.

Friend erythroleukemic cells (Friend cells) provide a useful system for a genetic study of erythroid differentiation in vitro. Non-inducible variant clones can be isolated and characterized for their ability to undergo a number of developmentally regulated events characteristic of erythroid differentiation. Using a radioactive binding assay, a surface marker of erythroid differentiation, termed erythrocyte membrane antigen (EMA), has been identified on both mature mouse erythrocytes and dimethylsulfoxide (DMSO)-induced Friend cells but not undifferentiated Friend cells. To examine the control of expression of these two events in Friend cell differentiation-hemoglobin synthesis and the appearance of EMA-variant Friend cell clones altered in their response to DMSO were studied. The first variant, M18, appears to be blocked in heme synthesis as it can be induced to produce hemoglobin only in the presence of both DMSO and heme. In contrast, the defect in another variant, TG-13, was not alleviated by the addition of heme and DMSO to the growth medium. These two variant clones also differ in their ability to express EMA after growth in DMSO alone: M18 is inducible for membrane antigen while TG-13 is not. These results suggest that the appearance of membrane antigen may be coordinately controlled with globin mRNA production and that heme synthesis is not required for the expression of EMA. The expression of EMA, as well as hemoglobin synthesis should therefore be useful markers for the characterization of other variant Friend cell clones and in the elucidation of the genetic and biochemical mechanisms controlling erythroid differentiation. (supported by grants from the National Cancer Institute and the Medical Research Council of Canada)

412 REGULATION OF ERYTHROID COLONY GROWTH, Pamela N. Porter, University of Nebraska, Lincoln, NE 68588

Culture conditions for erythroid colony forming cells (CFU_E) were manipulated in order to determine if standard conditions were optimal for erythroid differentiation or if additional factors could enhance the growth of mouse bone marrow cells. The addition of 0.05-0.2 mM hemin to methyl cellulose cultures of marrow cells significantly increased the number of erythroid colonies observed but did not significantly change colony size. In cultures containing 100 mUnits/ml erythropoietin (EPO), the addition of hemin (0.1 mM) resulted in the growth of twice as many colonies as obtained with EPO alone. At nonsaturating levels of EPO, EPO and hemin had a synergistic effect on colony formation. Serum-bound iron also increased colony number two-fold in the presence of 100 mUnits/ml EPO. The hemin precursor, δ-aminolevulinic acid was ineffective. No difference in the sensitivity of CFU_E to hydroxyurea (HU) was observed between bone marrow cells cultured with EPO and those cultured with both EPO and hemin. In both cultures there was a 75% reduction in the number of erythroid colonies obtained from a donor which had been injected with 900 mg/kg HU 2 hrs prior to the establishment of cultures. The similarity in response to HU suggests that the additional colonies observed when hemin is included in cultures are not the result of recruitment of additional cells into the cell cycle from G_0. These results show that hemin and iron promote the differentiation of erythroid cells in culture and indicate that additional cells are included in the CFU_E population when either iron or hemin are included in the medium. The mechanism by which this increase occurs is under investigation. (Supported by NIH Grant No. 86-131-272-03 and Am. Cancer Soc. Grant No. 85-131-402-01).

413 PURIFICATION AND CHARACTERIZATION OF HUMAN RED CELL MEMBRANES, Roberto Ravazzolo Cecilia Garrè, Franco Ajmar, Dept. of Hematology, University of Genova, Italy.

Human red cells contain membrane bound NAD(P) glycohydrolase. The function of the enzyme in the red cell is not known but a polymorphism in different populations was described and an inherited deficiency has been reported. NAD(P) glycohydrolase is also a convenient membrane marker, beeing located on the external surface of the membrane.

We have previously described a method of partial purification, biochemical characterization and electrophoretic demonstration of the enzyme prepared from Triton X 100 solubilized hemoglobin free membranes.

We have now improved the purification procedure with the use of hydrophobic chromatography on the solubilized preparation.

Comparison among preparations from different specie is also presented.

414 CELL COOPERATIVITY IN HEMOGLOBIN SYNTHESIS BY MOUSE MARROW CELL BURSTS, J.F. Eliason and E. Goldwasser, The Franklin McLean Memorial Research Institute and Department of Biochemistry, University of Chicago, Chicago, IL 60637

We have recently developed a method to measure hemoglobin synthesis associated with the growth of mouse erythroid bursts *in vitro*[1]. These measurements indicate that increasing the cell concentration has both positive and negative influences on hemoglobin synthesis. The positive effect is evident in the slope of the log hemoglobin synthesis vs. log cell concentration curve, which is always greater than the slope of the curve for log burst number vs. log cell concentration. This difference is not due to culture conditions, since in one experiment, where the measurements were made in the same cultures, the slope for hemoglobin synthesis was 1.51, while the slope for burst number was 1.13. Dispersing the cells from bursts into fresh medium greatly reduced the rate of hemoglobin synthesis as compared to cells which remained in the colony configuration, indicating that the structure of the burst is necessary for maximum hemoglobin synthesis *in vitro*. These positive effects of cell number may well represent cooperativity between cells. The negative effect involves the time and the rate of decline in hemoglobin synthesis after maximum burst formation. Both are cell concentration dependent, being much earlier and more rapid at high cell concentrations. Experiments in which cells were exposed to fresh medium showed that hemoglobin synthesis continued through 14 days, with a slowing in the rate of decline. The negative effects of cell concentration, since they can be overcome by the addition of fresh medium, are probably not the result of direct cellular influence. [1]Exp. Hemat. 5(Suppl.), 79(1977)

415 SPONTANEOUS AND LIGAND-INDUCED REDISTRIBUTIONS OF MEMBRANE PROTEINS OF ENUCLEATING MOUSE ERYTHROBLASTS, Joyce B. Geiduschek and S. J. Singer, University of California at San Diego, California 92093

The distributions and redistributions of three types of membrane proteins in the developing erythroblast, taken from mouse bone marrow, has been observed by immunofluorescence microscopy: 1) receptors for concanavalin A (Con A); 2) the H-2 histocompatibility antigen; and 3) spectrin. At stages preceding expulsion of the nucleus, Con A receptors are uniformly distributed over the surface of the erythroblast plasma membrane, as is spectrin on the inner surface. However, during the process of enucleation, the majority, but not all, of the Con A receptors are asymmetrically segregated and concentrated in the membrane that surrounds the emerging nucleus (E. Skutelsky and M. G. Farquhar, J. Cell Biol. 71:218-231 1976); the H-2 antigen remains uniformly distributed over the entire plasma membrane; whereas spectrin is completely segregated to the reticulocyte-forming portion of the membrane. If unfixed enucleating cells are incubated at 37° with Con A followed by anti-Con A antibodies, the Con A receptors are now withdrawn from the nuclear region and collected into the reticulocyte portion of the cell membrane. A similar collection of the H-2 antigen into the reticulocyte portion occurs upon incubation of the unfixed cells with anti-H-2 alloantibody followed by rabbit anti-mouse IgG. These experiments not only confirm the greater mobility of components in the membrane of the erythroblast than the mature erythrocyte, but suggest the existence of several categories of trans-membrane interactions across the membrane of the enucleating erythroblast. These will be discussed.

416 ERYTHROPOIESIS IN CONGENITAL ERYTHROID HYPOPLASIA (DIAMOND-BLACKFAN SYNDROME): EVIDENCE FOR T-CELL MEDIATED ERYTHROID SUPPRESSION AND A SERUM BLOCKING FACTOR ASSOCIATED WITH COMPLETE REMISSION. M.B. Coleman and M.H. Steinberg. VA Hospital and Department of Medicine, University of Mississippi, Jackson, Mississippi, 39216.

The technique of cloning peripheral blood erythroid precursor cells (BFU-E) in culture was employed to study the effects of cellular immune mechanisms on erythropoiesis in two patients with Diamond-Blackfan Syndrome (DBS)who were in prolonged, complete remission.

Normal erythroid colonies were not present when DBS mononuclear cells were cultured in fetal calf serum (FCS). Controls showed 15.3±5.2 BFU-E. When cultured in autologous serum, DBS cells proliferated normally, as did controls (21.4±6.9, 7.3±2.3, 19.3±7.5 BFU-E respectively). DBS serum supported BFU-E growth by either patients cells while AB serum did not. In mixing studies, DBS mononuclear cells inhibited BFU-E generation by normal controls. Normal control mixing studies were not inhibitory. The removal of T-cells from DBS mononuclear cells prior to either mixing studies or culture alone in FCS promoted BFU-E growth. An immunoglobulin preparation from DBS serum appeared to permit BFU-E generation when DBS cells were cultured in FCS.

T-cells, which can suppress autologous and heterologous BFU-E generation were present during remission in both our patients. A factor present in DBS serum,but not AB serum or FCS, allowed normal BFU-E to form. This factor may be antibody or an immune complex; may block the suppressive effects of DBS T-cells on erythroid progenitor cells and be responsible for the complete remission in our patients.

417 EXTRARENAL ERYTHROPOIETIN (Ep) PRODUCTION: ROLE OF ERYTHROGENIN, S.A. Rothmann Hamburger, M.J. Derelanko, R.C. Meagher, A.S. Gordon, and B.C. Del Villano, New York University, New York, N.Y. 10003 and Cleveland Clinic, Cleveland, Ohio 44106.

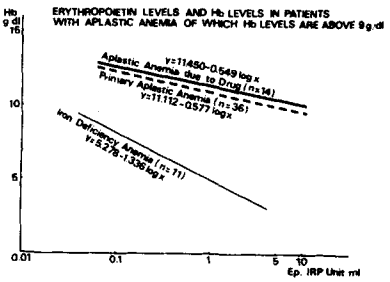
The kidney is well established as the primary site of origin or activation of Ep; however, the precise mechanism of Ep formation has not been unequivocally established. We have presented evidence to show that in response to hypoxia, the kidney elaborates a renal erythropoietic factor termed erythrogenin, which interacts with a serum substrate to produce Ep. Recently the liver and spleen have been implicated as the major sites of extrarenal Ep production. Because Ep often cannot be detected in the serum of anephric rats despite exposure to intense hypoxia, experiments were designed to determine whether erythrogenin is produced by extrarenal sites at times after nephrectomy when serum Ep is low or undetectable. Young male rats were nephrectomized 6, 12, 18, or 24 hours before exposure to 6 hours of intense hypoxia (0.35 atm). Serum Ep and liver and spleen erythrogenin were assayed in exhypoxic polycythemic mice. Erythrogenin from livers and spleens of nephrectomized rats was initially high (~0.1 U of Ep generated), decreased (~0.05 U) at 12 hours post-operatively, and rose to a high level (≥ 0.14 U) at 24 hours after surgery. A low level of erythrogenin (~0.04 U Ep generated) was observed in ureter-ligated control animals at all time intervals. Despite increased tissue erythrogenin in nephrectomized rats, only small amounts of serum Ep were detected (≤ 0.06 U). The results suggest that in the anephric animal, release of erythrogenin from extrarenal sites may be prevented, the activity of erythrogenin-mediated Ep production may be inhibited by undetermined mechanisms, or an additional unidentified factor required for normal Ep production is not present.

418 ERYTHROPOIETIC PRECURSORS IN FLASK CULTURES OF HUMAN BONE MARROW. R.K. Humphries, A.C. Eaves and C.J. Gregory. Dept. of Biophysics. B.C. Cancer Foundation. Vancouver, British Columbia, Canada.

The present studies were designed to explore the behaviour of human marrow cells cultured in tissue culture flasks and maintained under conditions which have been shown to support the first stages of murine erythropoietic cell differentiation. Cultures were initiated with $3x10^7$ cells from 9 different human bone marrow buffy coat specimens placed in 15ml of 20% FCS, 1% BSA, 10^{-4}M-mercaptoethanol and alpha medium. At weekly intervals 7.5ml and 1/2 the nonadherent cells were removed and 7.5ml fresh medium was added back to each flask. The cells removed were assayed for erythropoietic progenitor cells at three stages of development: CFU-E, Day12 BFU-E and Day18 BFU-E. At 2 weeks the recovery of Day18 BFU-E, the most primitive class, was still significant in 7/9 experiments (22-150% of input, mean 75%). By 4 weeks Day18 BFU-E were still detected in 1 experiment (14%). The other erythroid progenitors assayed were also detected for at least 2 weeks but the recoveries relative to input numbers were lower. In 2 experiments the number of Day18 BFU-E at 2 weeks was greater than the input number and in 3 other experiments the number of Day18 BFU-E showed an increase post culture during the 2nd or 3rd week. Thus simple persistence is unlikely to account for all the primitive BFU-E recovered. These results parallel those previously obtained in the mouse and suggest the usefulness of this approach to the analysis of early hemopoietic differentiation events in man.

419 REGULATION OF ERYTHROPOIETIN PRODUCTION IN APLASTIC ANEMIA. Keisuke Toyama, Hiroshi Suzuki, Junzo Kaneko and Masayoshi Negishi. Department of Medicine, School of Medicine, Keio University, Tokyo, Japan

Erythropoietin(Ep) levels were measured by ex-hypoxic polycythemic mouse method in 52 cases with aplastic anemia and 11 with iron deficiency anemia. Ep levels were expressed as logarithmic curve fit in relation to Hb levels. Ep levels were higher at 72 occasion in primary aplastic anemia(y=9.158-1.325 logx) and at 28 in secondary(y=9.207-1.347 logx) than at 11 in iron deficiency anemia(y=5.278-1.336 logx). The elevation of Ep in aplastic anemia was suppressed by blood transfusion. Ep was undetectable at the level of Hb above 9 g/dl in iron deficiency. The figure illustrates Ep levels in the patients with aplastic anemia of which Hb was sustained at the level of more than 9 g/dl without blood transfusions. Ep in aplastic anemia was markedly increased at the level of higher Hb than undetectable Ep. These results indicate that Ep is not merely regulated by hypoxia of the kidney tissue, but by hematopoietic stem cells per se which might be hyposensitive to Ep.



420 DOUBLE-LABEL IMMUNOFLUORESCENCE STUDIES OF GLOBINS EXPRESSED IN SINGLE ERYTHROID CELLS, Barbara S. Chapman and Allan J. Tobin, Dept. of Biol., University of California, Los Angeles, CA. 90024

At least seven globin genes are expressed during chick embryonic erythropoiesis. Four of these globins (2 α-like and 2 β-like) are no longer detectable by electrophoresis in the circulation after the twelfth day of embryo development. The adult β is not detected before 5-6 days, while the 2 adult α-like globins are made continuously. In order to study globins present in single differentiating cells, we prepared rabbit antibody against chromatographically purified hemoglobins. Antibodies were selected and absorbed on affinity columns of pure hemoglobins coupled to cyanogen bromide activated Sepharose. Specificity for unique early embryonic or adult globins was established by radioimmunoassay. Fluorescein- or tetramethyl rhodamine-labeled specific antibodies were used to stain erythroid cells from 4-8 day embryos (during the switch) and from hatched chick bone marrow. Fluorescence staining was correlated with erythropoietic stage by May-Grunwald Giemsa stain. In preliminary experiments the 'early embryonic' globins appear in early stages of erythropoiesis, both in embryonic circulation and in hatched chick bone marrow. These early results suggest that sequential expression of 'early embryonic' and unique adult globin genes is a feature both of embryonic and adult erythropoiesis.

421 ERYTHROID PROGENITORS IN PERIPHERAL BLOOD OF NORMAL AND POLYCYTHEMIC SUBJECTS. J.F. Prchal, Divisions of Histology and Medicine, University of Toronto and Toronto General Hospital, Toronto, Canada.

Peripheral blood of normal humans contain erythroid progenitors. Both BFU-E (the cells giving rise to colonies in 14 day cultures) and CFU-E (the cells giving rise to colonies in 7 day cultures) are present, as they are in the marrow. However there are considerable differences between blood and marrow erythroid progenitors: 1) in the peripheral blood there are more BFU-E than CFU-E; 2) the blood CFU-E and BFU-E cannot be separated by unit gravity sedimentation (STAPUT). The sedimentation rate for both is 4.5 mm/hr; 3) thymidine suicide data indicate that neither of the peripheral blood erythroid progenitors cycle.

In subjects with polycythemia vera and with secondary erythrocytosis, the peripheral blood CFU-E and BFU-E can be separated on STAPUT gradient. A proportion of both types of these progenitors are in cell cycle. In addition, in subjects with polycythemia vera, some of the progenitors form colonies in the absence of erythropoietin. However these epo "independent" progenitors do not differ from the epo responsive ones by either cell size or by cell cycle.

422 CHANGE IN ERYTHROPOIETIN SENSITIVITY OF CFU_e DURING THE ONTOGENY OF THE MOUSE, Bernhard Kubanek, Wolfgang Heit and Ivan N. Rich, Dept.Haem., University Ulm (SFB 112/A 2), 7900 Ulm/Germany

The erythroid colony forming technique was used to study the sensitivity of early and late precursors during the ontogeny of the mouse. Throughout hepatic erythropoiesis, fetal liver cells were not only responsive to the same erythropoietin concentrations, but that this concentration was significantly lower than that obtained for either adult bone marrow or spleen. A transitional CFU_e erythropoietin sensitivity was observed for early neonatal bone marrow and spleen. Realizing that the transition in erythropoietin responsiveness could be due to non-steady state conditions in the fetal and neonatal animal, or even perhaps to interactions in the populations under test in the in situ culture system, perturbation experiments were performed in order to see if the erythropoietin response could be changed. The erythropoietin response of heavily bled mice did not show a displacement in the dose response curve. Experiments in which 14 day fetal liver cells were irradiated with 850 r and mixed with normal adult bone marrow cells, or visa versa, also failed to shift the erythropoietin dose response for CFU_e either to the left or the right. Finally, CFU_e of bone marrow and spleen from pregnant mothers, a naturally perturbated state, from the 11th. to the 19th. day of pregnancy, did not show any change in the responsiveness to erythropoietin. The results suggest that the change in erythropoietin sensitivity of CFU_e can be correlated with the change in the erythropoietic organ during development.

423 *HEMATOPOIETIC EFFECT OF 5β STEROID METABOLITES*, *Frank H. Gardner, The University of Texas Medical Branch, Galveston, TX 77550*

Our laboratory has had a long-term interest in the effects of androgenic steroids in the treatment of refractory anemia. In the past 6 years animal and clinical data has indicated that erythroid responses may be obtained with a variety of 5β steroid metabolites. Etiocholanolone has been used for clinical studies to show that all committed stem cell compartments respond more rapidly than the usual testosterone esters. In the rodent and primate models betapregnanolone are more effective. Current interest are oriented towards assessing the response of human bone marrow in vitro CFUe to a variety of such 5β steroid metabolites.

424 INDUCTION OF ERYTHROPOIETIN RESPONSIVENESS IN PRIMITIVE ERYTHROID PROGENITOR CELLS IN VITRO, G. Wagemaker, S. J. L. Bol and M. F. Peters, Radiobiological Institute TNO, Lange Kleiweg 151, Rijswijk, The Netherlands.

Murine bone marrow contains a small population of primitive hemopoietic cells which in vitro form colonies in the presence of erythropoietin (EP) containing up to 10^4 reticulocytes. The cells giving origin to these colonies have been termed erythroid burst-forming units (BFU-e). Evidence exists that BFU-e are not controlled by EP in vivo. It was consequently demonstrated in vitro that expression of the erythroid differentiation potential of BFU-e requires the presence of a bone marrow associated activity, which has been termed burst feeder activity (BFA). Both number of erythroid bursts detected and sensitivity of BFU-e to EP appeared to be a function of the BFA-concentration of the cultures. BFA was shown to be associated with a distinct population of viable bone marrow cells, being characterised by a buoyant density of 1.083 $g.cm^{-3}$ and a heterogeneous sedimentation rate distribution with peaks of activity at 4.7 and 6.1 $mm.h^{-1}$. Density centrifugation resulted in almost complete separation of BFA-associated cells and BFU-e, thus allowing independent studies. Kinetic data indicate that erythropoietin responsiveness becomes apparent in BFA induced progeny of BFU-e.

Studies on the nature, specificity and physiological role of BFA-associated cells are in progress. Provisional evidence indicates that BFA and BFA-associated cells are not restricted to the bone marrow.

425 A URINARY FACTOR WHICH STIMULATES ERYTHROID BURST FORMATION MORE EFFECTIVELY THAN ERYTHROPOIETIN, Peter P. Dukes, Andrew Ma and Gregory DiRocco, Depts. of Biochem. and Peds., USC Sch. Med. and Childrens Hospital of Los Angeles, Los Angeles, CA 90054

We have previously briefly reported (Fed. Proc. 36:926, 1977) that by chromatography of proteins from the urine of anemic patients a factor (RP) was separated from the bulk of erythropoietin (EPO). RP by itself was stimulatory in the exhypoxic polycythemic mouse (in vivo) assay, but its maximal response fell short of that of standard EPO in this assay. Administration to the mice of mixtures of small or large amounts of RP and EPO caused less red cell formation than expected from a summation of their respective effects. Similar studies on the effects of RP and EPO on erythroid colony formation have now been performed. The methyl cellulose technique of clonal culture of mouse marrow erythroid cells originating from BFU-E and CFU-E was employed. It was found that the potency of RP (U/mg) relative to standard EPO was:

	in vivo	CFU-E	BFU-E
Mean ±SE (n)	1.24 ±0.13 (13)	2.71 ±0.14(14)	42.9 ±5.6(13)

Whereas in the CFU-E system equal maximal colony numbers were obtained with EPO and RP, the maximal response to RP in the BFU-E system was below that of EPO. Mixtures of RP and EPO added to the clonal culture systems had less than additive effects. RP seems to be a factor produced in response to anemia, perhaps a modified form of EPO, which can compete with EPO for its cellular target sites and is more effective than EPO in causing erythroid burst colony formation.

426 INHIBITION BY HYDROCORTISONE OF HEME, HEMOGLOBIN AND VIRUS SYNTHESIS IN DIMETHYL SULFOXIDE-STIMULATED FRIEND LEUKEMIA (FL) CELLS. William Scher, Deane Tsuei, Shigeru Sassa*, Peter M. Price and Charlotte Friend, Mt. Sinai Sch. Med., CUNY, New York, NY 10029, and *Rockefeller Univ., New York, NY 10021

When FL cells are grown in medium containing dimethyl sulfoxide (DMSO), erythrodifferentiation and oncornavirus production are enhanced. The effects of hydrocortisone (HC) on hemoglobin and virus synthesis in this system were studied. Dose-response studies demonstrated that HC, at concentrations between 10^{-8} and 10^{-6}M, inhibited DMSO stimulation of heme and hemoglobin synthesis as determined by spectrofluorometry and by counting the percent of benzidine-positive cells, respectively. No cytotoxicity due to HC was observed. After 5 days of treatment with a combination of DMSO and HC, the syntheses of heme and hemoglobin were inhibited by over 90% as compared to cultures treated only with DMSO. There was no significant decrease in DMSO-stimulated globin mRNA levels due to treatment with HC, as determined by hybridization to DNA complementary to mouse reticulocyte globin mRNA. Virus production, as measured by reverse transcriptase activity in the medium, was inhibited by HC by over 40%. It was similarly dependent upon the dose of HC. Whether the inhibition by HC is related to the production of defective mRNA, to a defect in translation of mRNA or to a defect in heme metabolism is under study.

This work was supported in part by NCI grants CA 10,000 and CA 13,047, ACS grant BC 180A and NIH grant T32 GM07036.

Granulopoiesis

427 INHIBITION OF MURINE BONE MARROW COLONY GROWTH IN VITRO BY VARIOUS NUCLEOTIDES. Riccardo Ghio, G. Bianchi Scarrà, Franco Ajmar, Mario Sessarego, University of Genova, Dept. of Hematology, Genova, Italy

Extracellular NAD has been reported as cytostatic agent in HeLa cells proliferation (1). A similar inhibitory effect on granulocytic colony growth was also demonstrated using cAMP and various other adenine nucleotides (2).

In the present report we have tested in vitro, on granulocytic and erythroid colonies from mouse bone marrow, the effect of the following compounds: NAD, NADP, ADPR, Nicotinamide, Ribose 5 P, Adenine, Adenosine, cAMP, cGMP. In one set of experiments the compounds were added directly in the semi-solid culture system and the number of colonies were scored. In a different set of experiments the compounds were first preincubated with the bone marrow cells in liquid culture for various times, then removed and the cells plated for colony growth.

The results indicate that the inhibitions effect could be traced to the adenine molecule. The effect is concentration dependent and the progenitor cells show different sensitivity to the inhibition.

1. S. Nolde, H. Hilz - Hoppe - Seyler's Z. Physiol. Chem. 353, 503-513, 1972
2. A. Morley et al. - P.S.E.B.M. 138, 57-59, 1971

428 DYNAMICS OF HAEMATOPOIESIS IN CYCLIC NEUTROPENIA: A MODELING STUDY, Michael C. Mackey, Department of Physiology, McGill University, Montreal, Canada.

In a previous study (Mackey, submitted to Blood) I explored the quantitative implications of the hypothesis that the primary defect in cyclic neutropenia (CN) is cell death during proliferation within the pluripotential stem cell (PPSC) population. It was demonstrated that in response to a loss of PPSC's during proliferation, the cellular flux from the PPSC into differentiated cell lines will either be: 1) depressed and constant; or 2) oscillatory about a greatly reduced level. Using data for humans and dogs, it was estimated that when the oscillatory behaviour occurs it will have a period of c.a. 20 and 12 days respectively.

In the present study, I have combined the model for PPSC dynamics with known and hypothetical mechanisms for the control of erythro-, granulo-, and thrombopoiesis. Estimates of parameters for these three cell lines in the normal range yield total system behaviour in the face of ineffective PPSC proliferation qualitatively identical with that observed in humans with CN and in the grey collie. It was not possible to reproduce the dynamics of CN with a normal PPSC (no loss from proliferation) and a defective feedback control of granulopoiesis.

Thus, the most parsimonious explanation for the dynamics of haematopoiesis in CN is that they are due to the effects of a proliferation defect within the PPSC, and that the control of erythropoiesis, granulopoiesis, and thrombopoiesis is normal.

This research is supported by the National Research Council of Canada.

429 DIFFERING CHARACTERISTICS OF HUMAN COLONY FORMING CELLS (CFU-C), Susumu Inoue, Mark J. Ottenbreit and Joseph Kaplan, Wayne State Univ. Schl. Med. Detroit, Mich. 48201

We examined various characteristics of human CFU-C using the marrow cells aspirated from children with acute leukemia in remission or with other miscellaneous disorders. Cultures were done using two types of conditioned media, culture supernatant of, 1) human fibroblasts (FCM) and 2) of PHA stimulated peripheral blood mononuclear cells (LCM). CFU-C enrichment by velocity sedimentation disclosed 2 peaks, the smaller cells with a modal sedimentation velocity (s.v.) of 5.6-6.6 mm/Hr.(4 specimens), and the larger cells with a modal s.v. of 8.0-10.5 mm/Hr. The former produced a significantly larger number of colonies with LCM stimulation (3-5 fold) (LCM responsive CFU-C) and formed either pure macrophage or mixed macrophage-neutrophil colonies. The latter formed more colonies (> 80% pure neutrophil) with FCM stimulation (3-12 fold) (FCM responsive CFU-C). Unseparated marrow cells exposed to a selected freeze-thaw procedure and cultured with LCM, formed the same number or more colonies than their unfrozen controls, while a marked reduction in the colony number occurred with FCM stimulation, indicating the cryosurvivability difference between FCM and LCM responsive CFU-C. To examine whether both types of CFU-C express lymphocyte differentiation markers, we preincubated marrow cells in the presence of complement with either normal rabbit serum (NRS), rabbit anti-human B lymphocyte antigen (HBLA) antiserum, or rabbit anti-HTLA antiserum, and cultured with FCM or LCM. Regardless of the conditioned medium, marked reduction in the number of colonies was observed in the anti-HBLA antiserum treated cultures but not in the other two. We conclude that human CFU-C consist of cells with heterogenous physical and growth characteristics, but that both types of CFU-C express B lymphocyte antigen.

430 THE EFFECT OF LITHIUM UPON HUMAN NEUTROPHILS (NEUT). Gerald Rothstein, David R. Clarkson, Wayne Larsen and John W. Athens, U. of Utah, Salt Lake City 84132
Li can increase the blood neut concentrations of neutropenic patients, but it has not been determined whether this is because of demargination or a true increase in the total neut pool. Neither has the effect of Li upon neut production or migration into inflammatory lesions been studied. We studied 12 Li treated psychiatric patients with the $DF^{32}P$ leukokinetic method and found that they had increased blood neut counts and neut production rates (NPR) and enlarged circulating (CNP), marginal (MNP) and total neut pools (TNP). (medians shown, *denotes p <.05).

Subjects	Neut Count ($cells \times 10^9/L$)	TNP	CNP ($cells/kg \times 10^7$)	MNP	NPR ($cells/kg/day \times 10^7$)
Li	4.7	105*	38*	80*	230*
Normal	3.6	61	31	29	160

Neut migration was measured by quantifying the movement of cells into chambers placed over standard skin lesions. Migration was 8.5 (1.7-68) $\times 10^5$ neut/24 hours (median and range) in 10 control subjects as compared with 150 (35-470) $\times 10^5$ neuts/24 hours in 4 lithium treated patients; thus neut migration was enhanced in Li treated subjects. This observation and that of increased neut production and pool sizes in Li treated patients provide physiologic support for the possible utility in Li in the treatment of neutropenia.

431 QUANTITATION OF NEUTROPHILIC GRANULOCYTE (NG) DIFFERENTIATION IN VITRO: THE EFFECT OF INCREASING CONCENTRATION AND WITHDRAWAL OF COLONY STIMULATING ACTIVITY (CSA), Elliott F. Winton, William R. Vogler, Kathryn L. Kellar, Margie B. Parker, Katherine C. Barnes, Joseph M. Kinkade, Jr., Depts of Med and Biochem, Emory University, Atlanta, Ga. 30322
By culturing mouse marrow cells in liquid media on glass slides, we have been able to more precisely quantitate proliferation and differentiation occurring in clones of NG during the first three days of culture. We have previously reported that increasing concentrations of CSA resulted in a decrease in the cell cycle time of clonally oriented NG as determined by ^{3}HTdR autoradiography (Blood, 50:289-302,1977). In marrow cells, the protein lactoferrin (LF) is found only in the secondary granule of the NG. Hence, immunofluorescent quantitation of the proportion of clonally oriented NG containing LF can be used to assess the relative state of differentiation of these clones. When CSA from endotoxin treated mouse lung conditioned media (CSA_{MLCM}) was increased over a 16 fold range, the proportion of cells containing LF decreased from 0.719 ± 0.037 (SE) to 0.341 ± 0.027 ($p < 0.001$) after 72 hours of culture. This apparent CSA_{MLCM} related delay in appearance of LF might be explained by the selection of more immature progenitor cells by CSA_{MLCM} at higher concentration. To investigate this possibility, we initiated cultures with equal concentrations of CSA_{MLCM}, and then withdrew CSA_{MLCM} from some cultures after 24 hours. CSA_{MLCM} withdrawal resulted in an increase in the proportion of LF containing cells after 72 hours of culture from 0.353 ± 0.027 to 0.658 ± 0.036 ($p<0.001$). Thus, CSA_{MLCM} in vitro appears to stimulate proliferation while postponing the appearance of a specific differentiation associated protein. Supported by ACS grants CH-24, PDT-3, and NIH grants CA22294, RR5364.

432 RECOGNITION OF HUMAN GRANULOCYTE BY SPECIFIC ANTINUCLEAR ANTIBODY. R.C. Briggs, J.F. Chiu, L.S. Hnilica, F. Chytil, L.W. Rogers, D.L. Page and M. Montiel. Depts. of Biochemistry and Pathology, School of Medicine, Vanderbilt Univ., Nashville, Tn. 37232 and Dept. of Pathology, Univ. of Texas Medical School, San Antonio, Texas 78284
When New Zealand white rabbits were immunized with nuclear nonhistone protein-DNA complexes prepared from a sample of human lung tissue, a granulocyte specific antinuclear antibody was produced. Horseradish peroxidase and fluorescence immunocytochemistry revealed antiserum recognition of the granulocyte nucleus in human lung tissue sections and in peripheral blood smears. In preliminary experiments using normal and pathologic blood smear and bone marrow specimens the antiserum appeared to react with nuclei during early stages of granulocyte development. The nuclei of the parenchymal cells in human lung, spleen, kidney, breast, placenta, and liver tissues including cancer cells from some of these tissues were not recognized. Nuclei of either rat or rabbit leukocytes also did not react. Current studies are aimed at determining the presence of nuclear antigens within three myeloid (neutrophilic, eosinophilic and basophilic) lineages, its presence under various pathologic conditions, as well as its earliest detectability in granulocyte development. Previous studies employing conventional biochemical techniques have revealed the existence of tissue specific chromosomal nonhistone proteins. This study has revealed a nuclear antigen which is apparently cell type dependent. The development of this immunocytochemical probe should facilitate the characterization of a nuclear component(s) involved in cellular differentiation. In addition it may be a useful diagnostic tool for identifying abnormalities in granulocyte development. Supported by Public Health Service contract NO1-CB53896 and grant HD-05384.

433 BUOYANT DENSITY ANALYSIS OF CFU_c IN GERMFREE AND CONVENTIONAL MICE: RELEVANCE OF THE GNOTOBIOTIC STATE, Wolfgang Heit, Peter Byrne, Hildegard Heit and Bernhard Kubanek, Dept.Haem., University Ulm, 7900 Ulm/Germany

Granulocyte-macrophage colony forming cells (CFU_c), in the bone marrow of germfree and conventional CBA mice, were quantitatively and qualitatively compared. Cells were separated on the basis on their buoyant density by equilibrium centrifugation in continous albumin density gradient. CFU_c in the density subpopulations were detected by culture in agar under stimulation by three different types of colony-stimulating factor. The sources of the CSF were post-endotoxin mouse serum, mouse lung conditioned medium (CSF_{MLCM}) and human urine. Mice were removed from the germfree environment and the buoyant density status of CFU_c was examined at one, four and eight weeks afterwards. No difference was found between germfree and conventional mice in the number of nucleated cells per femur nor in their modal-density. The number of CFU_c per femur, detected with CSF_{ES}, was also not different. The cell cycle status of CFU_c, as determind by ^{3}H-TdR showed no significant difference. Functional heterogeneity was found among the density subpopulations of both groups of mice which depended on the type of CSF. The density distribution of CFU_c was significantly different in germfree mice. There were proportionately more low density CFU_c. The mean model density of CFU_c under CSF_{ES} stimulation was less dense to the extent of 0.0045 g/cm^3 in germfree mice. The removal of mice from the germfree environment resulted in a shift in the distribution to higher densities. The trend was towards the conventional state. The significance of the buoyant density status of CFU_c and the possible role of endogenous CS-factors are discussed.

434 ACTION ON PROLIFERATION OF COLONY FORMING CELLS (CFC) OF INHIBITOR RELEASED FROM NEUTROPHILS AND BLAST CELLS, P.R.Galbraith, F.L.Baker, Queen's University, Kingston, Ont.

Normal neutrophils (PMN) contain inhibitor(s) which under appropriate conditions reduces proliferation of normal CFC(non-adherent light density marrow cells). Inhibitory molecules are: (1) released into culture medium such that equilibrium between free and cell bound inhibitor is established within 10 min. (2) dialysable (MW < 12,000) (3) labile at 37°C, but stable at -25°C. They appear to act by an allosteric mechanism to reduce the action of CSF on CFC because: with a given concentration of CSF the inhibitory effect is concentration dependent (log:log) to a saturation point while, Lineweaver-Burke analysis of the effect on colony formation of CSF concentration in the presence of inhibitor excess, gave evidence of competitive inhibition. In this study, levels of inhibitor released from blood leukocytes (2×10^6/ml.) were assayed at 10% in CSF stimulated cultures. A single density separation (Ficol ^{R} density 1.070 gms./ml.) was used to enrich leukocyte suspensions with high density PMN or lighter density blast cells. Inhibitor levels were estimated semi-quantitatively from a normal PMN inhibitor dilution curve (activity detected to a dilution of 1:64). In a spectrum of myeloproliferative and myelodysplastic disorders inhibitor released from neutrophils varied even within diagnostic categories, results being bimodally distributed. Leukemic blast cells released higher levels of inhibitor than normal PMN. Results suggest that different regulatory abnormalities may exist in different stem cell disorders and that inhibitors released from leukemic blast cells are able to reduce proliferation of normal CFC.

435 PURIFICATION AND PROPERTIES OF COLONY STIMULATING FACTOR (CSF). R.K.Shadduck, A. Waheed. Montefiore Hospital, University of Pittsburgh School of Medicine, Pgh., Pa.

We have previously reported a 5-step purification scheme for L-cell CSF which yielded two major fractions: non-adherent CSF (25%) which did not bind to Con-A-Sepharose and adherent CSF which was bound and subsequently eluted with α-methylglucoside (75%). Both fractions had an apparent molecular weight of 190,000 on Sephadex G150 and showed 1-3 minor contaminants by acrylamide gel electrophoresis. These fractions were further subjected to centrifugation in 5-15% sucrose at 100,000 G for 18 hours. CSF was recovered in the low density fractions indicating a M.W. of ~30,000. The CSF appeared to be pure as judged by electrophoresis in 4-15% acrylamide gels and by Ouchterlony gel diffusion using a rabbit anti-serum to CSF. Both purified fractions had specific activities of $\sim 4 \times 10^7$ colonies per mg of protein. Despite the variable reactions on Con-A, both fractions stained avidly with PAS suggesting a significant carbohydrate content. Following incubation with neuraminidase, both CSF fractions showed a reduction in electrophoretic mobility but retained full biologic activity. Purified CSF (375 mcg) from 12,000 ml of starting material was coupled to CnBr activated Sepharose and used to selectively elute CSF antibody from rabbit antiserum. In 6 studies, 50% recovery of the antibody was obtained. The purified antibody markedly inhibited murine granulocyte colony formation (titer 1:32) and showed moderate cross reactivity with human CSF (titer 1:4). These observations indicate that CSF is a glycoprotein with heterogeneity in carbohydrate side chains. Moreover, they provide sources of purified CSF and anti-CSF for studies of granulopoietic control mechanisms.

436 STROMAL CELLS FROM BONE FRAGMENTS PRODUCE COLLAGEN MATRIX BUT NOT GRANULOCYTE DIFFERENTIATION-INDUCING FACTORS IN CULTURE, R.W. Anderson, W. Stinson, and J.G. Sharp. Department of Anatomy, University of Nebraska Medical Center, Omaha, Nebraska 68105.
Stromal cells from femoral bone fragments obtained from guinea pigs, rats, and mice have been grown in vitro employing monolayer, gelfoam sponge and artificial capillary culture systems. Cultures were maintained in a fully humidified atmosphere of 5% CO_2 and air at a temperature of 37°C. Histochemical, light, and electron microscopic studies demonstrated that the cultured cells had the ability to produce collagenous matrix. Ultrastructurally, some cultured stromal cells demonstrated morphological characteristics which have previously been associated with endocrine hormone secretion in other systems (Jordan et al., J. Anat. 119:235, 1975). It has been suggested on the basis of circumstantial evidence that bone marrow stromal cells may be a source of granulocyte differentiation-inducing factors and this might be a mechanism of expression of the hematopoietic microenvironment. Consequently, these cells and their supernate were tested for the presence of such factors. The supernate was concentrated, dialyzed and added, by itself or in combination with one of several CSF preparations to 75,000 C57B1/6J mouse bone marrow cells in agar for determination of CFU-c numbers. The same assay was performed using the bone fragment derived stromal cells as a feeder layer. Although granulocytes were observed after many days in primary cultures, no significant granulocyte differentiation-inducing factors could be demonstrated in the cultures. Thus, the nature of endocrine product(s) which ultrastructural observations suggested may be associated with the cultured cells remains uncertain. (Supported by NCI Grant #CA18548).

437 GRANULOCYTE GROWTH MODULATORS ELABORATED BY HUMAN CELL LINES. John F. DiPersio, James K. Brennan, and Marshall A. Lichtman, University of Rochester School of Medicine, Rochester, New York.
Two human monocyte-like cell lines have been established which elaborate colony stimulating activity (CSA) for CFU-C of man and other species (DiPersio et al., Blood 48:970, 1977). Cell line CSA is sensitive to pronase, α-chymotrypsin, periodate, and to reduction and alkylation suggesting a glycoprotein containing disulfide bonds. Filtration of cell line CSA through Sephacryl S-200 yields a mouse CSA of 145,000 daltons and a human-mouse CSA of 40,000 daltons. A portion of the cell line CSA which stimulates mouse marrow is inactivated by antisera directed against purified urinary CSF. Cell line conditioned medium concentrated between 500 and 10,000 daltons by Amicon ultrafiltration contains a non-cytotoxic inhibitor of CFU-C growth (CIA). CIA does not inhibit the growth of human or mouse marrow erythroid colonies (CFU-E, BFU-E) or the incorporation of ^{3}H-Tdr and ^{3}H-Udr into resting and PHA stimulated lymphocytes. CIA is heat stable, resistant to proteases, adsorbs to activated charcoal and cannot be extracted into chloroform or ether. Purification of CIA by ultrafiltration, methanol extraction, SM2 chromatography, DEAE cellulose and TLC yields purified material with a molecular weight on Sephadex G-15 of ∿600 daltons. Monocytes elaborate similar CSA and CIA in culture. These cell lines provide an unlimited source of two potent modulators of granulopoiesis for further purification and for studies of their elaboration and biological effects.

438 DEPENDENCE UPON COLONY STIMULATING FACTOR FOR SYNTHESIS OF PROSTAGLANDIN E BY NORMAL AND NEOPLASTIC MONONUCLEAR PHAGOCYTES. J.I. Kurland, L. Pelus, R. Bockman and M.A.S. Moore, Sloan-Kettering Institute for Cancer Research, New York, NY 10021
It has been reported that the positive feedback drive of Colony Stimulating Factor (CSF) on committed granulocyte-macrophage progenitor cells is limited in vitro by the accumulation of macrophage derived Prostaglandin E (PGE). Utilizing three cellular systems, we have established that the synthesis and release of PGE by macrophages (MØ) is determined by an afferent feedback mechanism involving MØ surveillance of local CSF concentrations. Experiments using MØ from strains of mice differing only in their responsiveness to endotoxin (LPS), it was found that LPS stimulated both CSF and PGE by MØ from normal C3HeB/FeJ, but not LPS-nonresponsive C3H/HeJ mice. However, both CSF and PGE synthesis by MØ from C3H/HeJ was stimulated by zymosan or Concanavalin A, and methylmannoside selectively prevented the effect of only Con A on both CSF and PGE. In all cases, both constituitive and stimulated production of CSF preceeded the active synthesis of PGE, and the addition of a soluble source of CSF alone stimu- PGE synthesis and circumvented the LPS-nonresponsiveness of C3H/HeJ MØ. A number of continuous MØ cell lines constituitively synthesized PGE only with concomitant CSF production. Neoplastic MØ cell lines which were not constituitive producers of either CSF or PGE could be induced to active synthesis following treatment with zymosan, LPS or PPD. In all cases, CSF production was followed by the rapid induction of PGE synthesis, and in the absence of CSF-promoting agents, PGE was induced simply by a soluble source of CSF. Reduction of endogenous human monocyte CSF production by a cell-free granulocyte extract, coincidently decreased PGE synthesis, but the latter effect was reversed by the addition of exogenous CSF. These findings indicate that CSF is obligatory for the synthesis of MØ PGE, and establish the existence of a sensitive afferent mechanism in the contol of myeloid stem cell proliferation.

439 COLONY STIMULATING FACTOR RADIOIMMUNOASSAY. E.R. Stanley, Albert Einstein College of Medicine, Bronx, New York, 10461

A radioimmunoassay (RIA) has been developed for the colony stimulating factor (CSF) from mouse L cell conditioned medium sharing identity with macrophage growth factor (1) using purified L cell conditioned medium CSF (2). The RIA can be used to measure CSF at concentrations as low as 1.0 unit (3) (or 0.1 femtomoles of purified CSF) per tube within 24 h. There is a complete correlation between CSF levels determined by RIA and those determined by bioassay for most murine sources of CSF tested, including embryo cell conditioned medium, extract of pregnant uterus, mouse endotoxin serum and yolk sac conditioned medium. Notable exceptions were purified mouse endotoxin lung condition medium CSF (4) (not detected by the RIA) and mouse serum, for which the RIA yielded higher values, probably due to the presence, in mouse serum, of inhibitors of the bioassay (5). By modification to a 2 step, 48 hr. procedure, the RIA can also be used to measure this type CSF in human sources, although less sensitively (down to 100 units/tube). From preliminary studies, it appears that, irrespective of the species of origin of the source of colony stimulating activity or the target bone marrow cells (murine or human), the RIA detects only those CSFs which stimulate macrophage rather than granulocyte production.

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440 THYMIC REGULATION OF HEMATOPOIESIS:MYELOID DIFFERENTIATION IN THE STEM CELL DEFECTIVE W/W^V MOUSE. S.J. SHARKIS, A. Ahmed, and K.W. Sell, Johns Hopkins oncology center,Balt. Md., and NMRI and NIAID,NIH, Bethesda Md.

In addition to the stem cell defect of W/W^V mice these animals exhibit defects in the erythoid myeloid and lymphoid cell lines. Thus, we have shown that W/W^V mice appear to be missing a subpopulation of cells which are responsible for the maturation of erythroid precursors and in the self-renewal of stem cells. This cell, found in normal mouse bone marrow, spleen and thymus,is sensitive to the cytotoxic action of anti-Thy 1.2 serum and complement. The purpose of the present study was to determine the effect of antiserum treatment on myeloid differentiation. A mutant mouse possessing a transplantation marker(enlarged granules in the PMNs) was utilized as the bone marrow or thymocyte donor for W/W^V recipients. If the recipients recieved bg^J/bg^J (marked) bone marrow cells treated *in-vitro* with normal serum and complement, 70-87.5% of the PMNs contained the marker granules. If however, this marrow was treated with the antiserum and complement only up to 20% of the PMNs contained the marker. Addition of bg^J/bg^J thymocytes to unmarked anti-Thy1.2 serum treated unmarked($bg^J/+$) bone marrow produced 40-86.6% marked PMNs. The most interesting observation was that thymocytes from bg^J/bg^J(marked) donors when given alone to anemic recipients will result in marked granulocytes shortly after transplant in the peripheral blood of anemic recipients. As previously shown, we did not observe increases in RBC values unless both stem cells and the regulatory cells are injected together. We conclude that thymocytes besides their regulatory function in erythropoiesis can provide a committed granuloid precursor or cooperate with stem cells to differentiate into mature granulocytes.

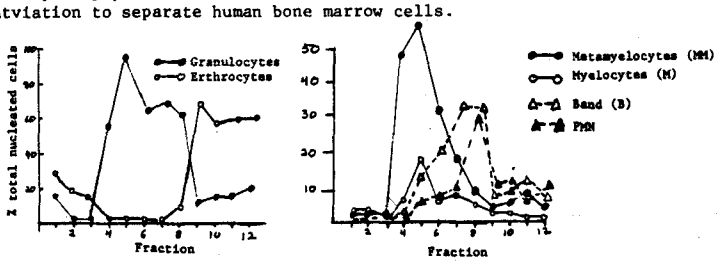
Hematopoietic Stem Cells

441 ANTIGENIC CHARACTERIZATION OF PLURIPOTENT STEM CELLS FOR PURPOSES OF AUTOMATED CELL SORTING, Richard L. Krogsrud and Gerald B. Price, The Ontario Cancer Institute, Toronto, Ontario, Canada M4X 1K9

The study of hematopoietic regulation is now at the stage where the isolation of pure populations of cells is necessary to determine relationships among stem cells, known soluble mediators and other cells in the hemopoietic system. We have approached this problem immunologically. Our previous studies have demonstrated that the hemopoietic stem cell of the mouse exhibit antigens which can be recognized by anti-human brain or anti-human sperm sera. Using a fluorescence sandwich technique for labelling these cells, we have obtained a 10 to 15 fold enrichment for pluripotent stem cells (CFU-S) by sorting out fluorescent cells in a fluorescence-activated cell sorter. The fact that anti-sperm activity and concurrently the anti-stem cell activity could be adsorbed from these antisera by a human teratoma cell line (Tera 2) brought to mind the possible involvement of the T/t locus of the mouse. Therefore, an anti-F9 serum has been investigated along with antisera to other possible markers of the stem cell such as Thy-1. The same technology and antisera have been used to investigate the possibility of enrichment of stem cells and the mixed colony-forming cells previously described by Johnson and Metcalf from fetal mouse liver.

442 SEPARATION OF HUMAN BONE MARROW CELLS BY CENTRIFUGAL ELUTRIATION, Frank L. Meyskens, Dept. of Hem/Onc., University of Arizona, Tucson, Arizona 85724

Delineation of the biochemical events that accompany differentiation of bone marrow (BM) cells will require pure populations of the relevant cell types. We report here the use of centrifugal elutviation to separate human bone marrow cells.



Compared to unfractionated BM the percentage of cells in the peak fraction were concentrated: M (x2.2), MM (x3.9), B (x2), and PMN (x2). Rare cell types also showed significant concentration: rubriblasts (x6), eosinophils (x6.6), and basophils (x10). Further subfractionation of the myelocytic series and details of this procedure will be presented.

443 Mutual suppression of the responses of marrow cells to erythropoietin and colony stimulating factor in vitro, Gary Van Zant and Eugene Goldwasser, Dept. of Biochemistry and The Franklin McLean Memorial Institute, University of Chicago, Chicago, IL 60637

We have previously shown that colony stimulating factor (CSF) derived from several sources caused a dose-dependent suppression of erythropoietin (epo)-stimulated erythroid burst formation and hemoglobin (Hb) synthesis in vitro. Moreover, epo caused a dose-dependent suppression of CSF-stimulated granulocyte-macrophage (G-M) colony formation. We have subsequently found that both of these types of competitive interaction are dependent on cell number. Epo (0.1-5 U/ml) caused suppression of CSF-stimulated G-M colony formation when mouse marrow was cultured at cell concentrations above approximately 2 X 10^5/ml. CSF (56-560 U/ml) caused suppression of 7-8 day Hb synthesis when mouse marrow was cultured in semi-solid medium at cell numbers above approximately 2 X 10^5/ml. We have found that maximum numbers of erythroid bursts are present between days 6 and 8 in similar cultures of marrow at several cell concentrations. Epo-stimulated Hb synthesis measured between 1 and 2 days, corresponding to the growth of erythroid colonies from CFU-E, was suppressed by similar amounts of CSF (56-560 U/ml) at cell concentrations at least 10 fold higher (above 2 X 10^6/ml). These data are consistent with the results of numerous studies in vivo showing that increased granulopoiesis is often associated with a concomitant decreased level of erythropoiesis, and vice versa. Hemopoietic cell differentiation in vivo takes place in a milieu in which cells are closely associated within the marrow space. Our findings suggest that relatively high cell concentrations may be required in vitro to study some of the normal mechanisms regulating hemopoietic differentiation.

444 AN ANTIGEN CHARACTERIZING CFU-S AND HEMATOPOIETIC THYMOCYTE PRECURSORS. Ross S. Basch, Thomas Panagiotatos and Joel Buxbaum, Departments of Pathology & Medicine, New York University School of Medicine, New York, N.Y. 10016.

Rabbit antisera against mouse brain contain antibodies which react with multipotential stem cells (CFU-S) and the hematopoietic precursors of thymic lymphocytes. These antibodies are not removed by absorption with T cells or cortical thymocytes, but are present on some thymic lymphomas. We have established and cloned a cell line of RL $\sigma^{\vee}$1 (a Balb C thymoma) which contains these antigens. Using an immunoselection technique, we have obtained a variant cell line which does not react with anti-mouse brain sera. Using this line and the parent cells, an antiserum has been prepared which appears to be specific for the antigen on thymocyte precursors. This serum retains the capacity to kill CFU-S, indicating that the antigen(s) present on these cells are identical to those on the thymocyte precursors. The serum has proven to be a useful immunofluorescent reagent for identifying these cells. Antigen-bearing cells are present in large numbers in the hematopoietic tissues of young mice. Their numbers decline with advancing age. They are present in essentially normal numbers in "nude" mice (which lack T cells). Their distribution in S/Sld anemic mice (whose defect is thought to reside in the inductive microenvironment) and in W/W^{V} anemic mice (which have reduced numbers of CFU-S) are under study.

445 **THE EFFECT OF AKR ONCOGENIC VIRUS ON HEMOPOIESIS IN LONG TERM MARROW CULTURES.** **Esther F. Hays, M. D. University of California, School of Medicine, Los Angeles, California, 90024.**

These experiments were initiated to evaluate the effect of AKR viruses on stem cell (CFU-S), progenitor cell (CFU-C), and granulocyte proliferation using the in vitro marrow culture system described by Dexter, et al (J. Cell. Physiol. 91:335-344, 1977). We have found that marrow cells from 6 week old AKR (AKR-N) mice are productively infected with N-ecotropic non-oncogenic virus. Marrow cells from 6 week old AKR (AKR-V) mice inoculated as sucklings with Gross virus or an N-ecotropic oncogenic AKR virus (Hays) are productively infected with oncogenic virus as well. Stroma were derived from AKR N and V mice and overlayed with N and V marrow cells at three weeks. The cultures were maintained in Fischer's medium with 22% horse serum at 33° with half medium change each week. Both AKR-N and V marrow cells maintained on AKR-N stroma showed active granulopoiesis for 10 weeks. CFU-C and CFU-S were present in cultures at 8 and 9 weeks respectively. In contrast, AKR-V cells cultured on AKR-V stroma showed granulopoiesis for 5 weeks. Cell numbers were lower than those from the cells cultured on N stroma. CFU-C were present in low numbers at 4 weeks, CFU-S were low or absent at 5 weeks and CFU-C were absent in 8 week cultures. These findings suggest that stroma from mice infected with oncogenic murine leukemia virus is defective in supporting stem cell maintenance and granulopoiesis in this system. However, stroma infected with the non-oncogenic endogenous virus of AKR is capable of supporting granulopoiesis by cells derived from both N and V marrows.

446 ONTOGENY AND CHARACTERIZATION OF HEMOPOIETIC COLONY-FORMING UNITS IN THE CHICK EMBRYO SPLEEN, Gordon Keller, Erwin Diener and Michael Longenecker, Department of Immunology, University of Alberta, Edmonton, Alberta, T6G 2H7.

Inoculation of chick embryo spleen cells onto the chorioallantoic membrane (CAM) of other chicken embryos led to the formation of white (granulocytic) and pink (mixed erythrocytic and granulocytic) hemopoietic colonies. The number of CAM colonies formed was a linear function of the number of cells inoculated, indicating that each colony originated from a single colony-forming unit (CAM-CFU). CAM-CFU's consist of an aggregate of primitive, undifferentiated cells which penetrate the ectodermal layer of the CAM, then proliferate and differentiate into mature, hemopoietic cells. Deaggregation of the CAM-CFU's to a complete single cell suspension results in the loss of colony-forming activity. This activity is restored if the deaggregated CAM-CFU's are allowed to reaggregate in vitro prior to inoculation. The fact that cell aggregation is a requirement for colony formation indicates that cell interaction may be involved in hemopoiesis within the colonies. Current studies using density centrifugation, as a means of cell separation, are aimed at characterizing the cell type(s) involved in colony formation. Preliminary results reveal that in a fifteen to thirty per cent BSA gradient, the cells from a ten-day-old embryonic spleen fall into three major density ranges. However, it appears as if only one of these groups of cells is involved in the formation of colonies. Ontogeny studies suggest that the CAM-CFU cells are a transient population. The peak colony-forming activity of the spleen is found between fifteen and seventeen days of incubation, declining thereafter to undetectable levels at two days post-hatching.

447 MURINE LEUKEMIA VIRUS INFECTION OF DIFFERENTIATING HEMATOPOIETIC CELLS Natalie M. Teich and T. Michael Dexter, Imperial Cancer Research Fund, London, and Paterson Laboratories, Manchester, England.

Long-term murine bone marrow cultures which support the proliferation of pluripotent hematopoietic stem cells (CFU-S) are observed to undergo changes in the proliferation of particular cell lineages after infection with pathognomonic variants of murine leukemia viruses (MuLV). Infection with the erythroleukemia-inducing Friend virus complex potentiates the survival of the CFU-S and of the committed granulocytic precursor cells (CFU-C) and extensive granulopoiesis is noted in the cultures. Injection of the cells into adult mice leads to erythroleukemia of recipient cell origin. These changes in the cultured cells are not seen in bone marrow cells derived from mice genetically resistant to Friend viral erythroleukemia, although replication of the nonerythroblastic component of the virus complex occurs. On the other hand, infection with the lymphoma-inducing Abelson virus with its helper Moloney MuLV leads to the proliferation of undifferentiated blast cells with neither θ antigen nor surface immunoglobulin; CFU-S and CFU-C disappear from the cultures at a rate faster than that of uninfected controls. The latter effect may be a consequence of the Moloney MuLV as cultures infected with Moloney virus alone also show rapid depletion of stem cells. Injection of the Abelson-infected cells into adult mice gives rise to null cell lymphomas, whereas those infected with Moloney MuLV do not induce tumors.

In Vitro Lymphocyte and Megakaryocyte Growth

448 A MONOCLONAL MODEL OF B-CELL DIFFERENTIATION. Richard G. Lynch and James W. Rohrer, Washington University School of Medicine, St. Louis, MO. MOPC-315 is a BALB/c myeloma which produces an IgA anti-DNP antibody (M315). When grown in peritoneal millipore diffusion chambers MOPC-315 cells undergo a progressive differentiation. A stem cell-rich population of lymphocytoid cells containing cytoplasmic M315 but little or no surface M315 is present for the first 2 days. Following this there is a progressive increase in both the frequency of cells with surface M315 and the amount of surface M315 expressed per cell. Beginning at day 7 there is a progressive increase in the frequency of MOPC-315 cells that actually secrete M315, and this is accompanied by a morphological shift to a plasmacytoid character. When presented with DNP-carrier the MOPC-315 cells are susceptible to carrier-specific help and suppression. These effects are manifested by changes in MOPC-315 cell proliferation and differentiation. The carrier-specific regulatory factors operate across a 0.2μ millipore membrane and are clearly of T-cell origin. MOPC-315 cell proliferation and differentiation can also be regulated by specific immunologic responses to idiotypic antigens (Id^{315}) of M315. Part of this regulation appears to be mediated by anti-Id^{315} antibodies. The responsiveness of MOPC-315 cells to immunoregulatory signals provides a unique opportunity to analyze the mechanisms which regulate the differentiation of B-cells and may provide a new therapeutic approach to myeloma.

449 ISOLATION OF A DNP-BINDING IgA LYMPHOMA LINE FROM MOPC-315 A DNP-BINDING PLASMACYTOMA OF BALB/c MICE, H.M. Gebel, J.R. Autry & R.G. Lynch, Dept. Pathology, Washington Univ. Sch. Med., St. Louis, MO 63110
MOPC-315 is a BALB/c myeloma which produces an IgA anti-TNP antibody (M315). Cell surface expression (M) and secretion (S) of M315 are detected, respectively, by immunocytoadherence and immune hemolysis using TNP-erythrocytes. When MOPC-315 cells from an established tumor are examined for M315 expression 3 types of cells are found: ($C^+M^-S^-$) which have cytoplasmic M315; ($C^+M^+S^-$) which have cytoplasmic and surface M315; and ($C^+M^+S^+$) which have cytoplasmic and surface M315 and secrete M315. We presented evidence (J. Imm. 119: 861, 1977) that MOPC-315 cells differentiated *in vivo* from small, non-secreting lymphocytoid cells to large M315-secreting plasmacytoid cells ($C^+M^-S^- \rightarrow C^+M^+S^- \rightarrow C^+M^+S^+$). To analyze further M315 expression we have established MOPC-315 in culture and isolated a stable line (TC-1) which has the phenotype $C^+M^+S^-$. Since TC-1 cells do not differentiate to $C^+M^+S^+$ *in vitro* they mimic an IgA lymphoma. The inherent stability of the $C^+M^+S^-$ phenotype was assessed by the examination of tumors which developed in normal mice injected with $1x10^7$ TC-1 cells. Over 100 tumors have been examined thus far, some through the third transplant generation. We observed that most TC-1 tumors maintained the $C^+M^+S^-$ phenotype, and the tumors expressed growth and tissue localization patterns which were distinct from the wild-type MOPC-315.

450 THE 'PATCHY' IMMUNODEFICIENCY OF THE CBA/N MOUSE, José Quintáns, La Rabida-Univ of Chicago Inst, Chicago, IL 60649
CBA/N mice have an X-linked defect of B lymphocyte differentiation which affects certain subpopulations of B cells. Our studies on antigen-induced responses *in vivo* and limiting dilution analysis *in vitro* demonstrate that CBA/N mice have a 'patchy' immunodeficiency. Thus they lack thymus-dependent (T.D.) and thymus-independent (T.I.) phosphorylcholine (PC)-specific B cells and T.I. TNP-specific cells responsive to Ficoll and Dextran. However they can mount normal T.I. responses to TNP-Brucella, TNP-Bacteriophage T_4 and TNP-Streptococcus pneumoniae Cs. The T.I. clones responsive to TNP-LPS in CBA/N mice are characterized by a preponderance of precursor cells producing low avidity antibody. These various defects are related to the acquisition of immunocompetence by the relevant clones during ontogeny. Our studies on the ontogeny of immune responses to PC and TNP-antigens indicate that the X-linked defect of CBA/N mice leads to an arrest of B cell differentiation which affects clones acquiring immunocompetence 1 day after birth.

451 NUCLEIC ACID SYNTHESIS IN DIFFERENTIATING THYMOCYTES, Susan Wolf Evans and Ronald L. St. Pierre, Dept. of Anatomy, The Ohio State University, Columbus, Ohio 43210

In vitro thymocyte differentiation has been described with respect to mitogen reactivity and antigenic properties, but little attention has been given to the subcellular events which occur during the maturation process. We have examined the patterns of nucleic acid synthesis of high density, immature mouse thymocytes cultured in diluted supernatants from monolayers of thymic epithelial cells (TE), lung epithelial cells and macrophages (LU), kidney fibroblasts (K) and L-cell fibroblasts. Fresh medium controls were also included. DNA and RNA synthesis was measured over several time periods by incorporation of tritiated thymidine and tritiated uridine, respectively. Changes in reactivity to Con A and PHA were also assessed. Preincubation in TE supernatant significantly increased the Con A response of thymocytes, while the PHA response was greatly enhanced in both TE and LU cultures. Cell viability was higher in these cultures when compared with cells in K and L-cell supernatants. Values for tritiated uridine incorporation remained higher than medium controls in both TE and LU supernatant cultures. However, the pattern of DNA synthesis in these cultures was dissimilar. By 36 hours thymidine incorporation in TE and K cultures was well below media controls, approaching control values at high dilutions. By comparison, incorporation levels for cells in LU supernatants, at all dilutions, were close to media controls. These results indicate that nucleic acid synthesis could supply an additional valuable parameter in the analysis of thymocyte differentiation. (Supported by NCI Grant CA19346)

452 STIMULATION OF THE SELF-RENEWAL AND DIFFERENTIATION OF THE T PROGENITOR CELLS IN A SUSPENSION CULTURE BY T LYMPHOCYTE GROWTH STIMULATOR (TL-GS), Alan M. Wu, Dept. of Anatomy (Histology), University of Toronto, Toronto, Ontario M5S 1A8, Canada

A specific cell-factor interaction is an essential process for the maturation of both myeloid and lymphoid cells. Previously we reported that conditioned medium prepared from short-term cultures of PHA stimulated human T lymphocyte (PHA-LyCM) contained human granulocytic colony stimulators (G-CS). The same CM also contained some factors promoting a long term growth of lymphoid cells in a suspension culture. The growth of lymphocyte population in culture was dependent on a continuous presence of TL-GS. TL-GS was distinguishable from some G-CS by their molecular size (13,000 for TL-GS versus 24,000 for G-CS) and their charge property detected in column chromatographic studies. The majority of the cultured cells was capable of forming spontaneous rosettes with sheep erythrocytes but not with mouse erythrocytes. Some of them were capable of responding to stimulator cells in a one-way mixed leukocyte culture and capable of killing tumor cells in vitro. Furthermore, some culture cells were capable of forming T-lymphocyte colonies in methylcellulose medium and the cells harvested from individual colonies can also grow and differentiate in the suspension culture in the presence of TL-GS. This finding together with that of the long term growth of T cells suggested that TL-GS contained some regulatory activity capable of stimulating self-renewal of T progenitor cells. Additional evidence obtained with surface marker studies further supported this notion. Finally, by performing limiting dilution experiments, the frequency of the "putative" progenitor cells was estimated to be one in 10^3 mononuclear marrow cells at minimum.

453 Humoral Control of Mouse Megakaryocyte Progenitor Differentiation in Vitro (CFU-M) E. F. Saunders, T. P. McDonald and M. H. Freedman, Div. of Hematology, Hospital for Sick Children, Toronto, Ontario and University of Tennessee Memorial Research Center, Knoxville, Tennessee.

The humoral control of megakaryocyte progenitors from C57BL mouse marrow was studied in vitro using a plasma clot culture system. CFU-M were defined as colonies of 3 or more acetylcholinesterase positive megakaryocytes. Without humoral stimulation no colonies grew. Erythropoietin (EPO) from anemic sheep plasma (Connaught 4 u/mg) in doses from 1 to 5 u/ml induced a linear increase in CFU-M to a maximum of 20 colonies/10^5 cells plated. The same product in vivo increased ^{35}S-sulfate uptake into platelets in the thrombocythemic mouse assay. In contrast, very few CFU-M grew with human urinary EPO (NIH 75 u/mg), with maximum colony numbers less than half that achieved with sheep EPO, and no increase in colonies with human EPO concentrations above 3 u/ml. This product had no thrombopoietic activity in vivo in the mouse bio-assay. Thrombocytopoiesis stimulating factor (TSF) derived from human embryonic kidney cultures induced a dose responsive increase in CFU-M in concentrations from 0.005 to 0.35 mg/ml in the absence of EPO. TSF did not support the growth of CFU-C, CFU-E, or BFU-E from mouse marrow indicating absence of a CSA or EPO activity. TSF in vivo did not stimulate erythropoiesis in the polycythemic mouse assay. We conclude both TSF and EPO containing materials stimulate the growth of CFU-M. As TSF contains no EPO activity and purified EPO is less effective than impure EPO, it would appear the humoral control of megakaryocytic differentiation is not due to erythropoietin.

454 REDUCTION OF THYMOCYTE SURFACE ANTIGENS IN VITRO AS A MODEL OF INTRATHYMIC DIFFERENTIATION

Joan Abbott and Pamela J. Doyle, Sloan-Kettering Institute, New York, N.Y. 10021

Thymocytes were cultured 24 hours in the presence or absence of various inducing agents and assayed for TL and thy-1 surface antigens. Induction with tumor necrosis serum (TNS) or carbamylcholine clearly caused a reduction in TL and thy-1 antigen as demonstrated by cytotoxicity assays. Anti-TL serum absorbed with TNS induced cells was significantly more efficient in killing fresh thymocytes than serum absorbed with untreated cells. These results indicate a quantitative loss of TL antigen from the induced thymocyte population. Lipopolysaccharide and dibutyril cyclic-AMP did not induce reduction of TL antigen. Differential cell death or cell adhesivity were not responsible for these results since both induced and non-induced cells were equally viable after 24 hours (ca. 80%) and equal numbers of cells were recovered from the cultures. Since both TL and thy-1 are lost or reduced during normal passage of thymocytes through the thymus in vivo, it is possible that we are inducing this differentiation step in vitro.

455 ADOPTIVE TRANSFER OF THYMIC MICROENVIRONMENT. R.K. Jordan, D.A. Crouse, and L.W. Arnold, Dept. Anat., Univ. Nebr. Med. Ctr., Omaha, Nebr.

While the primordium of the thymus is derived from epithelium of the pharyngeal pouches, the lymphoid cells are derivatives of precursors which migrate into the epithelium from the blood. It is likely that intra-thymic maturation of precursor cells is an obligatory step in functional T cell differentiation. To investigate the nature of the interaction between immigrant stem cells and thymic epithelium, monolayer cultures of thymic epithelial cells free of lymphoid elements have been obtained from explants of embryonic or newborn mouse thymus. Such monolayer cultures have been previously characterized (Owen, J.J.T., et al. Proc. Symp. "Developmental Immunobiology", In Press); two major morphological cell types can be distinguished. The first is a spread cell, rounded in profile with a central nucleus. The other type has been termed the "dendritic cell", because it has long cytoplasmic processes. The functional capacity of such epithelial monolayers in terms of the maturation of immigrant precursors has been tested by transplanting spin-mediated reaggregates of cultured cells into syngeneic, intact hosts. In these preliminary studies a lymphoepithelial structure developed at the graft site in the majority of attempts. In the present study we have allowed the cultured epithelial cells to self-aggregate in a gelatin based matrix in organ culture prior to transplantation in an attempt to improve the repeatability of the transplantation procedure. Such matrix organ cultures of lymphoid free cultured thymic epithelium have been used in a series of reconstitution experiments using various experimental and natural T deficient models. The functional reconstitution of the cell mediated responses of these animals is currently being assessed. (Supported by UNMC GRS funds and NIH Grant #CA 18548).

456 DEVELOPMENT OF LONG-TERM SUSPENSION CULTURES OF HUMAN T-LYMPHOCYTES FROM SINGLE COLONIES. F.W. Ruscetti and R.C. Gallo, Bionetics Research Laboratories and NCI, NIH, Bethesda, Maryland 20014.

Lymphocytes isolated from normal human peripheral blood, bone marrow and spleen can be induced to form colonies in semi-solid medium. Phytohemagglutinin (PHA) can induce formation of thymus-derived lymphocyte colonies. However, using methylcellulose instead of agar for colony formation, the continuous presence of the lethin is not required for colony formation. At a seeding density of $5x10^5$ cells per plate, the average number of colonies formed is 2350 $\pm$ 573 with 200-400 cells per colony after 6-7 days of incubation. We recently reported that a factor present in medium conditioned by pooled normal PHA-stimulated lymphocytes (Ly-CM) initiated and supported the long-term growth of T-lymphocytes in suspension.[1] PHA can not substitute for this factor. In single cultures of these T-cells, there is considerable heterogeneity of surface markers. In order to develop more homogeneous T-cell cultures, cultures have been initiated from single colonies formed in methylcellulose. Single colonies are picked using micropipets, resuspended in media containing 20% Ly-CM and incubated in 4 mm diameter tissue culture wells. The culture is continuously expanded until it can be grown in flasks. These cultures are routinely 99% sheep erythrocyte (E) receptor positive and respond but do not stimulate in one-way mixed lymphocyte reactions indicating their T-cell nature. These cell cultures can be distinguished from transformed lymphoblastoid cell lines by their dependence for growth upon the continuous presence of a protein factor(s) in Ly-CM. This system should be of value in studying functions of homogeneous populations of T-lymphocytes.

[1]Morgan et al., Science 193:1008, 1976.

457 SPONTANEOUS BLASTOGENESIS AND INDUCTION OF PHA-RESPONSIVENESS OF SPLEEN CELLS FROM NEONATALLY THYMECTOMIZED RATS CO-CULTURED WITH THYMIC NON-LYMPHOID CELLS. C.M. Harper, T.W. Bauer, and J.G. Sharp, Dept. of Anat., Univ. of Nebr. Med. Ctr., Omaha, Nebr.

We have grown the non-lymphoid stroma of the thymus (thymic reticuloepithelial or TRE cells) in culture in an attempt to recreate the thymic microenvironment in vitro. The morphological characteristics of the TRE cells have been defined at the light and electron microscopic levels. In successful cultures the predominant cell was elongated or spindle-shaped with one or more nuclei which contained prominant, often multiple, nucleoli. The other major cell type was pyramidal or polygonal in shape and usually contained a single nucleus with one or two prominant nucleoli. When T cell deficient spleen cells from neonatally thymectomized rats were co-cultured with TRE cells they showed a small but significant increase in DNA synthesis in response to a T cell specific mitogen (PHA). Similar, but less pronounced results were obtained when the spleen cells were co-cultured with concentrated TRE cell supernatant. No significant responses were induced towards a B cell specific mitogen (LPS) or by co-culture of the spleen cells with control fibroblasts. Additionally, spontaneous blastogenesis of some spleen cells, in the absence of mitogen, was observed on the TRE cell monolayers, particularly in association with the cytoplasmic processes of the pyramidal or polygonal cell type. These results are consistent with the hypothesis that the intrathymic differentiation of prethymic T cell precursors into mature T lymphocytes is a two step process. The early event may be proliferation of the precursor cell population involving contact with TRE cells, whereas, the hormonal effect may be a later event primarily associated with T cell maturation. (Supported by NIH Grants #CA 18548 and #HD 07097).

458 STUDIES ON THE RADIATION SENSITIVITY OF CLONOGENIC CELLS ISOLATED FROM THE PERIPHERAL BLOOD IN MAN. Stephen L. Seagren, John E. Byfield, Ulf Karlsson, Div. of Radiation Oncology, Univ. of Calif., San Diego, 92103, and Department of Anatomy, University of Iowa, Iowa City, Iowa, 52240.

A preliminary investigation has been undertaken in order to determine (a) the effect of fractionated half-body radiation treatment on the population kinetics of committed granulocytic stem cells in man and (b) to determine the in vivo & in vitro radiosensitivity of clonogenic lymphocyte sub-populations. The former studies utilized procedures described by Richman, et al. (BLOOD, vol. 47:1031, 1976) while the latter employed a modification of this technique in lymphocyte colonies in methylcellulose. The lymphocyte assays yield consistent results & we are currently using this assay to determine the effects of therapeutic radiation on the clonogenicity of B&T lymphocytes as well as the radiation survival parameters in vitro. This approach should permit direct clonogenic analysis of lymphocyte damage after x-ray and drug exposure in man. This data, along with EM studies of colony forming cells will be shown. In culturing CFU-c's, we found it difficult to obtain consistent results even in controls; to date, we have not been able to show the expected increase in the number of CFU'c's following single fraction half-body radiotherapy exposure (100 rads lower, 800 rads upper). These studies are continuing. Supported by University of California Ca. Coor. Comm. funds.

459 THYMIC INFLUENCE ON T CELL PROGENITORS IN THE BONE MARROW, J. John Cohen and Sally S. Fairchild, University of Colorado Medical Center, Denver, Colo. 80262

Two steps have been identified in the development of thymus-derived lymphocyte (T cell) precursors. In the first, committment of a stem cell to the T cell line occurs, presumably randomly, in the bone marrow; the second takes place upon arrival of the committed pre-T cell at the thymus, and involves humoral factors secreted by thymic epithelial cells and contact interactions. We have now found that the thymic humoral effect is exerted on pre-T cells even before they leave the bone marrow. We used an assay for pre-T cell function (J. Exp. Med. 144: 456, 1976) which exploits the ability of pre-T cells to enhance the mitogenic response of mature T cells to concanavalin A. The bone marrow of neonatally thymectomized mice is deficient in this activity as compared to sham-thymectomized controls. Similarly, nude (athymic) mice have lower levels of activity than controls. When neonatally thymectomized mice were treated for 3 weeks with thymosin their bone marrow pre-T cell function was restored to normal levels. These experiments indicate that very early, pre-thymic stages of T cell maturation are under the thymus's hormonal control. Thus the thymus, in addition to providing a site for T cell maturation, is able to increase the size of the pool of progenitor cells which enter it; this may, in fact, be the major role of the thymus hormone.

460 THE EARLY DIFFERENTIATION EVENTS IN LYMPHOID DIFFERENTIATION ARE POORLY UNDERSTOOD, Elizabeth Vallance Jones, The Ontario Cancer Institute, Toronto, Ontario M4X 1K9
Although the lymphoid and myeloid systems have a common stem cell, no assays are available to study the early stage of lymphocyte differentiation or the factors which regulate the differentiation of lymphocytes. An in vitro system would facilitate the study of the early events in lymphoid differentiation. Dexter has described a culture system in which pluripotent stem cells are maintained for several months on an adherent layer derived from bone marrow. The population of cells floating in the supernatant medium contains stem cells and other cells at later stages of myeloid differentiation. Mature lymphocytes have not been observed in these cultures. Since it is possible that the culture conditions select for stem cells restricted to myeloid differentiation, we have studied the differentiation potential of cultured stem cells. Cultures were established using bone marrow from CBA mice carrying the T6 chromosome marker. After various intervals in culture, the CBA.T6/T6 stem cells were injected into irradiated CBA recipients. Two months after reconstitution, dividing myeloid and lymphoid cells were examined for the presence of the T6 chromosome. As expected, myeloid cells have the T6 marker. Con A reactive cells (T cells) are also marked; LPS reactive cells (B cells) have not yet been examined. These preliminary results suggest that the failure to detect lymphocytes in vitro reflects a defect in the culture system rather than a loss in the potential of cells to generate lymphocytes. (Supported by the Medical Research Council of Canada and the National Cancer Institute of Canada).

461 ATTEMPTS AT IMPROVING COLONY METHODS FOR HUMAN LEUKEMIC CELLS, B. LOWENBERG AND A. Hagemeijer,Hema. & Cell Bio. Genetics, Erasmus Univ. Rotterdam, The Netherlands
The observations that the standard in vitro colony assays for human myeloid precursor cells nly produce little or no growth of leukemic colonies, are generally considered a drawback for a convenient analysis in vitro of clonogenic leukemic cell proliferation and for application of the system for the detection of leukemic cells for diagnostic purposes. The finding that human acute leukemic cells in suspension culture can respond to phytohaemagglutinin (PHA)(1,2) seems to provide a lead for circumventing this problem. For the purpose of developing a specific leukemic colony assay, we have designed a culture system in which the cells are exposed to the stimulating influences of both PHA and (irradiated) leucocyte feeder cells (2). So far, the system has been employed for studying bone marrows from 2 patients with acute myeloid leukemia (AML) and also from 7 patients with chronic myeloid leukemia (CML). In order to prove the development of leukemic colonies, only patients who were Ph^1 postive were selected for study. The results indicate that a) the system is suitable for inducing the growth of large size acute leukemic colonies, although leukemic growth is contaminated with T-lymphocytic colony formation; b) selective outgrowth of leukemic colonies can be successfully obtained when lymphocytic colony formers are separated from the cell suspension prior to culture; and c) the system is considerably more efficient in supporting the growth of CML colonies than is the Robinson system.

1. K.A. Dicke, G. Spitzer and M.J. Ahearn, Nature, 259 (1976) 128
2. B.Lowenberg and A. Hagemeijer,Proc. 8th Int. Symp. Comp. Res. Leukemia Related Dis.Elsevier Amsterdam, 1977, in press.

462 MATURATION OF PRECURSOR B LYMPHOCYTES IN VITRO, Sally S. Fairchild and J. John Cohen, Div. of Clin. Immunol., U. of Colo. Med. School, Denver, CO 80262.
Bone marrow derived (B) lymphocytes sequentially acquire characteristic surface markers and functions during their development. In order to study requirements for B cell differentiation we looked at two maturation events in vitro: the expression of surface immunoglobulin (sIg) and the capacity to proliferate when treated with the mitogen lipopolysaccharide (LPS). Bone marrow from CBA/J mice was used as a source of precursor B cells. To look at sIg expression bone marrow was passed over an anti-Ig column to remove sIg+ cells and then cultured with or without agents shown to be inductive in other systems: 8-p-chlorophenylthio-cyclic AMP, LPS, or a cAMP phosphodiesterase inhibitor RO20-1724. At different times after initiation of culture the number of sIg+ small lymphocytes was determined by immunofluorescence. Expression of sIg occurred spontaneously and was accelerated by cAMP, LPS, or RO20-1724; at 20 hr approximately 35% of the small lymphocytes were sIg+ in induced cultures compared to 11% in controls. The final number of sIg+ small lymphocytes was approximately 50% in both induced and control cultures, and sIg expression did not require cell division. We conclude that expression of a differentiative program that had been initiated in vivo could be accelerated but not actually induced in vitro. Acquisition of LPS responsiveness was studied by culturing cells for 2 days with or without inducer and then for 2 days with LPS; tritiated thymidine incorporation was measured on day 4. Maturation required cell division during the first 2 days; it was induced by LPS itself or other mitogens and was inhibited by BUdR and light. After mitosis, expression of LPS responsiveness could be accelerated by cAMP, LPS, and RO20-1724.

463 REGULATION OF THE PROLIFERATION OF MURINE MEGAKARYOCYTE PROGENITOR CELLS BY CELL CYCLE Neil Williams. Sloan Kettering Institute for Cancer Research,Rye,NY 10580

The extent to which mouse megakaryocyte progenitor cells (colony forming unit-megakaryocyte:CFU-M) can proliferate in semi-solid cultures proir to endomitosis, and conditions which may regulate that differentiation step have been investigated. The proliferative capacity of CFU-M was estimated by determing the number of megakaryocytes per colony. Colonies of upto 250-300 acetylcholinesterase positive cells were obtained. A bimodal distribution in the number of cells per colony was observed (modal values: 10-15 and 25-30 cells per colony), indicating that separate megakaryocyte progenitor cells may be differently biased in their capacity for proliferation versus endomitosis. The number of CFU-M cloned per femur was not influenced either by multiple transfusions of mice with platelets and platelet extracts, or by adding them directly to the semi-solid culture assay. Differences were observed in the cell cycle characteristics of CFU-M as determined in vivo and in vitro, which suggests that the maturation of CFU-M into megakaryocytes may be regulated within the marrow by control of the cell cycle of the megakaryocyte precursor cell.

464 COMPARISON OF HYDROXYUREA AND ^{3}H-TdR IN VITRO AND IN VIVO FOR THE MEASUREMENT OF THE KINETIC PROPERTIES OF HAEMOPOIETIC PRECURSOR CELLS, Bernhard Kubanek, Wolfgang Heit and Ivan N. Rich, Dept. Haem., University Ulm (SFB 112, A 2), 7900 Ulm/Germany

The effects of hydroxyurea in vivo and suicidal dose of tritiated thymidine (^{3}H-TdR) of high specific activity in vivo and in vitro were used in simultaneous experiments to characterize the kinetic status of the different haemopoietic progenitor cells of fetal and adult haemopoietic tissue. A similar propertion of agar colony forming cells was killed by hydroxyurea and by the ^{3}H-TdR suicide in vivo and in vitro. Comparable results were obtained with these methods, as expected, for the kinetic status of CFU_s. The effect of HU and ^{3}H-TdR in vivo were different for CFU_e derived from adult and fetal haemopoietic tissue. Treatment with HU killed 72 % of CFU_e from adult bone marrow and 80 % of CFU_e from 14 day old fetal liver, whereas a significantly smaller fraction of CFU_e 50 % resp. 71 % was estimated in S-phase by the ^{3}H-TdR suicide in vivo. The effect of ^{3}H-TdR in vitro on the CFU_e was comparable to the effect of HU in vivo in simultaneous experiments. A similar trend was seen when the different methods were applied for estimating the kinetics status of the BFU_e resulting in the high fraction killed by HU (~60 %). This results caution the view that the methods for estimating the kinetic status of cells population are interchangable and comparable for all progenitor populations even when comparable in two assay system (CFU_s and CFU_c).

Leukemia Cell Proliferation and Differentiation

465 EQUAL EFFECTS ON GRANULOCYTIC COLONY FORMATION (CFU-C) OF MULTIPLE COMBINATION CHEMOTHERAPEUTIC REGIMENS FOR CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA (ALL). Lois W. Dow, Rhomes J.A. Aur and Alvin M. Mauer. St. Jude Children's Research Hospital, Memphis,TN. 38101

This study evaluated the effects on marrow CFU-C of different numbers of myelotoxic agents given simultaneously as combination chemotherapy (CT) to children with ALL. After remission induction with prednisone, vincristine and asparaginase and cranial irradiation with intrathecal methotrexate (MTX), patients were randomized to receive the first 2, the first 3 or all 4 of the following: MTX, mercaptopurine, cyclophosphamide and cytosine arabinoside. Drug dosages were adjusted to maintain leukocyte counts of 2,000-3,500/mm^3 with proportional increase or decrease in all agents. Following 30 months of CT, all therapy was discontinued and bone marrow aspirations for morphology and cell culture studies were repeated serially thereafter. Marrow cells were plated in 0.3% agar over leukocyte feeder layers and colonies greater than 50 cells were scored at 12 days. The median CFU-C level of 23 patients assayed at the time of cessation of CT was 49/2 X 10^5 cells, with median levels and ranges for the 2, 3, and 4 drug treated groups of 49 (0-90), 63 (30-204), and 40 (4-92) respectively, and for 21 patients tested 14 weeks after the cessation of CT was 48/2 X 10^5 cells with median levels and ranges for the 2, 3 and 4 drug groups of 48 (9-88), 61 (36-68), and 57 (25-9[illegible]) respectively. Despite clinical evidence of increased immune suppression with increasing number of drugs as evidenced by an increased incidence of Pneumocystis carinii pneumonia, we conclude that adjustment of drug dosage by the leukocyte count in patients receiving these drug combinations resulted in equal effects on CFU-C.

466 Ia-LIKE ANTIGENS IN HUMAN NON-LYMPHOID LEUKEMIA. A.K. Sullivan, R. Ikeman, G. Rowden, L.M. Jerry. Dept. of Hematology, Royal Victoria Hospital; McGill Cancer Research Unit, McGill Univ. Montreal, CANADA.

It has been suggested that Ia-like antigens in man may be useful differentiation markers denoting lymphoid and other hematopoetic subpopulations where cell-cell recognition occurs. Previous work from this group has shown that Langerhans cells of the skin, possibly derived from the macrophage system, also express the antigen. Moreover, several groups have reported its presence to a variable degree on leukemic cells of the myeloid series.

We have extended past efforts to show: 1) In acute non-lymphoblastic leukemia a variability of expression that was not correlated with serum muramidase 2)In chronic myelomonocytic leukemia (CMML) it was found in only 1/3 cases. 3)Antigens purified from a patient with Ia(+) AML shows the two chain structure and immunologic identity (double immunodiff.) to those extracted from cells of CLL.4) Work in progress suggests it is present on megaloblastic pronormoblasts.

These studies furthur support the notion that the Ia-like antigen may be a product of eary hematopoetic cells which progressively disappears when the final differentiated form no longer requires specific cell-cell recognition for normal function. They also cast doubt on the validity of the monocytic derivation of CMML implied by morphology.

467 IN VITRO CULTURE PATTERN IN PRELEUKEMIA (PL) AND OLIGOBLASTIC LEUKEMIA (OL): THEIR DIAGNOSTIC & PROGNOSTIC VALUE. Gary Spitzer, Dharmvir Verma, Karel Dicke & Kenneth B. McCredie, Dept. Developmental Therapeutics, Univer of Texas System Cancer Ctr, M.D. Anderson Hospital and Tumor Institute, Houston, Texas 77030

Preleukemia and OL are a heterogenous group of disorders with a variable clinical course. Some progress rapidly to frank leukemia and others remain stable for several years. We examined 85 patients (pts) with these disorders (19 PL, 66 OL). The diagnostic value of in vitro agar cultures in PL and their prognostic value in OL for survival, leukemic infiltrate progression, impending deteriorating hematopoiesis and response to therapy were analysed. Also sequential agar cultures were performed to determine if changes in culture results were predictive of changing clinical behavior. Three in vitro culture patterns were recognized, 1) residual colonies with normal colony to cluster ratio and no leukemic growth (Category (Cat) I), 2) differeniated colonies of granulocytes or monocytes with leukemic clusters (Cat II) and 3) leukemic growth only with clusters of <20 cells (Cat III-A) and clusters of >20 cells (Cat III-B). Of PL pts, only Cat III (5/5), have progressed to leukemia. In OL the majority had a Cat III in vitro pattern. Survival was longest and both progression and hematopoietic compromise slower in Cat I and Cat II. Response to therapy was significantly higher in Cat III-A than Cat III-B. Serial cultures showed two basic changes, a) loss of differentiated colonies and conversion to Cat III and b) increase in cluster incidence in Cat III pts. These changes heralded rapid leukemic progression. Agar culture is of 1) diagnostic value in PL, 2) can detect subgroups of OL with different survival, progression and response to therapy and 3) can predict in advance rapid progression of OL.

468 ROLE OF COLONY STIMULATING FACTOR (CSF) IN GROWTH OF HUMAN NORMAL AND LEUKEMIC MYELOID COLONIES. C.H.Park, M.A.Savin, M.Amare, and B.Hoogstraten, University of Kansas Medical Center, Kansas City, Kansas 66103

CSF is required for optimal growth of myeloid colonies in culture, but its precise role has not been elucidated. CSF may be a triggering agent required only for initiation of colony growth, or it may be needed continuously to sustain growth. In order to distinguish between these two possibilities, the following studies were performed: Normal and leukemic human bone marrow cultures were performed using the modified agar culture method in which old culture medium is removed and fresh medium fed to the cultures each day. This technique permits growth of normal and leukemic myeloid colonies using phytohemagglutinin-stimulated human leukocyte conditioned medium (LCM) as the source of CSF (Park et al, Cancer Research, in press Dec. 1977). Using either normal bone marrow or bone marrow from a patient with acute myelocytic leukemia, groups of cultures were set up. The control group was fed daily with medium containing LCM. In the remaining groups, LCM was deleted from the feeding medium after 1, 2, or 5 days of growth, thus limiting exposure of myeloid cells to CSF to the early period of colony growth. In normal marrow cultures, deletion of LCM after 1, 2, and 5 days resulted in colony counts of 45%, 79% and 95% of control. In leukemic marrow cultures, no colony growth occurred with deletion of LCM after 1 day of culture. Thus, in normal bone marrow cultures CSF appears to function as an initiator of colony growth, and its continued presence is less important after the first day of culture. Leukemic marrow cultures, on the other hand, appear to have an absolute requirement for CSF to maintain continued myeloid colony growth.

469 Isolation and Partial Characterization of the Human Acute Lymphocytic Leukemia Associated Antigen, John E. Smart, Robert Sutherland, and Melvyn Greaves, Imperial Cancer Research Fund, London, England. We have isolated and partially characterised on antigen synthesised by a lymphoid cell line (Reh - derived from an acute lymphocytic leukaemia), which is specifically recognized by an ALL cell surface specific antiserum. The antiserum was raised in rabbits by injecting ALL cells which had been coated in vitro with rabbit antibodies directed against normal lymphocyte antigens. The resultant anti-serum was absorbed with various normal tissues and tested by immunofluorescence for its ability to discriminate between ALL and other leukaemias, and between ALL and normal cells. Immunoprecipitation of medium (cell secreted) or cell surface components yields a single component of ca. 100,000 dalton upon SDS PAGE. The cell secreted (medium) ALL antigen is not disulphide linked to other cellular components, nor does it possess much disulphide bond stabilized secondary structure, since it still migrates at ca. 100,000 daltons upon SDS PAGE under non-reducing conditions. In isoelectric focusing gel the cell secreted (medium) behaves as a single moiety with an isoelectric point of ca. pH 8. Sequential lectin affinity chromatography followed by immunoprecipitation shows that 100% of the antigen is bound to and specifically eluted from Ricinus communis (RCA_I) lectin; whereas, approximately 50% of the antigen is bound to and specifically eluted from Lens culinaris (LcH) lectin. We have recently fractionated the plasma membrane components of human B, T, and Null (non-T, non-B) cell lines by lectin affinity chromatography and are currently immunoprecipitating the various fractions with antisera to: (1) the acute lymphocytic leukemia associated antigen, (2) Ia antigens, (3) T cell specific antigens, (4) IgG, and (5) α_2-macroglobulin.

470 ANATOMOPATHOLOGICAL, HEMATOLOGICAL STUDY IN THE NEOPLASIA INDUCED BY PV-MSV VIRUS IN DBA_2 ADULT MICE:AN EXPERIMENTAL MODEL FOR HUMAN MYELOPROLIFERATIVE DESORDERS.

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The disease induced in mice by PV-MSV virus(Plasma Variant of Moloney Sarcoma Virus)has been classified by M.A.Chirigos as an undifferentiated sarcoma. Our study shows that,in fact,this neoplasia is defined by 2 main successive phases:1)a preleukemic phase with an hyperplasia of red pulpe in the spleen: the spleen weight increases exponentially and reaches 1g;2)a"tumoral"phase characterized by the appearance of"tumoral nodules"first in spleen and thymus and after in liver,lungs,kidneys and heart.These nodules which are composed by 3 types of cells:granulocytes,large undifferentiated blast cells and a few fusiform cells,destroy the normal tissular parenchyma.At this terminal phase,the amount of reticulins increases largely in the bone marrow,provoking a myelosclerosis.The peripheral blood is invaded with myelocytic and erythroblastic cells,the concentration of uncleated cells reaching 50.000/m3 and mice become anemic(hematocrit:22%).In conclusion,PV-MSV induced leukemia seems to be a good model to study the human myelosclerosis and the relations between the early hyperplasic phase and the late tumoral phase.

471 PRODUCTION OF MESENCHYMAL TUMORS IN NUDE MICE BY PH^1 NEGATIVE "FIBROBLASTS" OBTAINED FROM A PH^1 POSITIVE CML PATIENT, Floyd D. Wilson, Bernard R. Greenberg, William L. Spangler, Moshe Shifrine, and M. Eric Gershwin, Radiobiology Laboratory and School of Medicine, University of California, Davis, CA 95616

Although experiments in rodents have suggested a major functional role of hematopoietic stromal elements in the regulation of hematopoiesis, essentially no knowledge of a similar stromal function in humans is currently available. The importance of understanding hematopoietic-stromal interactions in humans is illustrated by the association of myelofibrosis with myeloproliferative disorders. An experimental model system is presented for the investigation in humans of the role of hematopoietic stromal elements in the regulation of hematopoiesis as well as in the pathogenesis of myelofibrosis in myeloproliferative disorders. The model is based on the simultaneous application of three techniques: (1) growth of bone marrow fibroblastic colonies in vitro, (2) cytogenetic demonstration of leukemia associated marker chromosomes, and (3) the transplantation of isolated stromal elements into nude mice. Using this model, we describe the induction of mesenchymal tumors in nude mice by Ph^1 negative fibroblasts obtained from the bone marrow of a patient with a Ph^1 positive CML. Morphologic, cytogenetic and electron microscopic studies of bone marrow mesenchymal elements in culture and of tumors induced in nude mice indicate the cells composing the tumor are of human origin and are negative for the Ph^1 chromosome. The results provide the first in vivo morphological and cytogenetic support, using human materials, of the hypothesized relationship of progenitors of in vitro fibroblastic colonies to marrow stromal elements.