DIFFERENTIATION AND FUNCTION OF HEMATOPOIETIC CELL SURFACES

Vincent T. Marchesi, Robert Gallo and Philip Majerus, Organizers

February 15–February 20, 1981

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Biology of Leukemia Viruses and Their Interactions and Effects on Hematopoietic Cell Surfaces

270 DIFFERENT HEMATOPOIETIC TARGET CELLS FOR TRANSFORMATION BY REPLICATION-COMPETENT MURINE LEUKEMIA VIRUSES

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The mechanisms by which replication-competent type C RNA viruses cause leukemia are not well understood. In contrast to replication-defective type C viruses, most of which cause sarcomas in vivo and induce transformation of fibroblasts in tissue culture, leukemia viruses replicate in tissue culture cells without causing transformation. Mouse leukemia viruses (MuLV) have generally been thought to induce tumors predominately of the T lymphocyte lineage. We have studied the phenotype of tumors and tumor derived cell lines induced by clonal strains of Rauscher and Moloney MuLV. Moloney MuLV-induced tumors and lymphoma cell lines exhibited Thy.1 antigen in the absence of detectable Fc or C3 receptors, indicating their T cell origin. In contrast, Rauscher MuLV primary tumors and lymphoma cell lines of the same mouse strain invariably exhibited Fc receptors in the absence of Thy.1 antigen. Moreover, Rauscher MuLV lymphoma lines invariably expressed immunoglobulin heavy (μ) chain in the absence of detectable light (κ or λ) chains as determined both by biosynthetic and radioimmunologic techniques. These findings established that the target of neoplastic transformation in response to Rauscher-MuLV is an immature cell within the B lymphoid lineage.

To investigate the basis for the target cell specificity for transformation by Moloney and Rauscher-MuLV, we have compared the binding of purified viral glycoproteins (gp70) to lymphoid cell subpopulations and the expression of viral antigens in lymphoid cells of infected animals utilizing fluorescent activated cell sorting techniques. These studies indicate that virus specificity for transformation cannot be explained on the basis of a limited population of target cells for virus infection. In order to localize the region of the viral genome responsible for transformation of different lymphoid subpopulations, recombinant viruses have been generated between replication competent type C viruses and analyzed for their biologic activity in vivo. The results of these studies and efforts aimed at elucidation of host genetic factors that may influence the target cell specificity for transformation by these viruses will be presented.

271 TRANSFORMATION OF B-LYMPHOID CELLS BY ABELSON MURINE LEUKEMIA VIRUS

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Exposure of bone marrow or fetal liver cells to Abelson murine leukemia virus (A-MuLV) causes the immortalization and proliferation of a small fraction of the cells. Analysis of immunoglobulin-related proteins by immunoprecipitation techniques and of immunoglobulin-related genes by specific hybridization methods have shown that virtually all of the A-MuLV transformants are related to the B-lymphoid pathway of lymphocyte production. In the majority of cell lines induced by A-MuLV, there is immunoglobulin mRNA production and often mu chain production. Light chain production is rare. At the DNA level, there is rearrangement evident at the heavy chain J positions but no rearrangement evident at the light chain loci. Thus the cells appear to be blocked between the time that heavy chain rearrangement occurs and the time that light chain rearrangement occurs. Studies on the behavior of B-lymphocytes in fetal liver have indicated that during normal lymphopoiesis heavy chain expression precedes light chain expression.

The protein that causes transformation by A-MuLV is a cell surface protein encoded partly by sequences in the viral genome derived from the normal mouse genome. The equivalent normal cell mouse gene has been identified and at least a portion of it has been molecularly cloned from the mouse genome. How the Abelson protein causes transformation is unclear but a tyrosine-specific protein kinase activity associated with the protein suggests that the mechanism of A-MuLV cell transformation is similar to that of Rous sarcoma virus, feline sarcoma virus and others. Thus, although the cell type-specificity of transformation might indicate that A-MuLV should have a transformation mechanism closely attuned to the B-lymphoid pathway, the transformation process appears similar to that used by sarcoma viruses and, furthermore, A-MuLV can transform established fibroblastic cell lines. Thus we favor the notion at this time that the transforming activity of the virus is a consequence of an induction of cell growth rather than a specific block in cell differentiation. The transformed cells are, however, blocked in differentiation and we are at present considering the possibility that this block is a natural consequence of the pathway of development of B-lymphocytes and not a consequence of the transforming activity of the virus.

272 INDUCTION OF LYMPHOID LEUKOSIS BY AVIAN LEUKOSIS VIRUS: ACTIVATION OF A CELLULAR "ONC" GENE BY PROMOTER INSERTION.

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Lymphoid leukosis is a B cell lymphoma of birds which is caused by a class of RNA tumor viruses called avian leukosis viruses (ALVs). These viruses differ from the acute transforming viruses (sarcoma viruses and acute leukemia viruses) in several important ways. First, macroscopic tumors appear in infected birds only after a latent period of 4-12 months, compared with 2-3 weeks for the acute viruses. Second, ALV, unlike the acute viruses, does not transform cells in tissue culture at a detectable frequency. Third, ALV does not appear to encode a transforming protein.

To gain information about the mechanism involved in ALV-induced neoplastic disease we have analyzed the virus-related DNA and RNA in more than twenty ALV-induced lymphomas. These studies have led to the following observations: (1) Many, and perhaps most, of the integrated proviruses in lymphomas are defective. No viral 35S and 21S mRNAs were detectable in at least 50% of tumors. Thus expression of the viral genes is apparently not required for maintenance of transformation. (2) Tumors from different birds contained proviruses integrated at a limited number of common sites. (3) New tumor-specific RNAs, consisting of viral information covalently linked to cellular sequences, were found in all but two tumors.

The data suggest that ALV might, as a rare event, integrate adjacent to a potentially oncogenic cellular gene. Transcription, initiating on a viral promoter and continuing into the adjacent cellular gene, could induce elevated expression of this gene, resulting in cell transformation. To test the possibility that this putative cellular "onc" gene might be the cellular counterpart of a known viral transforming gene, we prepared cDNA probes corresponding to the transforming sequences of five different viruses. Analyses of the lymphoma RNAs and DNAs revealed that, in at least 85% of ALV lymphomas, the cellular gene involved was c-mac, the cellular homologue of the transforming gene of MC29 virus.

273 ONCOGENES OF AVIAN LEUKEMIA VIRUSES: THEIR PRODUCTS and THEIR ORIGINS,

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Naturally occuring tumors of chickens have yielded retroviruses that induce acute leukemias as well as other tumors, and that transform hematopoietic cells in tissue culture (1). Prototypes and their target cells include: myelocytomatosis virus (MCV)/fibroblasts, epithelial cells, and myeloid cells; avian erythroblastosis virus (AEV)/fibroblasts and erythroblasts; and avian myeloblastosis virus (AMV)/myeloid cells. These viruses require the assistance of a helper virus to replicate and are therefore known as "defective leukemia viruses" (DLV). Neoplastic transformation of hematopoietic cells by DLV is accompanied by - could conceivably may be mediated by - arrest of cellular differentiation. As a consequence, the transformed cell appears to embody a specific, immature stage in a hematopoietic lineage. Each strain of DLV possesses a unique genetic locus that is presumed to be responsible for oncogenesis. The situation of these loci (or oncogenes) within the viral genomes, and the proposed mechanisms by which they are expressed, will be reviewed (see also refs. 2 and 3). MCV gives rise to a single large protein whose pleiotropic effects apparently account for all forms of tumorigenesis by the virus. By contrast, the oncogene of AEV contains two separately expressed domains that may individually mediate transformation of erythroid cells and fibroblasts. In a third variation, the oncogene of AMV is expressed by a single, sub-genomic mRNA whose product has yet to be identified. The oncogenes of DLV (and of other retroviruses, as well) are apparently derived from unique loci in the genomes of vertebrate animals (4). Each of these "proto-oncogenes" has been highly conserved during the course of evolution, each has structural features that typify cellular genes, and each is expressed in uninfected cells. The proto-oncogenes for DLV are expressed at relatively high levels in characteristic spectra of hematopoietic tissues. These and other findings lead to several related speculations: that proto-oncogenes and their homologous viral oncogenes encode similar, even identical functions; that proto-oncogenes are determinants of normal growth and development; that viral oncogenes transform cells by introducing excessive amounts of otherwise normal proteins; and that the study of oncogenes and their cellular progenitors may provide insight into the control of normal cell division and differentiation.

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274 INTERACTION OF T CELL GROWTH FACTOR AND A NEW RETROVIRUS (HTLV) WITH HUMAN T CELLS,
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Normal human T cells with mature characteristics (e.g., E-rosette +; TdT -; and functional activities) can be grown in liquid culture for long periods by the periodic addition of T-cell growth factor (TCGF) to lectin activated cells (for recent summary see F.W. Ruscetti and R.C. Gallo, Blood (Editorial Review) 57:0000, 1981). It appears that these cells develop receptors for TCGF after their activation by antigen/lectin. Prior to activation TCGF does not interact with normal T-cells. Human TCGF has been recently purified. It is a small (12,500 MW) non-glycosylated protein (J.W. Mier and R.C. Gallo, Proc. Nat. Acad. Sci., U.S.A.77:6134-6138, 1980). When the purified or partially purified TCGF is added to transformed human T-cells from patients with some forms of T-cell leukemias and lymphomas they respond directly to the TCGF. In contrast to normal T-cells they do not require prior in vitro activation (Poiesz et al., Proc. Nat. Acad. Sci., U.S.A., in press). This suggests either: (a) these cells are activated in vivo by chronic antigen exposure; (b) in the process of neoplastic transformation alterations in cell membranes occur which lead to exposure of previously cryptic TCGr receptors; or (c) that an unrecognizable (too few in number) subset of T-cells normally contain TCGF receptors and are the targets for transformation in these disorders.

Some of the cultured growing neoplastic T-cells from patients with cutaneous T-cell lymphoma and leukemia (Sezary syndrome) produce a type-C retrovirus (Poiesz et al., in press). Some of the virus releasing neoplastic cells become independent of exogenous TCGF and constitutively release TCGF. These retroviruses (called HTLV) were analyzed in detail. They form a new and distinct retrovirus group which are not significantly related to known animal retroviruses (Reitz et al., submitted). HTLV is not an endogenous (germ-line transmitted) ubiquitous human virus because sequences of HTLV were not found in the DNA of many normal donors. HTLV is, therefore, either acquired or it is endogenous to specific families. These alternative possibilities have not yet been distinguished. Recent results indicate that some patients with these diseases have specific antibodies to structural proteins of HTLV, namely p19 and p24. Other results suggest that HTLV might be selectively transmitted to "normal" T-cells from family members of some patients with leukemia.

Some of these results suggest some models for the control of T-cell proliferation in man and a possible role of HTLV in abnormal proliferation.

Proteins Involved in the Regulation of Membrane Function I

275 SPECTRIN, Vincent T. Marchesi, Department of Pathology, Yale University School of Medicine, New Haven, Ct. 06510.

Spectrin is the major cytoskeletal protein of the human red blood cell. Confined entirely to the inner surface of the membrane, spectrin is easily extracted in soluble form by exposing erythrocyte ghost membranes to low ionic strength buffers in the presence of EDTA. Low temperature extracts of spectrin are composed of several oligomeric forms, but dimers and tetramers are most prevalent in dilute solutions. Spectrin dimers are composed of two chemically distinct polypeptide chains, designated α and β, with molecular weights of 250,000 and 225,000 daltons. Each spectrin subunit is itself composed of multiple chemical domains which have been defined on the basis of proteolytic sensitivity, refolding capacity, high resolution peptide mapping, and monoclonal antibody reactivity.

Spectrin binds to IOVs by specific associations with the protein ankyrin; the domain bearing this binding site has been localized to a specific region of the β subunit. Spectrin tetramers form by end-to-end association of dimers; this linkage is mediated by non-covalent interactions between an 80,000 dalton peptide on the α subunit and a complimentary segment on the β chain. Spectrin monomers also associate together via multiple non-covalent associations between their contiguous polypeptide chains.

Spectrin is widely believed to confer stability to the lipid bilayer of the red cell membrane; it may also be responsible for regulating red cell deformability and its visco-elastic properties. On the basis of evidence at hand it is possible to attribute these functions to the fact that spectrin is a flexible, multiple-domained, rod-like molecule which has both the capacity to bind with high affinity to protein receptors on the membrane and the capacity to associate with itself via low affinity interactions to form large oligomeric units. These latter forms may be modulated by other membrane proteins (possibly 4.1 and/or actin) to form a dynamic cytoskeleton.

276 REGULATION OF SURFACE RECEPTOR TOPOGRAPHY,

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Understanding membrane modulation requires a detailed molecular description of the connections between cytoskeletal elements and the cell surface. We have studied erythrocyte membranes and coated vesicles as two examples rich in connecting elements.

In erythrocyte membranes spectrin is the major element that links actin to band 3, the major transmembrane protein. Other proteins, including ankyrin (band 2.1) and band 4.1 participate in this linkage. By analyzing binding affinities and by using low angle shadowing, a detailed picture of the meshwork formed by these proteins can be deduced.

In coated vesicles, clathrin is the major polypeptide of the coat but little is known of its associations with itself or with other proteins. We have discovered that clathrin coats dissociate reversibly into triskelions, structures composed of three usually bent somewhat flexible legs radiating from a center. These triskelions contain trimers of clathrin together with approximately three light molecular weight polypeptide chains. Triskelions, whose molecular weight is 630,000, assemble to form the cage around coated pits and coated vesicles.

277 CLATHRIN: A PROTEIN WHICH REGULATES ENDOCYTOSIS

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Nineteen years ago Roth and Porter postulated that coated vesicles mediated specific protein transport. In the intervening years, numerous proteins have been demonstrated to be carried by their ubiquitous structures. However, the route these organelles take is not always the same since the receptor dictates the cellular fate of the protein. For example in oocytes a select set of serum proteins are stored for later use during embryogenesis. In contrast immunoglobulins are transferred directly across the cell in the tissues of the placenta, mammary, yolk sac and small intestine. In other cell types molecules like EGF, insulin, α_2-macroglobulin, asialoglycoprotein, and LDL appear to be targeted to the lysosomal system. Alternatively, other ligands choose none of the above routes but rather become localized in the perinuclear zone, a case in point being NGF. The fate of the receptor also varies. Some receptors are recycled while others are down regulated and degraded. The initial sites for receptor ligand interaction fall into two general classes, those that occur only in coated pit and those that occur dispersed over the surface of the cell with subsequent clustering into coated regions. In either case, the nature of the transmembrane events that result in the receptors being clustered in the coated pits are unknown. Both calmodulin and trans-glutaminase have been suggested as being involved in this process, but little if any substantive experimental evidence supports either contendor. Once the receptor ligand becomes highly concentrated in the coated regions of the membrane, the processes which give rise to the naked vesicles which transport the ligands are unknown. In some cases, receptors for ligands like asialoglycoprotein, LDL and vitellogenin are sorted out very near the cell membrane and return rapidly to the cell surface. What directs the vesicles containing the ligand to its specific fate is to be determined. The probable role of coated regions of the membrane in recycling through some portions of the Golgi-GERL seems likely. Coated vesicles also appear to be involved in the movement of specific ligands between many intracellular organelles. Certainly, the ER-Golgi and possibly the Golgi-PM shuttles utilize the coated vesicles for some aspects of selective movement of protein in the secretory pathway. Thus, the molecular organization, which dictates the architecture and recognition moieties organized in these structures, programs the ligand binding and eventual fate of the ligand in the cell. In doing so, the vesicles become important elements in the choreography of the cell, partners in the drama unfailingly programmed to ensure a developmentally timed and yet ligand specific interaction between disparate organelles.

Proteins Involved in the Regulation of Membrane Function II

278 FIBRONECTINS: INVOLVEMENT IN CELLULAR BEHAVIOR. Richard O. Hynes, Massachusetts Institute of Technology, Cambridge, MA 02139

Fibronectins are large glycoproteins present at cell surfaces, in extracellular matrices, and in plasma. The soluble and insoluble fibronectins differ somewhat but share many structural and functional properties. They affect a variety of aspects of cellular behavior including morphology, cytoskeletal organization, migration, and phagocytosis. These different phenomena all involve cellular adhesion to various extracellular materials and it is likely that fibronectins are involved as ligands between cell surface and other molecules.

Fibronectin forms fibrillar matrices beneath, between and around cells. These fibrils are frequently aligned with actin microfilament bundles. This and other evidence suggest a relationship between extracellular matrix and intracellular cytoskeletal fibrils. Such a relationship may also be involved in the behavioral properties mentioned earlier.

Structural analyses of fibronectin have shown that there exist within the protein multiple binding sites for different molecules including gelatin, collagen, fibrin, and heparin. There is also evidence for interactions of fibronectin with glycolipids, hyaluronic acid, DNA, bacteria, and factor XIII transglutaminase and by disulfide bonding with itself and/or with other cell surface molecules. The arrangement of these binding sites along the elongated fibronectin molecule must be an important factor in the biological roles of fibronectin.

Reviews

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279 PLATELET MEMBRANE GLYCOPROTEINS: ROLE IN AGGREGATION. David R. Phillips, Michael C. Berndt, Lisa K. Jennings and Joan E. B. Fox, Department of Biochemistry, St. Jude Children's Research Hospital, 332 North Lauderdale, Memphis, Tennessee 38101

Platelets possess a number of functional characteristics which are initiated at or mediated by the surface of the platelet plasma membrane. These include receptors for agents which activate or inhibit platelet function, plasma coagulation factors and components responsible for the specific interaction of platelets during aggregation. The present discussion will be concerned with surface membrane components involved in thrombin-induced platelet aggregation - a process initiated by a catalytic event on the platelet membrane surface that culminates with tissue-specific interactions of activated platelets. Two questions will be addressed: (i) the identity of the thrombin receptor; and (ii) the identity of the membrane surface component(s) mediating platelet-platelet interaction.

Several lines of evidence have accumulated implicating that the interaction of thrombin with its receptor involves a proteolytic event. Only one platelet plasma membrane glycoprotein, glycoprotein V (M_r = 82,000), is apparently cleaved by α-thrombin. Coincident with its disappearance, a hydrolytic fragment, glycoprotein V_{f1} (M_r = 69,500), was observed in the platelet supernatant. Glycoprotein V has been purified to >98% homogeneity[1] and verified to be a thrombin substrate. Several lines of evidence will be presented which suggest that glycoprotein V hydrolysis plays an important role in thrombin activation of human platelets.

Platelet aggregation receptors have been identified by two independent procedures: (i) thrombin-activated or thrombin-aggregated platelets were extracted with the nonionic detergent Triton X-100. While the Triton-insoluble cytoskeletons of thrombin-activated platelets were disperse, those of aggregated platelets remained associated in aggregates.[2] Two membrane glycoproteins, IIb (M_r = 142,000) and III (M_r = 114,000) remain associated with the actin filaments of aggregated cytoskeletons suggesting that these glycoproteins bridge the association of the cytoskeletal structures; (ii) platelets from patients with Glanzmann's thrombasthenia (a genetic bleeding disorder characterized by an absence of platelet aggregation) were analyzed for membrane glycoprotein content. The platelets from all patients examined were found to lack glycoproteins IIb and III[3], again implicating these glycoproteins as aggregation receptors. Glycoproteins IIb and III have now been purified to apparent homogeneity and have been found to be subunits of the same protein. The relationship of the glycoprotein IIb/III complex to platelet aggregation will be discussed.

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Role of Cyclic Nucleotides, Calmodulin, and Protein Phosphorylation in Stimulus-Secretion Coupling and Cell Motility

280 REGULATION OF CONTRACTILE PROTEINS BY CALCIUM, CALMODULIN AND cAMP, Robert S. Adelstein, Mary D. Pato, James R. Sellers, Primal de Lanerolle and Mary Anne Conti, NIH, Bethesda, Md. 20205.

The interaction of actin and myosin in vertebrate smooth muscle and non-muscle cells is regulated by the reversible phosphorylation of myosin (1). We have used the purified contractile proteins actin and myosin, the Ca^{2+}-binding protein calmodulin, as well as the purified enzymes, myosin kinase (2) and myosin phosphatase I (3) to study the phosphorylating-dephosphorylating regulatory system of smooth muscle and non-muscle cells.

Myosin kinase was purified from turkey gizzard smooth muscle and human platelets by calmodulin-Sepharose affinity chromatography (2,4). Homogeneous gizzard myosin kinase was used to produce antibodies in rabbits. The antibodies were purified by affinity chromatography using myosin kinase coupled to Sepharose. Immunofluorescence studies of fibroblasts indicate that myosin kinase is localized on stress fibers, probably in association with myosin (5).

Myosin phosphatase I was purified by affinity chromatography on a column of thiophosphorylated light chains bound to Sepharose. Phosphatase I is a trimer composed of three subunits (M_r=60,000; 55,000; 38,000) and dephosphorylates both myosin and myosin kinase (3).

Reversible phosphorylation of myosin: The purified enzymes myosin, myosin kinase and myosin phosphatase I were used to reconstitute a reversible myosin phosphorylating system. The actin-activated MgATPase activity correlated positively with the state of phosphorylation of the 20,000 dalton myosin light chain during phosphorylation, dephosphorylation and rephosphorylation (6). These results with purified smooth muscle proteins complement previous results with non-muscle myosins (1) and suggest that phosphorylation of myosin plays an important role in regulating contractile activity.

The Role of Calmodulin: Contractile activity may be initiated in both smooth muscle and non-muscle cells by a rise in Ca^{2+} from 10^{-7} to 10^{-5}M which results in the binding of Ca^{2+} to calmodulin. Ca^{2+}-calmodulin activates myosin kinase (7) which catalyzes the phosphorylation of myosin, resulting in contraction.

The Role of cAMP: Myosin kinases isolated from both smooth muscle and platelets are substrates for cAMP-dependent protein kinase. In the absence of bound calmodulin myosin kinase is phosphorylated at two different sites, resulting in a marked decrease in the ability of this enzyme to phosphorylate myosin (8). This effect is reversed by dephosphorylating myosin kinase with myosin phosphatase I. A decrease in myosin kinase activity following phosphorylation by cAMP-dependent protein kinase is one mechanism by which cAMP may act to decrease contractile activity in smooth muscle and non-muscle cells.

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281 THE MECHANISMS OF ACTIN FILAMENT ASSEMBLY AND CROSS LINKING, T.D. Pollard, Dept. of Cell Biology & Anatomy, Johns Hopkins Med. Sch., Baltimore, Maryland 21205.

We have used electron microscopy of two dimensional crystals of Acanthamoeba actin and image reconstruction techniques (Aebi, et al., Nature 288:296 (1980)) to determine the size and overall shape of the actin molecule. The dimensions are 5.5 x 4.5 x 3.3 nm and the mass is subdivided into a major and a minor lobe separated by a distinct cleft. We (Pollard and Mooseker, J. Cell Biol. (1981)) have measured the rate constants for actin polymerization. At the fast (barbed) end the association rate constant is 9 s^{-1} μM^{-1} (suggesting that the process is diffusion limited) and the dissociation rate constant is 2 s^{-1}. At the slow (pointed) end the association rate constant is 2 s^{-1} μM^{-1} and the dissociation rate constant is 1.4 s^{-1}. Cytochalasin B inhibits polymerization by reducing both of the barbed end rate constants to $\sim$0. We have purified a cytochalasin-like "capping protein" from Acanthamoeba which also blocks the barbed end of the filaments (Isenberg, et al. Nature (1980) in press). This remarkable protein also inhibits cytochalasin binding to actin (Grumet et al. (1981)), nucleates actin polymerization and inhibits the self-association of actin filaments. Actin filaments are capable of forming rigid isotropic networks held together by actin-actin bonds and by actin filament cross-linking proteins. One such protein from Acanthamoeba is highly asymmetric, has a subunit of 85,000 and cross-links actin in a Mg^{++}- and ATP-dependent, Ca^{++}, cytochalasin and capping protein inhibited fashion (Pollard et al., J. Cell Biol. 87:223a (1980)).

Changes in Membrane Proteins during Erythropoiesis

282 MEMBRANE PROTEIN CHANGES DURING RETICULOCYTE MATURATION, Daniel P. Witt, University of Colorado Health Sciences Center, Denver, CO 80262.

Using a specially developed apparatus to generate shallow discontinuous dextran gradients, circulating rabbit reticulocytes have been separated into fractions on the basis of specific gravity. This has been shown to correlate with age and degree of maturation of the reticulocytes. Analysis of these reticulocyte ghost membranes by SDS electrophoresis has revealed that dramatic qualitative and quantitative changes take place in the membrane proteins and glycoproteins during the relatively brief maturation period of the reticulocyte. For instance, spectrin, a major component of the mature erythrocyte membrane is found to be present only at low levels in the early reticulocytes. Other proteins, including several glycoproteins, are rapidly lost during maturation and are not present in the erythrocyte. Contamination of the preparations by lymphocytes or other cell types cannot account for the changes seen. In general, the changes are consisent with a transition occurring between membrane proteins of the developing erythroblast and membrane proteins of the mature erythrocyte. These findings suggest that these membrane protein changes occur during early reticulocyte maturation rather than in the later phase of erythroblast maturation and raise some interesting questions about protein synthesis and degradation in these enucleate cells.

283 CHANGES IN CELL SURFACE GLYCOPROTEINS DURING DIFFERENTIATION OF HUMAN ERYTHROID CELLS, Minoru Fukuda, Fred Hutchinson Cancer Research Center, Seattle, WA 98104

The cell surface glycoprotein profiles of human cultured erythroblasts and erythroleukemic cell line K562 (as representing proerythroblasts) have been characterized by cell surface labeling followed by endo-β-galactosidase (Fukuda et al, J. Biol. Chem. 254, 5458, 1979) or followed by immunoprecipitation with antibodies specific to Band 3 or glycophorin.

The obtained results were compared with those obtained on mature erythrocytes and the changes of cell surface glycoproteins during differentiation of human adult erythroid cells can be summarized as follows: 1) Band 3 and Band 4.5 are absent on proerythroblasts but increase gradually hereafter. 2) Glycophorin is present as a minor component in proerythroblast stage and increases significantly during further maturation. 3) Unique glycoproteins with molecular weights of 105K, 95K are major components in proerythroblasts but gradually decline and are absent in mature erythrocytes. 4) Carbohydrate chains susceptible to endo-β-galactosidase ("polylactosaminoglycan") are present minimumly in proerythroblasts then increase gradually in parallel to the increase of Band 3 and Band 4.5. 5) The number of branches in polylactosaminoglycan increases significantly during maturation of adult erythroid cells.

These studies show that each stage of erythroid cell differentiation and maturation can be characterized by a unique cell surface glycoprotein profile. The work is now in progress to compare these results with changes in the cell surface glycoprotein profile in other cell lineages such as granulocyte-monocyte.

284 CHANGES IN THE EXPRESSION OF MEMBRANE PROTEINS DURING THE DIFFERENTIATION OF CHICKEN ERYTHROBLASTS, Michael J. Hayman and Keith Savin, Viral Leukaemogenesis Laboratory, Imperial Cancer Research Fund, P.O. Box 123, Lincoln's Inn Fields, London WC2A 3PX.

Chicken erythroblasts can be transformed by the avian retrovirus, avian erythroblastosis virus, AEV. Studies have shown that the mechanism of transformation appears to involve a 'block' in differentiation, in that when erythroblasts are transformed by a temperature sensitive mutant of AEV, ts34, and incubated at the non-permissive temperature the cells start to differentiate and produce haemoglobin. In addition, initial studies had revealed that whereas chicken erythrocytes express a relatively restricted number of cell surface antigens the AEV-transformed erythroblasts express many more cell surface proteins. Therefore, we thought that this temperature-sensitive in vitro differentiation system would provide a system in which we could study changes in the expression of these cell surface proteins during differentiation. The approach taken has been to prepare monoclonal antibodies against the cell surface proteins of the erythroblasts and use these as reagents to monitor the expression of certain membrane proteins during the in vitro differentiation of the cells. Results will be presented that have been obtained using certain of these monoclonal antibodies which are directed against proteins which disappear on differentiation.

285 ROLE OF THE ERYTHROID-SPECIFIC SURFACE LECTIN EDA IN THE DIFFERENTIATION OF MURINE ERYTHROKEUKAEMIA CELLS, C. James Chesterton, F. Lynne Harrison and Susan Godsave, Department of Biochemistry, King's College London, U.K.

During their development in vivo, rabbit erythroid cells bear on their surface a 13,000 Mol. Wt. β-galactoside-specific protein lectin which we have termed erythroid developmental agglutinin (EDA) [Nature, 286, 502-504, 1980]. Our studies with this molecule, including immuno-labelling to determine localization in vivo, in vitro agglutination tests and blocking experiments with anti-EDA Fab fragments, point to a role for this lectin in the strong inter-erythroblast recognition and adhesion seen in bone marrow tissue. Anti-EDA antibody cross reacts with a component on the surface of mouse erythroblasts as detected by indirect immunofluorescent labelling. In addition, both rabbit and mouse erythroblasts are readily agglutinated in vitro by the lectin, the homologous reaction being more specific.

Murine erythroleukaemia cells are, however, not agglutinated by EDA unless induced to differentiate by dimethyl sulphoxide. We have investigated the possibility that EDA is produced as part of the developmental programme triggered by induction and that the behaviour of the virus-transformed cell is a reflection of the lack of normal mechanisms for cellular recognition. Our current data suggest, however, that an EDA-like molecule is synthesized by uninduced cells. The change in agglutinability seems likely therefore to be a reflection of a change in the properties of the surface membrane following induction.

286 CHARACTERIZATION OF THE TRANSFERRIN RECEPTOR PROTEIN IN HUMAN RETICULOCYTE MEMBRANES, Caroline A. Enns, Jeffrey E. Shindelman and Howard H. Sussman, Stanford University, Stanford, CA 94305

The nature of the transferrin binding portion of the reticulocyte transferrin receptor was investigated using goat antibodies directed against human transferrin and against a purified human placental transferrin binding protein. A radioimmunoassay using the latter immune serum was developed. Antibody developed against the placental transferrin receptor crossreacts with a similar transferrin binding reticulocyte antigen. A 5% reticulocyte ghost preparation gives a value of 9.5 µg/mg protein. Normal red blood cell ghost membrane preparations show binding (0.57 µg/mg protein) proportional to the amount of reticulocytes normally present in blood (0.5-2.0%).

The transferrin binding protein of the reticulocytes is similar to that of the placenta. SDS polyacrylamide gel electrophoresis of immunoprecipitates of anti-human transferrin, transferrin and ^{125}I-iodinated reticulocyte enriched membranes reveal a 94,000 dalton polypeptide identical to that of the placenta. Proteolytic digests of both of these polypeptides with S. aureus protease show identical proteolytic patterns, indicating similar sequences.

Although normal erythrocytes do not bind transferrin, immunoprecipitation of these ^{125}I-iodinated ghosts with the anti-transferrin receptor antibody does bring down a band of 41,000 M_r. This band is not found in as high concentrations in reticulocyte enriched preparations and could represent a degradation product of the receptor.

287 EXPRESSION OF I SUBREGION ANTIGENS ON MURINE HEMATOPOIETIC STEM CELLS (HSC), John H. Fitchen and Soldano Ferrone, Wadsworth VA Hospital, Los Angeles, CA 90073 and Scripps Clinic and Research Foundation, LaJolla, CA

We used antibodies reactive with I-A or I-E antigens to study the representation of I region antigens on murine HSC. Monoclonal anti-I-A^k antibodies were produced by the hybridoma technique and anti-I-E antibodies were prepared by absorption/elution from murine lymphocytes of a rabbit anti-human Ia-like heteroantiserum. The specificity of both antibodies was demonstrated by immunochemical techniques. C3H mouse bone marrow cells were incubated with the respective antibodies and complement (C'), washed, and assayed for CFU-S, CFU-C and CFU-E. The results were as follows (mean ± S.E. of three experiments):

Treatment	CFU-S/10^5 cells	CFU-C/2 x 10^5 cells	CFU-E/2 x 10^5 cells
Control	22 ± 1.6	130 ± 14	338 ± 48
Anti-I-E + C'	3 ± 0.9	24 ± 6	287 ± 33
Anti-I-A + C'	23 ± 3.1	113 ± 14	ND

The inhibitory effect of anti-I-E antibodies on CFU-S was removed by absorption with Daudi or KG cells (Ia-positive) but not with Molt-4 cells (Ia-negative). These results suggest 1) that CFU-S and CFU-C express I-E antigens but CFU-E do not and 2) that I-A antigens are not represented on CFU-S and CFU-C.

288 THE 2-IMINOBIOTIN-AVIDIN INTERACTION AS A TOOL FOR THE SELECTIVE RETRIEVAL OF CELL MEMBRANE PROTEINS, R. Zeheb and G.A. Orr, Albert Einstein College of Medicine, Bronx, NY 10461

An analytical and preparative approach which provides information concerning the organization and function of cell surface components without prior isolation of plasma membranes has been developed. The basis of the technique is the covalent attachment of compounds containing 2-iminobiotin, the cyclic guanidino analog of biotin, onto cell surface proteins. The 'tagged' species are then isolated by virtue of the unique interaction between the covalently attached ligand and its binding protein, avidin. The pH dependent interaction of 2-iminobiotin with avidin makes recovery possible. At high pH, the free base form of 2-iminobiotin retains high affinity specific binding to avidin characteristic of biotin, whereas at acidic pH values, the salt form of the analog interacts poorly with avidin. The practicality of both the preparative (affinity chromatography using immobilized avidin) and the analytical (immunoprecipitation with anti-avidin antibody) selective retrieval systems has been established utilizing 2-iminobiotinylated fetuin. These studies showed that for efficient binding of 'tagged' proteins, the pH had to be greater than 8.5 (anal.) or 9.5 (prep.). Quantitative release could be achieved in either system by lowering the pH to 4.0 or by the addition of 1 mM biotin. We have demonstrated the potential of this technique for the study of cell surface proteins by 'tagging' and selectively retrieving the major sialoglycoproteins; PAS 1, PAS 2, and PAS 3 from intact human erythrocytes, uncontaminated by other membrane or cytoplasmic proteins.

Regulation of Growth and Differentiation

289 SPECIFIC HOMING OF A SUBSET OF BM CELLS TO THE THYMUS - F. Lepault and I.L. Weissman, Department of Pathology, Stanford University, Stanford, CA 94305

Pre-thymic cells represent a small subpopulation of bone marrow (BM) cells capable of undergoing thymic maturation to T-lymphocytes. The characterization of these cells has been, so far, made either in vitro, or in long term thymus homing experiments. We have developed an in vivo assay for the study of the homing and early differentiation of bone marrow cells in the thymus of irradiated animals. BM cells were labeled in vitro with a fluorochrome and injected IV into lethally irradiated animals. As soon as 3 hours after the reconstitution, a small number of the donor cells ($\leq$.1% of the input cells) were detectable in the recipient thymuses. The migration of BM cells was compared to that of cells from other lymphoid organs (thymus, MLN, spleen). The highest frequency of thymus homing cells was found in the BM, and the lowest in MLN and thymus, (for instance, 30 times as many BM cells as LN cells migrated to the thymus). In contrast, thymus, MLN and spleen cells homed as well as or better than BM cells to peripheral lymphoid organs. These data suggest that the thymus-homing is not random but specific. The thymus-homing BM cells were not susceptible to prior in vitro treatment with anti-Thy 1 serum plus C. Using congenic donor cells we studied the surface antigen phenotype of the immigrants 3 to 24 hours after reconstitution. The percentage of migrants expressing Thy 1 increased over the assay interval (9% at 3 hours, 20% at 24 hours). $H2K^k$, Ia^k, and Tla antigens are also expressed on a significant percentage of the cells. The Lyt antigens are currently under study.

290 PROSTAGLANDINS ACTIVATION OF ERYTHROPOIETIN (Ep) PRODUCTION AND ERYTHROID PROGENITOR CELLS, James W. Fisher, Heinz W. Radtke, William Jubiz, Peter Kim Nelson and Allen Burdowski, Tulane Univ., New Orleans, La. and Univ. of Utah, Salt Lake City, Utah

The E-type prostaglandins have been demonstrated to stimulate erythropoiesis in several experimental animal models. The mechanism by which prostaglandins activate erythropoiesis is two-fold: 1) increased kidney production of Ep; and 2) direct activation of the erythroid progenitor cell compartment in the bone marrow. We postulate from our model that prostaglandins of the E-type are involved in enhanced kidney production of Ep in response to beta-2 adrenergic activation and ischemia (reduction in renal blood flow); while prostacyclin (PGI_2) is involved in increased kidney production of Ep due to a decrease in blood oxygen tension. E-type prostaglandins also activate the erythroid progenitor cell compartment (CFU-E and BFU-E). Our discussion will center primarily around the kinetics of CFU-E and BFU-E activation by several E-type prostaglandins and the mechanism of prostanoid activation of kidney Ep production. The criteria which have been met to prove that prostanoids of the E-type activate erythropoiesis are as follows: 1) several E prostaglandins (PGE_2, 15-methyl prostaglandin E_2, 16,16-dimethyl E_2, 6-keto-E_1 and PGE_1) produce a significant increase in radioiron incorporation in red cells of exhypoxic polycythemic mice; 2) PGE_2 and arachidonate increase production of Ep in the isolated perfused kidney and arachidonate was blocked by the cyclooxygenase inhibitor drugs indomethacin and meclofenamate; 3) PGE_2 and arachidonate produced a significant increase in erythroid colonies (CFU-E) in vitro in normal mouse bone marrow; and 4) injections of 15-methyl E_2 daily for 6 weeks in normal and hypoxic mice produced a significant elevation in the total circulating red cell mass.

291 SPECIFIC BINDING OF IgE TO A HUMAN MACROPHAGE CELL LINE, U937. Clark L. Anderson and Hans L. Spiegelberg, University of Rochester, Rochester, NY, and The Research Institute of Scripps Clinic, La Jolla, CA.

Recent studies indicate that macrophages and monocytes have plasma membrane receptors for IgE and are able to lyse Helminths by means of an IgE-mediated mechanism. To study in more detail the interaction of IgE with macrophages, we evaluated the binding of radiolabeled human IgE myeloma proteins to cultured cells of a human macrophage cell line, U937. Binding was specific for IgE in that it was inhibited by 3 different human IgE myeloma proteins and by Fc fragments prepared from IgE, but not by IgE Fab fragments nor by myeloma proteins representative of the 4 IgG subclasses nor by a rat IgE myeloma protein. Dissociation and association rates of IgE with the plasma membrane were very rapid with an estimated $t_{1/2}$ of less than 1 min. By Scatchard plots the Ka was high, 4.75×10^8 L/mole. Between 53,000 and 93,000 sites per cell were calculated. The in situ U937 receptor for IgE was trypsin-sensitive, whereas the receptor for IgG on the same cell was trypsin-resistant. These findings corroborate previous observations that IgE mediates lysis of Helminths by macrophages and that human and rat macrophages bear Fc receptors for IgE. Furthermore, these studies suggest that the U937 cell line may be a useful model for further analysis of macrophage Fc receptor structure and function.

292 TRANSFERRIN RECEPTORS AND CELLULAR DIFFERENTIATION. Chang-Jing G. Yeh and W.P. Faulk. Blond McIndoe Centre for Transplantation Biology, Queen Victoria Hospital, East Grinstead, Sussex RH19 3DZ, England.

It is generally agreed that transferrin serves a central role in transporting iron to erythropoietic cells for use in hemoglobulin synthesis; however, recent experiments demonstrating transferrin receptors on human syncytiotrophoblast (Proc. Roy. Soc. (B) 204, 83, 1979), transformed cells (Cell. Immunol. 49, 215, 1980) and fresh malignant tissues (Lancet ii, 390, 1980) has provided a basis for implicating transferrin in functions in addition to iron transport. Indeed, the receptor may be a marker of transformation. In light of the suggestion that the regulatory mechanisms that control differentiation and malignancy are closely associated (Proc. Natl. Acad. Sci. USA, 74, 253, 1977), we have examined the ability of the human promyelocytic leukemia cell line (HL-60) to bind transferrin before and after exposure to dimethyl sulfoxide (DMSO). Using immunohistological methodology, we have shown transferrin receptors on HL-60 cells. DMSO induces HL-60 cells to differentiate to mature granulocytes displaying many normal functional characteristics. Consonant with this poorly understood process of differentiation, we have found that the HL-60 cells lose their transferrin receptors. Taken in conjunction with our previous report that normal fibroblasts develop transferrin receptors when they lose contact inhibition, the concept of transferrin receptors as a marker of transformation receives support. This prompts us to suggest that the regulatory mechanisms controlling the synthesis and expression of transferrin receptors may be associated with malignancy. Further study of transferrin receptors may produce results which shed new light on these regulatory mechanisms in fetal, normal and abnormal cells.

293 PROLIFERATION AND DIFFERENTIATION OF HSC IN LONG-TERM CULTURES OF ADULT SPLEEN, Eugene A. Arnold, Irena Katsnelson, Chandra Choudhury, and Jack Levin, Division of Biophysics, School of Hygiene & Public Health, and the Departments of Pathology & Medicine, The Johns Hopkins University School of Medicine, Baltimore, MD 21205

We have found that in liquid cultures (RPMI1640+20% horse serum) of spleen cells of adult Syrian hamsters or adult mice, hemopoietic inductive microenvironment formation is adequate to induce proliferation of spleen stem cells for periods of more than 4 months in hamster systems and up to 2 months in mouse systems, and to permit granulocytic, monocytic and megakaryocytic differentiation without secondary repopulation or addition of exogenous growth factors, other than those in the medium. Intimate topographical relations are established between spleen stromal cells and hemopoietic cell components of the culture in adherent "cell producing" islets. Some of these islets are associated with multiple hemopoietic cell types such as myeloid, monocytic and megakaryocytic cells. Other islets are associated with a single cell type such as megakaryocytes which suggest a limited potential of some adherent stromal cells to direct the differentiation of precursor cells. Cultures of this type provide a simple and convenient model for investigation of the mechanisms controlling differentiation of hemopoietic stem cells not only for granulocytic and monocytic differentiation but for megakaryocytic differentiation.

294 EFFECTS OF 4NQO and PDD on CFU-C FROM SYRIAN HAMSTER BONE MARROW IN LONG TERM CULTURE EXPERIMENT. Wen-Shing Liaw, Eugene Arnold and Paul O.P. Ts'o, Div. of Biophysics, Sch. of Hyg. and Pub. Hlth and Dept. of Pathology, Sch. of Medicine, Johns Hopkins University, Baltimore, Md. 21205.
In an effort to develop an in vitro neoplastic transformation system for hematopoietic tissue by chemical carcinogens, we wish to report the establishment of an in vitro long term (4 mos.) culture system of bone marrow cells obtained from Syrian hamsters, and the effect of 4 nitroquinoline oxide (4NQO) and phorbol-12,13-didecanoate (PDD) on the growth and differentiation. Untreated cultures were grown in RPMI 1640 plus 20% horse serum; the composition of supernatant cells changed from mostly myeloid cells to mostly macrophages and finally stabilized at about 25% myeloid cells at about 80 days; CFU-C content assayed with hamster spleen conditioned medium (SCM) fell and rose again to 2.6%, finally to 6.0% in 120 days. Freshly prepared bone marrow cells were exposed to 4NQO (10^{-5}M) for 2 hr. After an initial decrease during the first 5 days post treatment, the CFU-C content in the supernatant became 2-2.5-fold higher than the untreated counterpart at 37th day and 78th day, and fluctuated similarly in both cultures. The effects of PDD (0.1μg/ml) on CFU-C at 37 days to 120 days were examined. Without SCM, colonies can still be formed in the presence of PDD and the stimulating effects of SCM and PDD are nearly additive for both control and 4NQO treated cultures , though PDD tended to stimulate to produce monocytic colonies in CFU-C assay. In addition, the culture treated with 4NQO contained 2-3-fold more myeloid cells (up to 60%) than the control between 34-48 days. In summary, 4NQO treatment increased CFU-C and myeloid cell content significantly during certain periods. PDD showed an effect similar to that of SCM, with a promotion of monocytic colonies.

295 CHARACTERIZATION OF CULTURED HEMATOPOIETIC CELL LINES, Melinda Patterson and J.R. Kettman, The University of Texas Health Science Center, Dallas, Texas 75235

Long term culture of mouse spleen cells generates a population of factor dependent cell lines similar to those described by Dexter et al. (JEM 152:1036-1047). These cells, presently identified as arrested granulocyte precursors, appear to correspond morphologically and cytochemically with "cultured mast cells" described by Ginsburg and Sachs (JNCI 31:1-39) and others.

Murine hematopoietic cell lines are generated when low numbers of spleen cells are seeded on irradiated macrophage or Kirsten sarcoma virus transformed fibroblast feeder layers. Both initiation and continued viability of the cell lines depend on the presence of a factor found in WEHI-3 or Con A supernatant. Leishman-Giemsa staining of a typical cell line at 6 weeks after culture initiation demonstrates a population comprised primarily of mononulcear cells, many of which contain numerous vacuoles and/or cytoplasmic granules. The cells contain approximately 1 ug histamine/10^6 cells. FACS II analysis of the cell lines stained with monoclonal reagents show the cells to be H-2K+, IA-, and Thy 1.2-. Treatment of spleen cells with anti Thy 1.2+ C' prior to culture does not prevent the development of hematopoietic cell lines. Limiting dilution analysis of mouse spleen cells cultured with this system indicates that 1/9000 ± 3000 cells are stimulated to clonal proliferation. Preliminary studies indicate that 25% or more of subcultured clones will develop the characteristics of the hematopoietic cell lines described above.

296 LITHIUM EFFECTS ON HEMOPOIESIS. Peter J. Quesenberry and Michael Coppola, University of Virginia School of Medicine, Charlottesville, Virginia 22908
We have previously reported that lithium chloride (1-4 meq/L) augments in-vitro Dexter culture granulocyte, megakarocyte, CFU-C and CFU-S production during the early phases of culture and inhibits these cell types during more prolonged culture intervals. (N Eng J Med 302: 713, 1980). The effects of lithium were further studied in serially transplanted "stem cell limited" mice and in-vitro Dexter cultures. C57Bl/6J mice were injected IP with lithium chloride or acetate (60mg/kg) over schedules ranging from 6-15 days, at various points in serial marrow transplantation schedules (1-3 transplants, 42-114 days), and Hct, WBC, tibial cellularity, CFU-S, CFU-C, BFU-E values determined. In no instances was inhibition of hemopoiesis observed while lithium acetate markedly stimulated erythropoiesis and granulopoiesis. Dexter cultures were exposed to lithium chloride (1 meq/L) with demi-depopulation weekly for 3 weeks, supernatant cells removed, stromas extensively washed and subjected to 650R X-irradiation to sterilize residual stem cells. Normal supernatant Dexter cells (3 weeks or more) which were incapable of forming stroma were added to the lithium pre-exposed or control stroma and resulting in-vitro hemopoiesis assessed. Total cell recovery from flasks with lithium pretreated stroma 1-4 weeks after irradiation ranged from 131-773% of control. CFU-S recovery at weeks 1-2 was 170-301% of control and CFU-C were elevated at weeks 2-5. Stromal adherent cells were increased 144-156% of control by lithium treatment over 1-3 weeks before irradiation. A late decrease in hemopoiesis on the lithium stroma was seen in 3 of 4 experiments. These data indicate that lithium may exert stimulatory and inhibitory effects on hemopoiesis via an effect on adherent marrow stromal cells.

297 GENERATION OF ERYTHROPOIESIS IN LONG-TERM BONE MARROW SUSPENSION CULTURES, Christine E. Eastment and Francis W. Ruscetti, National Cancer Institute, Bethesda, MD 20205
Long-term cultures of hamster bone marrow (BM) differ from other systems studied in that there is no continuous requirement for an adherent monolayer to maintain proliferation of stem cells (Eastment et al., J. Supramol. Str. Suppl. 4:212, 1980). In recent experiments involving 2-3 month old supernatant cultures, we were able to generate erythropoiesis in suspension cultures in the presence of prostaglandin E_1 (PGE_1) and erythropoietin (epo). Continuous production of erythroid cells was maintained in these cultures for more than 4 weeks after the initiation of erythropoiesis. Cultures containing only RPMI-1640 and 20% horse serum consist of 80% myeloid cells, 15% macrophages, 5% blasts, and no benzidine-positive cells. In similar cultures containing PGE_1 at 10^{-5}M, there is a two-fold increase in cell number after 7 days with over 95% myeloid cells, few blasts, and no benzidine-positive cells. Epo at 1 U/ml stimulated the production of less than 1% benzidine-positive cells. Addition of both epo and PGE_1 results in the development of cell clumps of erythroid precursors within 48 hrs., and benzidine-positive cells in 4 days. By day 7, 60% of the cells are benzidine-positive. Clumps of up to 200 cells, some containing hemoglobin visible under phase contrast, can be seen in these cultures. Wright's stained preparations contain late stage normoblasts. Addition of PGE_1 and epo to hamster stem cells growing in the presence of a marrow adherent cell layer produces no such changes, suggesting that the adherent layer interferes in some way with erythropoiesis. In this system, erythropoiesis in vitro requires neither an adherent layer, fetal calf serum, nor a semi-solid matrix. This system provides a useful means to evaluate the effects of various factors that regulate the balance between erythropoiesis and myelopoiesis.

298 REQUIREMENTS FOR ERYTHROID INDUCTION OF K-562 HUMAN ERYTHROLEUKEMIA CELLS, Peter T. Rowley, Betsy M. Ohlsson-Wilhelm, Barbara A. Farley, Barbara Kosciolek, Sandra LaBella, and David Hicks, University of Rochester, Rochester, NY 14642
K-562 is a human leukemia cell line with two unusual properties: (1) certain agents induce erythroid maturation including hemoglobin synthesis (Andersson et al, 1979) and (2) the hemoglobin synthesized is embryonic and fetal despite the origin of the cells from an adult (Rutherford and Weatherall, 1979). We have investigated requirements for erythroid induction with regard to effective inducing agents, serum requirement, and alteration of cellular response by mutation. (1) Inducers. Of 39 agents tested for induction of benzidine positivity, 19 were effective including actinomycin D, bleomycin, butyric acid, cycloheximide, cytosine arabinoside, 5-fluorouracil, hemin, mithramycin, mitomycin C, and ouabain. DMSO and hexamethylene bisacetamide, effective inducers of Friend murine erythroleukemia cells, were ineffective. (2) Serum. Fetal bovine serum (FBS)(10% routinely used) cannot be replaced by an equivalent concentration of newborn bovine serum (NBS). Without inducer, the percentage of benzidine positive cells was higher in FBS (5%) than in NBS (1%). With 6 of 14 inducers, this percentage was much higher in FBS than in NBS. This FBS factor is heat labile and less than 6000 daltons by gel filtration. (3) Effect of mutation. We have isolated 37 clones differing phenotypically from the parental line. These presumptive mutants differ from the parent in 2 ways. First, 14 of the 37 are spontaneously induced, i.e. are benzidine positive in the absence of inducer. Second, 18 of 23 tested to date are not inducible by 1 or more agents which induce the parent. Analysis of globin synthetic pattern by PAGE in Triton X-acid-urea and fluorography reveals further differences between mutant and parental lines.

299 THE APPLICATION OF LECTIN PROBES IN THE STUDY OF DIFFERENTIATION AND PROLIFERATION OF CELLS DERIVED FROM HUMAN BONE MARROW (BM). A. Filipovich, C. Lannon, K. Smith, J. Kersey, and N.K.C. Ramsay, University of Minnesota, Minneapolis, MN 55455.
We have used the galactosyl-binding lectins peanut agglutinin (PNA) and soybean agglutinin (SBA) as probes for immature precursors in human BM and in the study of the regulation of CFU-c growth in vitro. PNA and SBA receptors are present on 6-35% and 18-45% of normal BM mononuclear cells and the majority of macrophages and neutrophils. 46 ± 20% of PNA^+ BM cells ingest latex. Utilizing double fluorochromes, small populations of normal BM cell bearing BA-1 and BA-2, monoclonal antibodies directed against common ALL, are PNA^+ (1-2%, ~½%, respectively).
PNA and SBA added to 14 day soft agar BM cultures increase CFU-c in a dose-dependent manner.[1] The "additional" lectin-stimulated colonies consist of monocyte/macrophages or mixed cells. Lectin-mediated increases in CFU-c are not seen in adherent BM fractions, although these are generally enriched for PNA^+ and SBA^+ cells. On the other hand, macrophage-depleted, nonadherent BM fractions still demonstrate lectin-mediated increases in CFU-c. An example of one such experiment is shown:

	CFU-c ± S.D. / 2 x 10^5 cells		
	Unseparated BM	Nonadherent BM	Adherent BM
No lectin	120 ± 3.0	163 ± 3.6	164 ± 1.5
+0.08 mg PNA	223 ± 2.4	259 ± 1.4	159 ± 1.7
+0.12 mb SBA	194 ± 3.2	232 ± 2.6	159 ± 1.0

[1]Filipovich, et al., Exp. Hematol., August 1980.

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300 THE ROLE OF THE PLASMA MEMBRANE IN CELLULASE ACTIVATION IN HIGHER PLANTS,
Lowell N. Lewis, University of California, Riverside, CA 92521

During the process of abscission or fruit ripening, ethylene causes the rapid synthesis of the enzyme cellulase. The enzyme is transported across the plasma membrane and released into the cell wall area where it hydrolyzes the cell wall material to allow abscission or, in the case of fruit ripening, to soften the walls of the fruit. In the abscission system, the isoenzyme of cellulase associated with the abscission process is nonexistent prior to the ethylene stimulation. It has been shown by the use of antibodies to the abscission cellulase that a form of this enzyme is embedded in the plasma membrane. This form can be detected only after solubilization of the membrane with Tritons X-100. The membrane bound cellulase has a larger molecular weight than the abscission cellulase which may suggest that an insertion protein is functioning in the transport of this enzyme across the plasma membrane. We are presently making monoclonal antibodies to the abscission cellulase to assist in following its transport from the cytoplasm through the plasma membrane into the cell wall area, and to better understand the relationship between the membrane bound cellulase and the form ultimately found in the cell wall area. Although the differentiation process being studied in my laboratory is found only in the differentiation of plants, the mechanism by which a specific enzyme is transported across the plasma membrane is of interest to all cell biologists.

301 SECRETION OF CSF BY THE ADHERENT LAYER OF LONG TERM BONE MARROW CULTURE IN MICE. Bruno R. Varet, Serge Fichelson, Jean-Michel Heard, U152 CHU Cochin, 75674 Paris, France.

The long term bone marrow culture (LTBMC) technique allows the continous differentiation of granulomonocytic precursors into mature cells. However CSF was never detected in LTBMC and the addition of CSF was recently shown not to modify the behavior of LTBMC. This rised the questions of whether CSF was of physiological significance and wheter the granulomonocytic differentiation in LTBMC reflected an abnormal and possibly preleukemic behavior. This last point was of majour interest since LTBMC is used as a tool for studying interactions between myeloid progenitors and oncogenic viruses. In an attempt to answer this question we applied a double agar culture technique for growing granulocytic colonies from fresh bone marrow over the adherent layer of LTBMC. During the first three weeks of LTBMC a high number of granulomonocytic colonies was observed in the upper layer of agar reflecting the secretion of high concentration of CSF. The mean number of colonies (- 150 / 7 x 10^4 fresh bone marrow cells plated) was as high as the higher number obtained after addition of growing concentrations of CSF. After recharging LTBMC with a fresh bone marrow moculum, which induced a sharp increase in the number of cell produced by LTBMC, the number of granulocytic colonies in the upper layer of agar dropped. A highly significant inverse relationship was observed between the number of cells produced in LTBMC and the number of granulomonocytic colonies formed over the adherent layer. This result might be explained either by the consumption of CSF by differentiating cells, either by the production by mature cells of an inhibitor (or inhibitors) for CSF. In both hypothesis this study demonstrates that the adherent layer of LTBMC produces CSF. Therefore the complete differentiation of granulocytic progenitors observed in LTBMC probably reflects the normal response to a physiological stimulus.

302 BIOLOGICAL OSCILLATIONS IN HUMAN RED BLOOD CELLS AND IN BLOOD CELL GHOSTS, Leah Peleg, Aviva Dotan and Israel E. Ashkenazi, Dept. Human Biology, Sackler School of Medicine, Tel Aviv University, Tel Aviv ISRAEL.

In in vivo studies, circadian rhythms in the activity of some enzymes were established. When the same red blood cells were incubated in suspensions without entraining signals, the rhythms were of shorter time periods.
In this study an attempt was made to evaluate the relevance of biological oscillations to circadian rhythms in mammals. Activities of the enzymes glucose-6-phosphate dehydrogenase (G6PD), glyceraldehyde-3-phosphate dehydrogenase (GAPD), and the binding of Ca^{++} were studied in erythrocyte suspensions and in suspensions of red cell membranes (ghosts). Measurements were made every two hours over a period of 36 to 48 hours. All parameters exhibited defined patterns of oscillations which differed significantly from random fluctuations. The period of the oscillations ranged from eight to twelve hours. For both enzymes, it was found that part of the enzyme was in an inactive, membrane bound form. Our results suggest that the membrane is involved in generating rhythmic oscillations in enzyme activity and Ca^{++} binding. This probably does not involve the cytoskeleton, since membranes from which significant amounts of spectrin and actin had been removed still showed the oscillations.

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303 ORIGINS OF THE PROTECTIVE EFFECTS OF NON-CYCLING ON NORMAL HUMAN STEM CELLS, John E. Byfield, Paula Calabro-Jones and Sallie Frankel, Univ. Calif. San Diego School of Medicine, San Diego, Calif. 92103

A fundamental source of the success of cancer chemotherapy (CT) in human malignancy lies in the partial resistance of resting bone marrow stem cells to the useful anti-cancer drugs. This is customarily ascribed to the increased sensitivity of replicating DNA to many agents. We have studied this in vitro using resting normal human peripheral lymphocytes (HLc). HLc are probably derived ontogenetically from bone marrow stem cell precursors and can be selectively recruited by appropriate stimulation (e.g. antigen or lectin). In this fashion they resemble marrow stem cells. Previous studies (Byfield et al., Proc. Amer. Assoc. Ca. Res. 20:136, 1979) showed that the classical alkylating agents (AA, e.g. melphalan, HN_2) are relatively excluded from resting HLc but are actively taken up by cycling (PHA-stim) HLc, leading to significant differences in the survival curves (soft agar colonies). Newer lipid soluble agents show no cycle-dependence of toxicity. All agents studied have fallen into one of two classes; those agents which show delayed marrow recovery in man are those whose toxicity is cycling-independent. PHA-stimulated HLc are very resistant to phosphoramide mustard independent of cycling suggesting that they lack a surface receptor for the agent. The toxicity of melphalan can be mitigated by coincident amino acid exposure confirming the transport dependence of its uptake applies also to normal cells. The studies suggest that relative exclusion of drug from resting normal cells is the basis for the better therapeutic ratio of transported agents and that more active uptake by the more rapidly dividing human tumors provides part of the basis of their greater responsiveness to chemotherapy.

304 POLYAMINES AND HUMAN PROMYELOCYTIC LEUKEMIC CELL DIFFERENTIATION* C.E.Weeks, T.J.Slaga and E.Huberman. Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN 37830.

Polyamine levels were evaluated in human HL-60 promyelocytic leukemia cells after treatment with inducers of terminal differentiation. Differentiation in these cells was determined by increases in the percent of morphologically mature cells and in lysozyme activity. Treatment of the HL-60 cells with phorbol-12-myristate-13-acetate (PMA), phorbol-12,13-didecanoate or other inducers of terminal differentiation, such as dimethylsulfoxide and retinoic acid, resulted in dose-dependent increased levels of putrescine. However, no increase in putrescine could be detected after PMA treatment of a HL-60 cell variant, which exhibited a reduced susceptibility to PMA-induced terminal differentiation. Similarly, no increase in putrescine was observed with two non-tumor promoters, phorbol-12,13-diacetate and 4-O-methyl phorbol-12-myristate-13-acetate or with anthralin, a non-phorbol type tumor promoter. In addition to enhancing putrescine levels, PMA also increased the amount of spermidine and decreased the amount of spermine. The increase in putrescine and spermidine levels preceded the expression of all the various differentiation markers. Furthermore, marked enhancement of polyamine levels occurs in these cells in which no new DNA is synthesized after PMA treatment. Unlike the changes observed in the polyamine levels after PMA treatment, the activities of ornithine (ODC) and S-adenosylmethionine (SAMD)-decarboxylases, which are polyamine biosynthetic enzymes, did not significantly change. Specific inhibitors of ODC and SAMD did not affect differentiation in the control or PMA treated cells. The differentiation specific change in polyamine levels may involve pathways other than the known biosynthetic ones. * Research sponsored by the Office of Health & Environ. Res., U.S. Dept. of Energy, under contract W-7405-eng-26 with UCC.

305 APPEARANCE OF BLEBS ON THE SURFACE OF DIFFERENTIATING FRIEND ERYTHROLEUKEMIA CELLS, Jay C. Brown and Kenneth L. Klotz, University of Virginia School of Medicine, Charlottesville, Virginia 22908.

The scanning electron microscope has been employed to examine the surfaces of Friend murine erythroleukemia MEL) cells induced to differentiate in culture with DMSO. Whereas immature, undifferentiated cells appear to be relatively smooth with a uniform density of small microvilli, fully differentiated cells are covered with large blebs or lumps having diameters in the range of 0.5-2.0 um. Greater than 85% of induced MEL cells have "bleby" surfaces after four days of growth in the presence of DMSO when significant hemoglobin synthesis has taken place. In contrast, only 10% of uninduced MEL cells exhibit surface blebs and hemoglobin synthesis is less than 10% of that observed in induced cells. A control, non-erythroleukemia cell line did not express cell surface blebs in the presence or absence of DMSO. Analysis of three variant MEL cell clones that fail to synthesize hemoglobin in response to DMSO showed that two clones express surface blebs while one does not. We conclude that the appearance of large cell surface lumps or blebs is a part of the normal program of erythroid differentiation expressed in induced MEL cells, but that appearance of surface blebs is not functionally or causally linked to hemoglobin synthesis. This work was supported by a grant from the American Cancer Society.

306 HUMAN ERYTHROID DIFFERENTIATION USING A MODEL SYSTEM, S.H.Cedar and M.A.Horton, St.Bartholomew's Hospital, London, EC1A 7BE, U.K.

Our work is involved in the study of differentiation of the erythroid lineage using a human erythroleukemia cell line, K562, which has been in suspension culture for eight years.Although isolated from a patient with chronic myelogenous leukemia in blast crisis, the presence of glycophorin, the major sialoglycoprotein of and exclusive to erythrocytes has been shown to be on its surface, demonstrating its erythroid nature.Investigations using glycophorin as a target antigen have employed heteroantisera, absorbed by En (a-) cells, as well as by monoclonal antiglycophorin antibodies.K562 is also inducible with Na-butyrate and with haemin, becoming positive for haemoglobin (which has been shown to be of a foetal nature) in the peroxidase-benzidine test.Inducing agents for the Friend virus transformed cell lines (MELC) such as DMSO or HMBA are not found to induce K562.

Future work with this cell line will centre on the changing pattern of surface antigens during induction, using our own monoclonals, with particular reference to glycophorin and spectrin, both major components of the red cell cytoskeleton.Other work will involve attempts to isolate the target cell of this form of leukemia, the potential stem cell.This will enable us to study its in vitro characteristics regarding its ability to proliferate and differentiate and the conditions required for these functions.Work will also include comparisons to normal bone marrow cells currently being cultured.

307 AVAILABILITY OF A MEGAKARYOCYTOPOIETIC CELL LINE FROM THE RAT, H. G. Hempling, Medical University of South Carolina, Charleston, South Carolina 29401

We would like to bring to the attention of participants a cell line of proliferating promegakaryoblasts which has been maintained in culture in our laboratory for almost four years. This line was isolated and cloned by Dr. A.D. Cicoria from the bone marrow of the Long-Evans rat. When cultured in 10% calf serum, it maintains a proliferative stage with a 30 hour division time. When allowed to reach numbers in excess of 1×10^6/ml or when cultured in fetal calf serum, a sizeable proportion undergo maturation to megakaryoblasts and to megakaryocytes, characterized by chromosome counts as high as 16N and cell volumes up to 6000 μ^3. Greater than 50% of the mixed population stains positively for acetylcholinesterase. In our hands, this population of cells has been used to investigate the effects of cellular maturation on membrane function and osmotic properties. (Cicoria and Hempling, J. Cell. Physiology, 1980) After receiving the cell line, Maciag, Weinstein, Steinberg and their associates at Beth Israel Hospital, Boston have confirmed our morphological findings and have also demonstrated Factor VIII by immunofluorescence. They have also identified a component in the culture medium which stimulates the proliferation of smooth muscle cells in culture. We welcome the opportunity to provide this cell line to investigators interested in megakaryocytopoiesis and in platelet-derived growth factors.

308 ADENOVIRUS 5 MUTANTS TEMPERATURE SENSITIVE IN FUNCTIONS REQUIRED FOR TRANSFORMATION, R.B. Gaynor and A.J. Berk, Molecular Biology Institute, UCLA, Los Angeles, CA 90024.

The objective of my research is twofold: first, to analyze the mechanisms by which an adenovirus positive regulatory element activates the expression of viral genes, and, second, to see how these regulatory elements influence the initiation and maintenance of cellular transformation. Methods for introducing mutations into early region I, the transforming region of Adenovirus 5, have been developed. One temperature-sensitive mutant has now been isolated, and we plan to isolate others. These temperature-sensitive mutants will be used in pulse labeling experiments after shifting from the permissive to the nonpermissive temperature. We hope to determine from these experiments whether early region functions are continuously required for expression of other viral genes. The mutants will also be used to transform rodent fibroblasts at the permissive temperature. We will determine if the mutant function is required for maintenance of the transformed state by shifting to the nonpermissive temperature and analyzing cellular properties. Those cellular properties that regress will be considered under continuous control of the temperature-sensitive viral product. This work is being performed with Dr. Arnold Berk.

309 PR-C II AVIAN SARCOMA VIRUS: Characterization of virion RNA and viral specific intracellular mRNA. Becky Adkins, Tony Hunter, and Karen Beemon, TVL, Salk Institute, P.O. Box 85800 , San Diego, Calif. 92138

PRC II is an avian sarcoma virus which, like Rous sarcoma virus (RSV), causes sarcomas in vivo and induces cellular transformation in culture. PRC II also resembles RSV in that cells transformed by PRC II contain elevated levels of phosphotyrosine in cellular protein. However, the PRC II transforming sequences appear to be unrelated to the RSV src gene. PRC II infected chick embryo fibroblasts (CEF) contain an immunoprecipitable 105K dalton protein which has ^{35}S methionine-tryptic peptides from a portion of the gag gene in addition to a number of peptides not shared with any of the known leukosis viral gene products and which are presumably derived from cellular sequences. These non-viral sequences are immunologically and structurally related to sequences in the putative transforming proteins of Fujinami sarcoma virus and two strains of feline sarcoma virus. We are interested in characterizing the PRC II transformation specific sequences in virion RNA and in viral specific intracellular mRNA. Two-dimensional fingerprint analysis has been performed on RNase T1 digested ^{32}P-labelled PRC II 70S RNA as well as on the 3.0 x 10^6 d helper and 1.5 x 10^6 d transforming genomes individually. Whole cell pA+ RNA and polysomal pA+ RNA from PRC II transformed CEF have been translated in vitro in parallel with PRC II 70S RNA. The major viral specific products have been characterized by immunoprecipitation and tryptic peptide mapping. In addition, the intracellular viral specific mRNAs have been characterized by Northern blot hybridization analysis.

310 SITE-SPECIFIC MUTAGENESIS IN THE POLYOMA MEDIUM T ANTIGEN, Dennis Templeton and Walter Eckhart, The Salk Institute, P.O. Box 1809, San Diego, CA, 92112.

The early region of polyoma virus encodes three immunoprecipitable tumor antigens having apparent molecular weights of 22K, 50-60K, and 90K daltons. These proteins are referred to as the small, medium, and large T antigens, respectively. The three T antigens share common N-terminal sequences. The large T antigen and the medium T antigen are translated in different reading frames over a common sequence of approximately 700 nucleotides. We have introduced mutations into a cloned polyoma genome in the region common to the large and medium T antigens. Several viable deletions have been isolated and characterized. We have also constructed a mutant containing a termination codon in the medium T reading frame which may help to clarify the role of medium T antigen in cell transformation. The characteristics of these mutants will be presented.

Receptors for Platelet Activation

311 ROLE OF GLYCOPROTEIN Ib IN FACTOR VIII/VON WILLEBRAND FACTOR (FVIII/vWF)-DEPENDENT PLATELET AGGLUTINATION, Michael C. Berndt and David R. Phillips, Department of Biochemistry, St. Jude Children's Research Hospital, Memphis, TN 38101

The initial event in hemostasis in response to vascular injury involves the FVIII/vWF-dependent adhesion of platelets to exposed subendothelium. While several lines of evidence implicate the involvement of the human platelet plasma membrane glycoprotein, GP Ib, in the adhesion reaction, previous attempts to examine the effect of hydrolytic removal of this glycoprotein on FVIII/vWF-mediated platelet agglutination and adhesion have been equivocal due to the lack of specificity of the proteases employed. In the present study, we treated washed human platelets with exogenous human platelet calcium-dependent protease to selectively hydrolyze only GP Ib from the platelet membrane surface. Protease-treated platelets possessed essentially normal aggregation responses toward collagen and the calcium ionophore, A23187, but were not agglutinated by either bovine FVIII/vWF or human FVIII/vWF-ristocetin. Protease-treated platelets with a residual maximal agglutination rate toward bovine FVIII/vWF of 26-28% have only 40% of the FVIII/vWF binding sites and ~40% of the level of GP Ib (43% of GP Ib_α, 37% of GP Ib_β) present on control platelets. The remaining FVIII/vWF binding sites, however, had the same binding affinity (K_d = 0.46 nM) as those on control platelets (K_d = 0.41 nM). With protease-treated platelets, an apparent direct linear relationship existed between the maximal agglutination rate to bovine FVIII/vWF and the residual level of intact GP Ib. The combined results provide strong support for the critical role of GP Ib in FVIII/vWF binding to and agglutination of human platelets and provide further evidence that GP Ib is the probable FVIII/vWF receptor on the platelet membrane surface.

312 DEVELOPMENT OF PHOTOAFFINITY ANALOGUES TO CHARACTERIZE THE PLATELET α-ADRENERGIC RECEPTOR, R.I. Handin, W.C. Horne, J.J. Mooney, and R.W. Alexander, Depts. of Medicine and Psychiatry, Harvard Medical School, Boston, MA

Alpha-adrenergic catecholamines, acting via membrane receptors, induce platelet aggregation and secretion. Para-aminoclonidine (PAC), a partial α-adrenergic agonist, has been used to develop a radioligand binding assay for the platelet α-adrenergic receptor (AAR) as well as to synthesize two photoactive analogues which covalently label platelet membrane protein(s). PAC binds to AAR in a specific, saturable, reversible manner with a K_d of 5 nM and inhibits both basal and prostaglandin-stimulated adenylate cyclase activity. Initially, 4-fluoro-3-nitrophenyl azide was directly coupled to PAC to produce a photoaffinity analogue. Subsequently, para-azido-clonidine was synthesized from PAC by sequential incubation with $NaNO_2$ and NaN_3. Both PAC derivatives competed with [^{3}H]-PAC for binding to platelet membranes and were covalently linked to membrane proteins after photoactivation. Over 70% of the platelet-associated radioactivity was solubilized by Triton X-100 extraction. When chromatographed on Sepharose 6B, the labeled material eluted as a single peak on the trailing edge of the major platelet protein peak. These studies suggest that photoaffinity analogues of PAC can be used to selectively label platelet AAR. This should greatly aid in the isolation and characterization of these receptors.

Abnormal Growth and Differentiation

313 PROLIFERATION AND DIFFERENTIATION OF HEMOPOIETIC PROGENITORS FROM MICE INFECTED WITH THE ANEMIC OR POLYCYTHEMIC STRAIN OF FRIEND LEUKEMIA VIRUS, G.B. Rossi and C. Peschle, Istituto Superiore di Sanita, Rome, and Medical Pathology Institute-ICHO, II Medical School, Naples, Italy

Pronounced perturbation of the kinetics of burst-forming and colony-forming units (BFU-E, CFU-E) and, to a lesser extent, of myelomacrophage colony-forming units (CFU-C) has been demonstrated in marrow and spleen of DBA/2 mice at sequential time intervals after in vivo infection with the anemic (FLV-A) or the polycythemic (FLV-P) strain of Friend leukemia virus. The cycling of BFU-E was markedly enhanced, whereas that of CFU-C was only marginally increased (Peschle et al, P.N.A.S. 77:2054, 1980). Expression of CFU-E from FLV-P-treated mice is unaffected in plates with fetal bovine serum but without added erythropoietin (EPO); it is also substantial (>50%) under serum-free culture conditions. Expression of CFU-E from FLV-A-treated mice is, however, fully dependent upon the presence of EPO. Growth of BFU-E from FLV-P-treated mice, while always dependent on EPO addition, reaches plateau values at lower concentrations of spleen-conditioned medium (containing burst-enhancing factors--BEF) than those required for maximal growth of BFU-E from uninfected animals. These data indicate that infection with FLV-P--which consists of the leukemogenic, replication-competent (F-MuLV), as well as the spleen focus-forming (SFFV), viral components of FLV--apparently results in a substantial loss of CFU-E EPO dependence and in a higher BFU-E sensitivity to BEF or closely related factors.

314 MOLECULAR CLONING OF AVIAN MYELOCYTOMATOSIS VIRUS (MC29) TRANSFORMING SEQUENCES, James A. Lautenberger, Robert A. Schulz, Claude F. Garon*, Philip N. Tsichlis, and Takis S. Papas, National Cancer Institute and *National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20205

Avian myelocytomatosis virus (MC29), a defective acute leukemia virus, has a broad oncogenic spectrum in vivo and transforms fibroblasts and hematopoietic target cells in vitro. We have used recombinant DNA technology to isolate and characterize the sequences which are essential in the transformation process. Integrated MC29 proviral DNA was isolated from a library of recombinant phage containing DNA from the MC29-transformed nonproducer quail cell line Q5. The cloned DNA was analyzed by Southern blotting of restriction endonuclease digests and by electron microscopic visualization of R-loops formed between the cloned DNA and MC29 or helper virus. It was found that the 9.2 kb cloned DNA insert contains approximately 4 kb of viral sequences and 5.2 kb of quail cellular sequences. The viral sequences contain all of the MC29-specific sequences and 5' helper related sequences as well as part of the envelope region. The size of the cloned EcoRI fragment is the same as that of the major band in EcoRI-cleaved Q5 DNA that hybridizes to viral sequences. Transfection of the cloned DNA into NIH 3T3 cells revealed that the MC29-specific sequences are functional in that they induce foci of transformed cells with high efficiency.

315 INFECTION OF ERYTHROID PROGENITOR CELLS WITH FRIEND MURINE LEUKEMIA VIRUSES, Candace S. Johnson and Philip Furmanski, Michigan Cancer Foundation, Detroit, MI 48201

Mouse erythroleukemias induced by Friend virus are characterized by proliferation of hematopoietic stem cells in the splenic red pulp, severe immunosuppression, massive splenomegaly and death. There are two prototype strains of conventional Friend virus, FV-P and FV-A. FV-A is associated with mild anemia, while FV-P causes a hypervolemic polycythemia. Another strain of Friend virus (RFV) was isolated in our laboratory which induces an erythroleukemia that spontaneously regresses in 30-70% of the infected animals. The FV-P, FV-A and RFV strains of virus all induce pathologically identical diseases. However only FV-P infection gives rise to CFU-E's that have lost their dependency on Epo for growth. When spleen CFU-E's from FV-A and FV-P leukemic animals were tested for productive virus infection by susceptibility to cytotoxicity by monospecific anti-virus-gp70 antiserum, both were found to be infected with virus, even though FV-A spleen cells remained Epo-dependent. However, CFU-E colony reduction by antiserum treatment was observed in only 50% of RFV-leukemic animals. The data suggest that RFV leukemic animals with uninfected CFU-E's are those that will experience leukemia regression (regressors), whereas the leukemia in mice with infected CFU-E's does not regress (progressors). Therefore, our studies show that there are several mechanisms by which animals develop or maintain the leukemic state and that the interaction of virus with hematopoietic stem cells is related to regression of leukemia. (Supported by grants CA14100, CA22453 and CA06419 and an institutional grant from the United Foundation of Detroit.)

316 PREFERENTIAL EXPRESSION OF ENDOGENOUS TYPE-C VIRAL ANTIGEN IN RHESUS PLACENTA DURING ONTOGENESIS, Kurt Stromberg, Rachel I. Huot, Laboratory of Viral Carcinogenesis, National Cancer Institute, FCRC, Frederick, MD 21701

A radioimmunoassay for the major internal structural protein (p26) from Macaca arctoides type-C virus (MAC-1) was used to score antigen expression in rhesus placenta and other fetal organs obtained at various times of gestation. Antigen expression was detected in 16 out of 16 placental specimens but not in 10 other different fetal organs from each of eight selected animals. The levels of antigen detected in placenta ranged between 2 and 218 ng/mg protein with a correlation between lower antigen expression with term gestation or parity greater than 10. A 10-fold higher level of antigen expression was detected at the external surface of placenta near decidua than in the remainder of the placenta towards the amniotic surface. Thus, even within the placenta, there was a preferred site for endogenous retroviral antigen expression. Lastly, substantial inter-individual variation exists in regard to the amount of placental p26 expression in preterm animals of low (1) or intermediate (2-6) parity.

The uniformily enhanced expression of major retroviral protein in placenta, with higher levels near maternal decidua, might imply a physiologic involvement for endogenous retroviruses in primate embryogenesis. Alternatively, following cross-species infection during species evolution, endogenous virogenes may simply be favored for expression in placenta because of the rapid proliferation of placental tissue.

317 MURINE LEUKEMIA-ASSOCIATED INHIBITORY ACTIVITY (LIA): EVIDENCE FOR TWO MOLECULAR SPECIES AND IN VIVO ACTIVITY, John Marcelletti and Philip Furmanski, Department of Biology, Michigan Cancer Foundation, Detroit, Michigan 48201.

Erythroleukemia induced in mice by the RFV strain of Friend virus (FV) is histopathologically indistinguishable from that induced by other strains of FV, but spontaneously regresses in 50% of the leukemic mice. As observed in patients with leukemia and patients with leukemia in complete clinical remission, bone marrow cells from all mice with RFV-induced erythroleukemia, and 50% of mice in which erythroleukemia has regressed, elaborate a soluble mediator that inhibits in vitro colony formation by normal granulocytic stem cells (CFU-C's). Like human LIA, murine LIA is specific for CFU-C's in S-phase and does not affect CFU-C's from leukemic mice or most mice with regressed leukemia. However, unlike human LIA, LIA derived from RFV-leukemic animals also inhibits colony formation by erythroid progenitor cells (CFU-E's). Normal CFU-E colony formation is reduced 40-60% by exposure of bone marrow cells to murine LIA, while CFU-E's from leukemic mice are unaffected. Identical preparations generated with bone marrow cells from regressed animals can be classified into four groups: those with no detectable LIA activity; those that inhibit only CFU-C colony formation; those that inhibit only CFU-E colony formation; and those that inhibit both CFU-C and CFU-E colony formation. Normal animals do not produce detectable quantities of either activity. CFU-E colony formation by bone marrow cells from RFV-leukemic mice is less than 5% of that of normal age-matched controls. We conclude that mice with RFV-induced leukemia produce at least two species of LIA, and that these molecules may function in vivo to suppress normal hematopoiesis. (Supported by NIH grant CA-14100 and an institutional grant from the United Foundation of Detroit).

318 POLYCYTHEMIA- AND ANEMIA-INDUCING STRAINS OF THE SPLEEN FOCUS-FORMING VIRUS DIFFER IN PROCESSING OF THEIR GLYCOPROTEIN PRODUCTS, Sandra Ruscetti and Edward Scolnick, National Cancer Institute, Bethesda, MD 20205

The Friend strain of spleen focus-forming virus (SFFV) induces a rapid erythroproliferative disease when injected as a pseudotype into susceptible mice. While some variants induce polycythemia (FVP), others are associated with a slight anemia (FVA). Both induce erythroid burst formation in vitro without added erythropoietin. However, FVA-induced bursts differ from FVP-induced bursts in that the former are poorly hemoglobinized while the latter contain copious amounts of hemoglobin. Both FVP and FVA strains code for similar primary translational products: $p45^{gag}$ and $gp52^{env}$. Studies with subgenomic fragments of molecularly cloned FVP-SFFV have shown that the expression of $gp52^{env}$, but not $gp45^{gag}$, correlates with biological activity. In this study, we compare the processing of envelope proteins encoded by FVP and FVA strains of SFFV.

Cells nonproductively infected with different strains of FVP synthesize a larger glycosylated form of the primary envelope gene product: $Gp52^{env}$ is processed to $gp65^{env}$ by the addition of complex carbohydrates, and the latter protein can be detected on the cell surface. Cells nonproductively infected with an FVA strain of SFFV were similarly analyzed for processed envelope gene products. In contrast to cells infected with FVP strains of SFFV, FVA nonproducer cells cells contained no detectable $gp65^{env}$ but did contain a low amount of a new protein, $gp60^{env}$. No env-related protein could be detected on the surface of FVA nonproducer cells. The same differences were seen when spleens of FVP- or FVA-infected mice were analyzed.

Possible mechanisms by which processing of viral glycoproteins could contribute to the differences in the biological effects of FVP and FVA strains of SFFV will be discussed.

319 CYTOMEGALOVIRUS-INDUCED ALTERATIONS TO MONOCYTE SURFACES. Helen M. Garnett, University of the Witwatersrand, Johannesburg, South Africa 2000.

Human peripheral blood monocytes from healthy adult donors, challenged with human cytomegalovirus (CMV) in vitro have altered cell surfaces. Monocytes obtained by hypaque-ficoll separation were allowed to adhere to glass or plastic coverslips for 90 min before washing to remove non-adherent cells and subsequent exposure to CMV at a virus:cell ratio of 2:1, an infectivity dose which does not statistically decrease the viability of the monocytes after 48h in culture. Mock infected and latex-challenged cells were treated similarly. After a 90 min adsorption period all cultures were washed and the cells cultured in M199 containing 30% heat-inactivated autologous serum. Rosetting techniques were used to assess the presence of Fc and complement receptors on the surfaces of these cells 4h, 8h, 12h and 24h after exposure to virus. At 24h there was a 65% reduction in the expression of Fc receptors and a 63% reduction in the expression of complement receptors in the virus-challenged cells as compared to latex-challenged controls. Scanning electron microscopy revealed noticeable differences in the topography of the virus-challenged cells as early as 4h after virus addition. At 24h, when the control cultures were spreading on the substrate, the virus treated cells showed little tendency to spread and this effect was even more noticeable at 48h by which time the control cells had flattened and demonstrated extensive webbed filapodia. The virus-challenged cells remained spherical. It is possible that the virus interferes with the cytoskeletal system of the cell, hence altering the surface topography and receptor distribution. This is currently being investigated.

320 SPECIFIC TRANSFORMATION OF MACROPHAGE LINEAGE CELLS BY AVIAN MYELOBLASTOSIS VIRUS AND AVIAN MYELOCYTOMATOSIS VIRUS MC 29. David Boettiger and Elisa M. Durban, University of Pennsylvania, Philadelphia, PA 19104

Functionally differentiated macrophages and embryonic yolk sac cells containing hemopoietic blast-cells were exposed to AMV and MC 29. Both viruses induced a morphological transformation of mature macrophages and transformed a sub-population of the hemopoietic blast cell pool which begin differentiation along the macrophage lineage. Analysis of the macrophage differentiation markers, including cell membrane ATP ase, and phosphatase, neutral lipid accumulation, phagocytosis, Fc receptors and substrate adhesion reveal that these functions are affected differentially by AMV and MC 29. Furthermore, infection at the blast cell stage or following functional differentiation to mature macrophages produces the same transformed cell phenotype.

These results imply that transformation by these viruses is not the result of a block or inhibition of normal macrophage differentiation but suggest instead that the transformation genes carried by AMV and MC 29 specify new, distinct cell phenotypes. The range of markers expressed in the transformed cells allows them to be easily distinguished from any of the normal stages of macrophage differentiation.

321 REPLICATION-DEFECTIVE GIBBON RETROVIRUS, Thomas G. Kawakami and Lily Sun, Comparative Oncology Laboratory, University of California, Davis, CA 95616

Although the gibbon retrovirus (GaLV) infection *in vitro* and *in vivo* has been found to result in productive infection, GaLV can persist as a replication-defective infection in a human lymphoid cell culture. This study was undertaken to determine the biochemical nature of the genome of a replication-defective GaLV. A human lymphoid suspension cell culture, UCD-895, with replication-defective GaLV has been established by culturing normal human lymphocytes with a strain of virus, GaLV-1L, isolated from lymphocytic leukemia. This cell culture shows no evidence of virus replication, but expresses internal viral antigen, and has 70-80% of the GaLV-1L viral sequences detectable by ^{3}H-cDNA to cellular DNA and ^{125}I-viral RNA to cellular DNA hybridization analysis. Since UCD-895 is non-permissive to the homologous virus or virus strains of lymphocytic leukemia origin, we attempted to rescue the replication-defective genome with virus from different disease origin. Super-infection of UCD-895 with a strain of virus, GaLV-4M, of myelogenous leukemia origin resulted in low levels of virus replication. After propagation in a susceptible human lymphoid cell culture, NC-37, the genome of the rescued virus was examined for sequence relationship to GaLV-1L and GaLV-4M. Based on ^{3}H-cDNA-DNA and ^{125}I-viral RNA-DNA hybridization analysis, the rescued virus contains genomic sequences related to genomes of both GaLV-1L and GaLV-4M. Similar results were obtained when the defective GaLV was rescued by a type-C virus endogenous to cats. The conclusion that can be drawn from this study is that replication-defective GaLV infection in human lymphoid cell culture can be rescued by replication-competent GaLV helper viruses of different origins or by type-C virus from heterospecies.

322 A FELINE LEUKAEMIA VIRUS WHICH PREVENTS DIFFERENTIATION OF ERYTHROID CELLS, Oswald Jarrett, David E. Onions and Masami Mochizuki, University of Glasgow, Glasgow, Scotland.

Feline leukaemia viruses (FeLV) occur in 3 subgroups A, B and C. FeLV-A and B, which are commonly isolated in nature, induce lymphoid and myeloid leukaemias while FeLV-C, which is more rarely isolated from cats, interacts specifically with erythroid cells to produce a unique pure red cell aplasia.

Following infection of newborn kittens with each of 4 separate isolates of FeLV-C a severe non-regenerative anaemia occurs after 5 weeks. The virus appears to interact with cells early in the pathway of erythroid differentiation since there is a rapid fall in the number of BFU-E in the bone marrow following virus infection. Myeloid or lymphoid cells are not noticably affected.

An activity which interacts with receptors on the surface of feline erythrocytes is found in the envelope of FeLV-C viruses and is expressed on the surface of FeLV-C-infected cells.

Experiments to define the determinants of FeLV-C specificity for erythroid cells will be described.

323 EFFECT OF FRIEND SPLEEN FOCUS-FORMING VIRUS (SFFV) ON GENERATION OF WEHI-3 GROWTH FACTOR DEPENDENT HEMATOPOIETIC PROGENITOR CELL LINES FROM CONTINUOUS MOUSE BONE MARROW CULTURE. Joel S. Greenberger, Mary Ann Sakakeeny, Thomas Novak, and Nancy Hoffman, Joint Center for Radiation Therapy, Harvard Medical School, Boston, MA. 02115.

Nonadherent cells removed from hydrocortisone-supplemented marrow cultures of C57BL/6J, C57BL/KsJ, DBA/2J, B6D2F1/J, NZW/J, C3H/HeJ, C57Br/J, or CD-1 Swiss; but not NIH Swiss, NZB, HRS/J, or 129/J established permanent lines of hematopoietic progenitor cells in a factor(s) found in WEHI-3 cell dialyzed conditioned medium (DCM). The effect of added virus on generation of factor-dependent (FD) cell lines and their in vivo leukemogenicity was tested. Cultures from C57BL/6J, C3H/HeJ, CD-1 Swiss, and NIH Swiss were uninfected or infected with: a cloned SFFV (from SFFV-BALB-1902Bc14) rescued with a clonal Rauscher (M52R) helper virus, or helper virus alone. Nonadherent cells harvested at days 7, 27, 56, and 140 were grown in WEHI-3 (DCM). Permanent lines cloned in 0.3% agar were induced in 80-100% of all cultures following SFFV(R) infection; 10-20% of RM52R infected or uninfected CD-1, C3H/HeJ, of C57BL/6J and <1% of NIH Swiss cultures. 8 of 23 FD-lines from SFFV(R)-infected cultures released both SFFV and R-MuLV and 10^{7} cells produced granulocytic leukemia in vivo. FD-lines releasing M52R or no virus were not leukemogenic. Thus, SFFV increases the efficiency of generation of FD-hematopoietic progenitor cell lines and correlates with leukemogenicity.

324 INTERACTION OF MAMMALIAN RETROVIRUSES WITH HUMAN MONONUCLEAR CELLS, M. Popovic[1,2], A. Ridgeway[1], I. Royston[3] and R. C. Gallo[1], [1]Laboratory of Tumor Cell Biology, National Cancer Institute, NIH, Bethesda, MD 20205
Mouse monoclonal antibodies (L_{22}) to "human activated antigen(s)" (HAA), which is expressed on the cell membrane of human T and B lymphocytes growing in vitro or phytohemagglutinin (PHA) activated mononuclear cells but not on resting lymphocytes, have been developed by I. Royston (in preparation). These L_{22} monoclonal antibodies were used in an indirect immunofluorescence (IF) technique for the study of HAA induction by various mammalian retroviruses. Mononuclear cells, separated from peripheral blood or bone marrow of healthy donors, were infected in 10 fold dilutions with mouse leukemia viruses (AKR and Rauscher-MuLV), feline sarcoma virus (FeSV), baboon endogenous virus (BEV), Mason-Pfizer monkey virus (MPMV), gibbon ape leukemia virus (GALV) and simian sarcoma-leukemia viruses (SiSV/SSAV); the induction of HAA in these cells were followed by IF. Major differences were found in the induction capacity of HAA by different retroviruses. The same number of viral particles of both GALV and SiSV/SSAV exhibited approximately 50 to 500-fold higher capacity to induce HAA then MPMV, baboon, feline and mouse retroviruses, respectively. IF positive cells for HAA ranged from 25% to 60%. The viral specificity of HAA induction by SiSV/SSAV and 3 different strains of GALV is indicated by: (1) lack of HAA induction by heat inactivated or antibody treated viruses, (2) dose dependence, and (3) host-cell independence of induction. Finally, preliminary experiments indicate that the new type-C retrovirus, isolated from cutaneous T-cell lymphoma and leukemia (HTLV) can induce HAA in human mononuclear cells. [2]M.P. is a recipient of UICC Fellowship. [3]I.R. is with Dept. of Hematology, Univ. of California, San Diego, Calif.

325 GENERATION OF ERYTHROLEUKEMIA CELL LINES FROM MICE INFECTED WITH FRIEND MURINE LEUKEMIA VIRUS (F-MuLV) Allen Oliff, Sandra Ruscetti, Edward Scolnick. NCI, Bethesda, MD 20205
Erythroleukemia cell lines can be isolated from the leukemic tissues of mice infected with Friend Virus complex (SFFV plus F-MuLV). Both SFFV and F-MuLV can produce rapidly fatal erythroproliferative diseases, but it is unclear if either virus alone can be used to generate these cell lines. We have focused on F-MuLV which induces a massive proliferation of early erythroid precusors. However these cells are not transplantable into syngeneic hosts. Thus it is unclear if the disease caused by F-MuLV is truly neoplastic or simply represents a hyperplastic proliferation of erythroid precusors in response to the F-MuLV infection and the severe anemia associated with this disease. We removed the anemic stimulus from F-MuLV infected mice by transfusions of packed RBCs, and thus extended the animals' lives. Erythroid precusors continued to proliferate despite the transfusion therapy. After six weeks of transfusions the blast cells from 3 of 4 mice were now transplantable into syngeneic mice. Unlike the leukemic tissue of mice infected with Friend Virus complex which grow as solid tumors upon sc transplantation, our F-MuLV induced cell lines only grow in the hematopoietic organs of the recipient mice following iv, ip, or sc inoculation. The donor origin of the transplants was confirmed by cytogenetic identification of "Y" chromosomes in the neoplastic tissue of female mice that received transplants from male donors. The neoplastic cells are uniformly positive for spectrin antigen using an immunoflouresence assay. Both F-MuLV and Friend MCF virus can be detected in these cells but no evidence of SFFV can be found. We conclude that F-MuLV induced disease is characterized by an early hyperplastic stage where erythroid precusor cells are not transplantable and by a late neoplastic stage which is seen only if the lethal effects of the anemia are suppressed.

326 CHARACTERIZATION OF GROWTH AND DIFFERENTIATION OF ABELSON MURINE LEUKEMIA VIRUS-INFECTED B LYMPHOCYTES IN LONG TERM BONE MARROW CULTURE, Cheryl A. Whitlock and Owen N. Witte, University of California, Los Angeles, CA 90024. Growth and differentiation of murine B lymphocytes is being studied using the RNA tumor virus, Abelson murine leukemia virus (A-MulV). The culture system employed for propagation of normal bone marrow elements is a modification of that described previously by Dexter and Lajtha (1976). We find that this system, in the absence of infection with A-MulV, results in the establishment of a heterogeneous population of adherent cells from which arise small numbers of nonadherent cells that synthesize immunoglobulin light and heavy chains and respond to the mitogenic effects of K235 lipopolysaccharide. Immunoglobulin synthesis of uninfected cultures can be demonstrated for up to two months, suggesting that these culture conditions provide the necessary environment for long term growth and differentiation of B lymphocytes and/or their precursors.

Infection of the bone marrow cells with A-MulV prior to establishment in culture results in the rapid expansion of a nonadherent population of blast cells which synthesize viral proteins, μ-heavy chains of IgM, and terminal deoxynucleotidyl transferase (TDT) in roughly equivalent amounts. Of interest is that growth and viability of a major portion of these nonadherent cells are dependent upon factors produced by the adherent population. Experiments are in progress to determine the heterogeneity of the mass population of nonadherent cells produced by A-MulV infection and to examine the nature of the growth dependency of this population on the presence of the adherent cells. In addition, the culture conditions described here are being employed to examine the capacity of A-MulV-infected bone marrow cells to express mature B cell characteristics.

Differentiation and Function and Hematopoietic Cell Surfaces

327 CELL-SURFACE GLYCOPROTEIN SYNTHESIS DURING DIFFERENTIATION OF CHICKEN ERYTHROBLASTS TRANSFORMED BY TEMPERATURE-SENSITIVE AVIAN ERYTHROBLASTOSIS VIRUS. Keith W. Savin. Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, U.K.

It was recently demonstrated that chicken erythroblasts transformed by a temperature-sensitive mutant of avian erythroblastosis virus (ts34 AEV) have a greatly increased haemoglobin content if they are allowed to grow for 3 to 5 days at the non-permissive temperature of the virus (41°C), instead of the usual 35°C. Cell-surface molecular changes during this differentiation were investigated here by examining the glycoproteins synthesized by a ts34-transformed erythroblast cell-line. These cells were shown to synthesize a geratly increased amount of a 94,000 MW erythrocyte-surface glycoprotein after a shift in growth temperature from 35°C to 41°C, consistent with the proposal that such a temperature shift releases these transformed cells from a differentiation block. A time-course analysis indicates that the synthesis of both the cell-surface glycoprotein and haemoglobin begins between 2h and 6h after shift from 35°C to 41°C. This AEV mutant provides a new system for studying differentiation-associated cell-surface molecular changes. Some aspects of the control of synthesis of this glycoprotein analysed using a monoclonal antibody, will also be discussed.

328 GENETIC STRUCTURE OF AVIAN MEYLOBLASTOSIS AND E26 VIRUS, TWO ACUTE LEUKEMIA VIRUSES THAT DO NOT TRANSFORM FIBROBLASTS. Peter H. Duesberg*, Klaus Bister*, and Carlo Moscovici+. *Department of Molecular Biology, University of California, Berkeley, CA 94720, and +Virus Lab, Veterans Administration Hospital, Gainesville, FL 32601. Chicken myeloblasts transformed by avian myeloblastosis virus (AMV) in the absence of nondefective helper virus (termed nonproducer cells) were found to release a defective virus particle (DVP) that contains avian tumor viral gag proteins but lacks envelope glycoprotein and a DNA polymerase. Intracellular Pr76 and Pr180, but no nonstructural, virion gene-related proteins were detected. The RNA of DVP measures 7.5 kilobases (kb). Comparisons based on RNA-cDNA hybridization and mapping of RNase T_1-resistant oligonucleotides indicated that DVP RNA shares with helper viral RNAs nearly isogenic 5' terminal gag and pol-related sequences of 5.3 kb and a 3'-terminal c-region of 0.7 kb that is different from that found in other avian tumor viruses. Adjacent to the c-region, DVP RNA contains a contiguous specific sequence of 1.5 kb which is unrelated to the specific sequences of fibroblast-transforming avian acute leukemia and sarcoma viruses of four different RNA subgroups. The specific sequence of the DVP RNA is present in infectious stocks of AMV from this and other laboratories, and it is about 70% related to nucleotide sequences of E26 virus, an independent isolate of an AMV-like virus [PNAS (1980) 77, 5120]. Preliminary experiments show DVP to be leukemogenic if fused into susceptible cells in the presence of helper virus. We conclude that DVP RNA is the leukemogenic component of infectious AMV and that its specific sequence, termed amv, carries genetic information for oncogenicity. Currently we are investigating the genetic structure of E26 virus and the oncogenicity of the AMV DVP fused into susceptible cells.

Structure of the Erythrocyte Cytoskeleton

329A SPECTRIN OLIGOMERS: A MAJOR STRUCTURAL FEATURE OF THE ERYTHROCYTE CYTOSKELETON, Jon S. Morrow and Vincent T. Marchesi, Dept. of Pathology, Yale University School of Medicine, New Haven, Ct. 06510.
Purified human erythrocyte spectrin forms large oligomeric species without the collaboration of any other proteins. This reversible process of self-assembly is concentration driven and becomes prominent at spectrin concentrations exceeding 10 mg/ml. At the concentration of spectrin expected in vivo spectrin should exist as large oligomeric complexes containing 20 or more spectrin dimers. Such oligomers can be found in fresh, low ionic strength extracts of red cell ghosts. A terminal 80,000 dalton peptide generated by trypsin cleavage of the spectrin α-chain competes effectively with the oligomerization process. This result, together with other data, suggests that oligomerization involves the same sites on the spectrin molecule that are involved with tetramer formation. These results force a re-examination of the proposed roles of actin and band 4.1. Rather than linking tetramers of spectrin together these two proteins may serve to link large "islands" of oligomeric spectrin; alternatively either could play some as yet undefined role in supporting the spectrin cytoskeleton.

329B IMMUNOCHEMICAL IDENTIFICATION OF PROTEIN CONSTITUENTS OF MEMBRANE POLYMERS IN HUMAN ERYTHROCYTES, Ole J. Bjerrum, Paul E. Swanson, Martin Griffin and Laszlo Lorand
Dept. of Biochemistry & Molecular Biology, Northwestern University, Evanston, IL 60201

The increase of intracellular concentration of Ca^{2+} in human erythrocytes causes the formation of membrane protein polymers cross-linked by γ-glutamyl-ε-lysine side chain bridges (Lorand et al. in Membrane Transport in Erythrocytes, Alfred Benzon Symp. 14, p.285 Munksgaard, Copenhagen, 1980). Following solubilization in detergent in the presence of thiols, various classes of polymers were isolated by centrifugation in sucrose gradients containing Triton X-100. Flat-bed SDS-electrophoresis in agarose gel was used for estimating molecular weights. A class of polymers averaging 6×10^6 Mw was taken for further studies.

By rocket-line immunoelectrophoresis (in presence of Triton X-100), employing antibodies against several or against individual membrane proteins, the presence of spectrins (band 1 and 2), of ankyrin (2.1) and of band 3 protein in this polymer could be demonstrated thus far. There was no reaction between the polymer and anti-glycophorin (PAS I and II) antibody. Rabbit antibodies raised against membrane polymer could be shown to react with the spectrin, ankyrin and band 3 protein components of the normal erythrocyte ghost.

Similar immunochemical studies are being pursued with the γ-glutamyl-ε-lysine cross-linked polymer obtained from sickle cells. - Aided by NIH AM-25412

330 HIGH RESOLUTION ELECTROPHORETIC TECHNIQUES FOR THE ANALYSIS OF MEMBRANE PROTEINS UNDER DENATURING AND NON-DENATURING CONDITIONS. R.C. Steinfeld and G.A. Vidaver, Dept. of Chemistry, University of Nebraska at Lincoln, Lincoln, Nebraska 68588 (USA).

One- and two-dimensional polyacrylamide gel electrophoretic techniques have been developed. Under non-denaturing conditions, pigeon erythrocyte plasma membrane was solubilized at 4-6°C by sonication in the presence of the zwitterionic detergent, Zwittergent 3-14 (Calbiochem). The soluble extract was then treated with an admixture of that detergent, Triton X-100 and dibutyl formamide and then resonicated. Isoelectric focusing in gel rods was performed at 8°C followed by a survey of enzymatic activities using histochemical staining techniques. The presence of ATPase, GTPase, and protease activities were observed. The greater utility of the technique was demonstrated by showing the presence of NADH dehydrogenase activity in an extract of mitochondrial membrane. Combining this focusing procedure with a 2nd dimension electrophoresis modified from the method of Davis (Annals N.Y. Acad. of Science (1964) p. 404-427.) has also been done. The technique has been used to identify detergent binding proteins in the membrane extract. The native focused gels were also crossed into a 2nd dimension separation under denaturing conditions modified from O'Farrell (J. Biol. Chem. (1975) 250, 4007-4021). This enhancement of resolution employs a combined pH and acrylamide gradient with the addition of urea and n-Butyl urea as denaturing agents. (This work was supported by Research Grant HE-13256 from U.S. Public Health Service, the University of Nebraska-Lincoln Research Council and NIH Biomedical Research Support Grant RR-07055.)

331 FURTHER STRUCTURAL ALIGNMENT AND PARTIAL SEQUENCE OF SPECTRIN DOMAINS, David W. Speicher, Peter Yurchenco, Jon S. Morrow, and Vincent T. Marchesi, Yale University School of Medicine, Department of Pathology, New Haven, Ct. 06510.

Trypsin digestion of human erythrocyte spectrin at 0°C produces intermediate-size peptides which are relatively resistant to further proteolysis. Nine unique domains had previously been identified and aligned in the two spectrin subunits providing a low resolution topographical model (DW Speicher, et al., PNAS [1980] 77, 5673). This model has been confirmed and extended by using chemical cleavages to provide additional domain overlaps. Chemical cleavages also permitted identification of β subunit regions, including phosphorylation sites, which had not been identified previously. Monoclonal antibodies confirmed the unique nature of the spectrin domains, identified the origin of smaller tryptic peptides and were used to purify peptides by affinity chromatography. Several peptide domains have been used to examine spectrin interactions. The most extensively studied interactions involve an 80,000 dalton domain from the α subunit which mediates dimer-dimer interactions (J.S. Morrow, et al., PNAS [1980], 77, In Press). This peptide binds to spectrin dimer and competitively inhibits tetramer formation. 79,000 and 74,000 dalton peptides derived from the 80,000 dalton domain are functionally inactive. Amino acid sequence analysis suggests that these smaller fragments are generated by removal of the amino terminal region of the 80,000 dalton domain. It appears that structural integrity of the amino terminal region of the 80,000 dalton domain may be necessary for the formation of spectrin tetramers and higher oligomers.

332 POLYMERIC STATE OF ACTIN IN THE HUMAN ERYTHROCYTE, Mark A.L. Atkinson, Jon S.Morrow, and Vincent T. Marchesi, Yale University School of Medicine, New Haven, Ct. 06510.
Actin is a dynamic molecule, existing in the pure form in an equilibrium between monomeric G-actin and a double stranded helical polymer of indeterminate length reflecting the concentrations of monomer, salt and divalent cations in the solution. In vivo the state of actin polymerization may be further complicated by the interaction of monomer and polymer with other cellular constituents which serve to stabilize one form or the other without regard to the G → F equilibrium. Reports on the state of actin in the red cell have been diverse. We have used Phalloidin to stabilize the actin in erythrocyte ghosts prior to extraction in low ionic strength media. Two-dimensional electrophoresis of these extracts shows that actin is primarily in the form of a high molecular weight complex with spectrin. Mild proteolytic digestion will degrade the spectrin preferentially and a high molecular weight actin polymer can be isolated by Sepharose 4B gel filtration. This molecule can be visualized by low angle rotary shadowing and appears to be essentially similar to rabbit muscle F-actin. Estimates of the size of this molecule can be gained by measuring the platinum-carbon replicas in electron micrographs and from migration in a non-denaturing gel electrophoresis system; both indicate that the principal form of actin is a polymer 110 nm in length which has a molecular weight of 10^6 Daltons. The similarity in dimensions to the spectrin dimer and the preponderance of oligomeric actin associated with the spectrin high molecular weight complex both explain why F-actin is not clearly visualized prior to Trypsin treatment.

333 DISSOCIATION OF ERYTHROCYTE MEMBRANE SKELETONS BY POLYPHOSPHATES - LACK OF HYDROLYSIS AND pH DEPENDENCE, Michael P. Sheetz and J. Casaly, University of Connecticut Health Center, Farmington, CT 06032
The erythrocyte membrane skeleton is an extensive lamellar complex at the cytoplasmic face of the membrane which can be isolated from intact erythrocytes in a one-step extraction procedure (Sheetz and Casaly, J. Biol. Chem. 255:9955 (1980)). Unlike some other skeletons the erythrocyte skeleton must accommodate the extensive deformations which can occur in erythrocytes. A likely explanation for this deformability is lability of interactions in the skeleton which can be increased by 2,3 diphosphoglycerate (2,3 DPG) (present in erythrocytes at concentrations from 4-6 mM). The dissociation does not involve hydrolysis of spectrin phosphates which have been labeled by preincubation of cells with ^{32}P. Likewise, 2,3 DPG is not hydrolysed during dissociation. 2,3 DPG dependent dissociation is maximal at pH 7.7 because 2,3 DPG is protonated at lower pH's and the skeleton itself becomes unstable at higher pH's. Other polyphosphorylated and polysulfonated anions also cause dissociation. Further, the binding of column purified spectrin to rabbit skeletal actin is inhibited by 2,3 DPG. We postulate that the cell can control lability in the skeleton complex by controlling the levels of certain phosphate compounds such as 2,3 DPG and polyphosphoinositides.

334 SPECTRIN-ACTIN BINDING IN VITRO: EFFECT OF PROTEIN 4.1 AND POLYPHOSPHATES, L.C. Wolfe, S.E. Lux and V. Ohanian, Children's Hospital Medical Center, Boston, MA 02115
Red blood cell (RBC) membrane skeletal function may be influenced by effects on the interaction of spectrin, actin, and 4.1. Therefore, we developed a new microassay of the binding of spectrin to actin and the influence of 4.1. Specifically, we wanted to study the effects on this system of polyphosphates (e.g. 2,3-DPG and ATP) which have already been shown to increase the lateral mobility of band 3. We added varying amounts (5μg-35μg) of ^{125}I-spectrin dimer (S_D) to a final volume of 80μl (pH 7.6) containing 20μg F-Actin (A), 5mM KCl, 4mM PO_4, 10mM Tris, .2mM $MgCl_2$, .5mM DTT, .1mM ATP, .01mM $CaCl_2$, 1 mg/ml albumin, and 0-600mM NaCl ± 6μg 4.1; incubated the samples for 1 hr at 0°C or 23°C; layered 50μl onto a sucrose cushion; collected the A pellet by centrifugation and counted the bound S_D. Binding of S_D to A in the absence of 4.1 (-4.1) was observed at 23°C but not at 0°C. In the presence of 4.1 (+4.1) S_D to A binding was markedly enhanced at both temperatures and was cooperative. When S_D was .4 mg/ml the molar ratio of 4.1 to S_D in the complex was 1.15:1. As [NaCl] rose from 0 to physiologic, S_D to A binding fell to a low plateau while S_D to A binding induced by 4.1 rose and leveled off at its maximum. Binding of S_D to A ± 4.1 was totally inhibited by 15mM 2,3-DPG. ATP also inhibited S_D to A binding ± 4.1 but the effect plateaued at 60% inhibition at 5 mM. At physiologic concentrations of 2,3-DPG and ATP the inhibition of complex was 85% and 45% respectively. 2,3-DPG had no effect on the binding of S_D to 4.1 (analyzed by sucrose gradients). A similar assay failed to detect any 4.1 to A binding. Hence, polyphosphates must affect the S_D-A-4.1 complex at the S_D to A interaction site.

335 ROLE OF MEMBRANE PROTEIN PHOSPHORYLATION BY ATP-^{32}P SYNTHESIZED IN INTACE RED CELLS ON VINBLASTINE-INDUCED STOMATOCYTOSIS AND INCREASED SODIUM TRANSPORT, Yoshihito Yawata, Kosuke Miyashima and Shunsuke Koresawa, Kawasaki Medical School, Kurashiki 70101 Japan
It is known that vinblastine (VB, 0.25mM) induces marked stomatocytosis (upto 95%) and increased sodium transport (upto 4 times) in intact red cells (RBCs). Membrane protein phosphorylation has been suggested to be one of determinants in shape change and membrane functions in intact RBCs. Thus, a metabolic correlation between these changes and membrane protein phosphorylation was examined in intact RBCs incubated with VB (0.25mM). Methods: RBCs were incubated with inorganic ^{32}P (^{32}Pi) in tris-HCl glucose buffer (pH 7.4) with or without VB at 37°C upto 18 hours. ATP-γ-^{32}P synthesized in intact RBCs was separated from ^{32}Pi. ATP-γ-^{32}P incorporation into membrane proteins was determined in ghosts prepared from VB-treated RBCs. Results: (1)^{32}Pi transport was reduced upto 42±13% in VB-treated stomatocytes. (2)ATP-γ-^{32}P, however, was synthesized normally from ^{32}Pi in intact RBCs with VB (ATP-γ-^{32}P/^{32}Pi:38±5%, control 34±3%). (3)Membrane proteins were phosphorylated normally by ATP-γ-^{32}P which was synthesized from ^{32}Pi in VB-treated RBCs (295±25 cpm/mg ghost proteins, control 305±20). (4) Specific activities of membrane protein phosphorylation by ATP-γ-^{32}P synthesized in VB-induced stomatocytes were almost identical to those in discocytes without VB. In summary, membrane protein phosphorylation was not decreased in VB-treated RBCs. Thus, phosphorylation of membrane proteins may not be required to produce stomatocytosis and increased sodium transport under the experimental conditions as described above.

336 PROTEIN PHOSPHORYLATION AND DEPHOSPHORYLATION IN HUMAN ERYTHROCYTES, E.W. Westhead, P.A. Kiener and B. Roth, University of Massachusetts, Amherst, Mass. 01003

Understanding of protein phosphorylation and dephosphorylation in the erythrocyte is still rudimentary. There are many reports on the phosphorylation of membrane proteins but few on the phosphorylation of cytosolic proteins. There is very little information on the dephosphorylation process. We have recently described the control of pyruvate kinase activity by phosphorylation and dephosphorylation (1,2). We now report substantial purification and characterization of a phosphoprotein phosphatase active against p-nitrophenyl phosphate (PNPP), and the phosphorylated forms of spectrin, casein, and pyruvate kinase. The enzyme has a molecular weight near 160,000 D. and shows a significant activity in 10 mM EDTA. Activity is only little enhanced by Mg^{2+}, is completely inhibited by Zn^{2+}, and is stimulated 10-fold by Mn^{2+}. With PNPP, Mn^{2+} shows a K_A value of 80 μM. The enzyme appears to be distinct from the one reported previously (3). Control of pyruvate kinase activity implies control not only of energy metabolism, but also of 2,3 diphosphoglycerate levels and reduced pyridine nucleotide levels.

In vitro, we find phosphorylation stimulated by cAMP. It has been reported that endothelial cells can secrete cAMP (4). Consideration of the intimate contact between erythrocytes and the endothelial cells of capillaries suggests the possibility of direct cell-to-cell communication with cAMP as "first messenger".

(1) P.A. Kiener, C. V. Massaras & E.W. Westhead, B.B.R.C. 91, 50 (1979). (2) P.A. Kiener & E.W. Westhead, B.B.R.C., in press. (3) G. Fairbanks, J. Avruch, J.E. Dino, & V.P. Patel, J. Supr. Str. 9, 97 (1978). (4) L.A. Boxer, J.M. Allen & R.L. Baehner, J. Clin. Invest. 66, 268 (1980).

337 COMPARISON OF ANION EXCHANGE IN K562 ERYTHROLEUKEMIC CELLS AND HUMAN RED BLOOD CELLS, Philip A. Knauf and Foon-Yee Law, Hosp. for Sick Children, Toronto, Canada M5G 1X8
The human leukemic cell line, K562, has recently been shown to exhibit several erythroid characteristics, including synthesis and surface expression of the major red cell sialoglycoprotein, glycophorin. K562 cells also express a surface glycoprotein known as GP105, which has been reported to resemble the fetal form of band 3, the anion transport protein of the mature red cell. To determine whether or not GP105 is functionally equivalent to band 3, we have examined the anion exchange characteristics of K562 cells. The rate constant for chloride-36 exchange in these cells at 25°C in RPMI-1640 medium was only 0.052±0.004 min^{-1}, 0.03% of the value (181 min^{-1}) for mature red cells at the same temperature (Brahm, J. Gen. Physiol. 70: 283-306). On the basis of their chloride flux, K562 cells have less than 5,600 fully functional band 3 monomers per cell, in comparison to 1,000,000 per red cell. A variety of inhibitors of red cell chloride exchange, including furosemide, DIDS, H_2DIDS, DNDS, NAP-taurine and niflumic acid, all inhibited chloride exchange in K562 cells, but at concentrations 8 to 40 times higher than those effective with red cells. These data indicate that there are less than 300 red cell-like band 3 transport sites per K562 cell. Unlike red cells, K562 cells displayed very little chloride/sulfate selectivity. From these data, it appears that there are striking differences in the anion transport characteristics of K562 cells and red cells, and that GP105 in K562 cells is not equivalent to a functional form of band 3. Glycophorin and band 3 seem to be independently expressed during red cell maturation, with glycophorin being synthesized at an earlier stage of maturation than is band 3. Supported by the Medical Research Council (Canada).

338 WHY IS BAND 3 A DIMER? Ian G. Macara, Lewis C. Cantley, The Biological Laboratories, Harvard University, Cambridge, MA 02138.

Every transport protein so far characterized appears to be oligomeric. There are several possible reasons for this arrangement: (1) formation of the oligomer could drive insertion of the subunits into the membrane; (2) the transport sites could be within a cleft between the subunits; or (3) transport might require allosteric interaction between subunits. In order to test the last two hypotheses, we have examined the interaction between aromatic disulfonates binding to the external transport sites of Band 3. Affinities of a fluorescent probe were measured for both "empty" Band 3, and "half-filled" Band 3 (possessing one subunit to which a stilbene disulfonate had been covalently attached). Binding was found to exhibit negative co-operativity, "empty" sites displaying an affinity for the probe of about one order of magnitude greater than the "half-filled" sites. However, covalent attachment of an aromatic disulfonate to Band 3 had no effect upon the K_m for influx of ^{35}S-sulfate into red cells, suggesting that subunit interaction is not necessary for transport. The distance between the external transport sites was found by fluorescence resonance energy transfer to be 30-50Å. The true distance is probably closer to 30Å. We suggest that the binding sites for large aromatic disulfonates on the subunits of the Band 3 dimer overlap. This arrangement explains the observed negative co-operativity of aromatic disulfonate binding, and supports the hypothesis that the external transport sites exist in a cavity situated between the two subunits.

339 THE ALDOLASE BINDING SITE OF THE HUMAN ERYTHROCYTE MEMBRANE. PRIMARY STRUCTURE OF THE AMINO-TERMINAL DECAPEPTIDE OF BAND 3, S.N. Prasanna Murthy, Theresa Liu, Heinz Kohler and Theodore L. Steck, La Rabida-University of Chicago Institute and Department of Biochemistry, University of Chicago, East 65th Street at Lake Michigan, Chicago, IL 60649

Band 3 is the predominant membrane-spanning polypeptide and the mediator of anion-transport in the human red cell. In addition, it provides the site(s) of association for frutose 1,6-bisphosphate aldolase and other cytoplasmic proteins with the erythrocyte membrane. Aldolase catalytic activity was reversibly inhibited upon binding to band 3. The aldolase binding activity of band 3 was recovered in the amino-terminal 23,000 dalton fragment generated by cleavage via S-cyanylation of its cytoplasmic pole. Among tryptic peptides only those were inhibitory which contained the amino-terminal region of the 23K polypeptide. Prolonged exposure of the 23K fragment to formic acid liberated an amino-terminal decapeptide by hydrolysis of an Asp-Pro bond. Its amino acid sequence was: X-Met-Glu-Glu-Gln-Leu-Asp-Glu-Tyr-Glu-Asp. This peptide inhibited aldolase activity. The absence of positive charges in the highly acidic decapeptide is consistent with the strongly electrostatic character of the band 3 - aldolase interaction. A nonapeptide generated by cyanogen bromide cleavage of the decapeptide lacked the amino-terminal blocked methionine. Its affinity for aldolase was ten times less than the decapeptide. From these findings we conclude that the association of aldolase with band 3 involves a site containing the amino-terminus of band 3. (Supported by grants GM-25687 and American Cancer Society BC-95).

340 STEADY AND TRANSIENT STATE KINETICS OF ERYTHROCYTE ANION EXCHANGE. EVIDENCE FOR COOPERATIVITY IN SUBSTRATE AND INHIBITOR BINDING SUGGESTING SITE-SITE INTERACTIONS WITHIN THE BAND 3 PROTEIN DIMER, J.M. Salhany, Elizabeth D. Gaines and Randy Sullivan, VA Medical Center & Depts. of Internal Medicine & Biomedicinal Chem. Univ. of Nebraska Medical Center, Omaha, Nebr. 68105

We investigate the origin of the non-hyperbolic transport kinetics characteristic of the erythrocyte membrane anion exchange system. Steady state measurements of reversible single and multiple inhibition, as well as presteady state measurements, are presented. "Zero-trans" heteroexchange measurements which show negative cooperative transport, reveal that the degree of negative cooperativity is non-linearly dependent on stilbene disulfonate concentration, such that at low concentrations, the inhibitor enhances the degree of negative cooperativity. This is a classic result for a two site (i.e. two transport site) system, with site-site interactions and the inhibitor mimicking the substrate. A partially concerted dimeric model for band 3 protein function is presented and tested which explains how these cooperative effects can occur despite the presence of linear Dixon plots and a linear dependence of $K_{i\text{-}app.}$ on substrate concentration. Transient state measurements of divalent anion transport are also shown which confirm the presence of site-site interactions within a dimeric functional unit.

Surface Changes in Aggregation and Hemostasis

341 ISOLATION AND QUANTITATION OF THE PLATELET MEMBRANE GLYCOPROTEIN DEFICIENT IN THROMBASTHENIA USING A MONOCLONAL HYBRIDOMA ANTIBODY. R. P. McEver, N. L. Baenziger and P. W. Majerus, Washington University, St. Louis, MO 63110

We fused spleen cells from Balb/c mice immunized with human platelets to mouse myeloma cell line Sp2/0-Ag14. Spleen cells from Balb/c mice immunized with human platelets were fused to mouse myeloma cell line Sp2/0-Ag14. Hybridoma lines producing a variety of antiplatelet antibodies were isolated by HAT selection and cloned, and purified monoclonal IgG from six lines was prepared. One of these lines, 8aB5-9, produced an antibody, *Tab*, that binds to a protein on normal but not thrombasthenic platelets. We isolated this protein from Triton X-100 solubilized normal platelet membranes by affinity chromatography on *Tab*-Sepharose. As determined by SDS polyacrylamide gel electrophoresis, the isolated protein is a complex of glycoproteins IIb and IIIa, because the two subunits comigrate with glycoproteins IIb and IIIa of whole platelets and show identical changes in mobility after disulfide bond reduction. We prepared ^{125}I-*Tab* to determine the number of glycoprotein IIb-IIIa complexes on normal and thrombasthenic platelets by a direct binding assay. Platelets from 17 normal donors bound 39,000 ± 4,600 (SD) *Tab* molecules/platelet. Platelets from four patients with thrombasthenia lacked *Tab* binding sites (<5%). Five obligate and four presumed heterozygotes for thrombasthenia bound 24,500 ± 5,800 *Tab* molecules/platelet. The platelet alloantigen, Pl^{A1}, is not that recognized by *Tab*, because platelets from three Pl^{A1}-negative subjects bound *Tab* normally. The *Tab*-producing clone has allowed development of an assay to detect thrombasthenic homozygotes and heterozygotes and has provided a technique for isolation and quantitation of a membrane protein complex that is required for normal platelet aggregation and clot retraction.

342 THE FATTY ACID COMPOSITION OF PHOSPHATIDYLINOSITOL FROM THROMBIN-STIMULATED HUMAN PLATELETS, Stephen M. Prescott and Philip W. Majerus, Washington University, St. Louis, MO 63110

The level of phosphatidylinositol (PI) in human platelets falls abruptly when they are stimulated with thrombin and then returns to baseline within 15 min. PI is enriched in arachidonate and our recently proposed pathway for arachidonate release (Bell, R.L., Kennerly, D.A., Stanford, N., and Majerus, P.W. [1979] Proc.Natl.Acad.Sci.USA 76, 3238-3241) provides a link between the unique fatty acid composition of PI and the increased turnover of PI seen in many secretory tissues when they are stimulated. We measured the fatty acid composition of PI at time points following stimulation with thrombin as a means of defining the route of PI resynthesis which occurs, and the mechanism for the arachidonate-enrichment. In resting platelets the fatty acid content (% of total fatty acid contributed by each) of PI is: palmitate 5.1, stearate 39.9, oleate 8.6, linoleate 5.6, and arachidonate 40.8. At 30 sec after thrombin treatment, when the mass of PI has fallen from 18.7±2.3 to 12.5±2.4 nanomoles/10^9 platelets, there are no significant changes in % fatty acid composition. However, at 8 min, when PI has returned to 16.8±2.9 nanomoles/10^9, the % fatty acid composition is: palmitate 7.8 ($p > 0.05$), stearate 34.0 ($p < 0.005$), oleate 10.7 ($p < 0.05$), linoleate 16.1 ($p < 0.001$), and arachidonate 31.3 ($p < 0.05$). The altered fatty acid composition excludes the "PI cycle" as the mechanism for resynthesis as it would result in an unchanged pattern. The evidence suggests that PI is synthesized without a specific fatty acid composition, and then undergoes a deacylation-reacylation cycle to result in the unique 1-stearyl-2-arachidonyl pattern.

343 SPECIFIC AND SATURABLE BINDING OF PLASMA FIBRONECTIN TO THROMBIN-STIMULATED HUMAN PLATELETS, M.H. Ginsberg, E.F. Plow, Res. Inst. of Scripps Clin. La Jolla, CA

Upon thrombin stimulation, human platelets adhere to each other and to vessel wall surfaces in a plasma milieu containing 6×10^{-7} M fibronectin (fn). To assess fn as a potential modulator of platelet function, we have studied the effect of thrombin on platelet interaction with plasma fn. Suspensions of washed human platelets were incubated at 37°C with radioiodinated purified human plasma fn. Platelet bound fn was separated from free by centrifugation through 20% sucrose. In the absence of thrombin stimulation, no binding of fn was detected, but thrombin stimulation resulted in significant binding. Fn binding was time-dependent reaching a maximum within 20 minutes, and radioiodinated and non-labelled fn were bound to thrombin stimulated cells with similar affinities. Addition of excess cold plasma fn inhibited binding of radiolabelled fn >98% but unrelated proteins did not suggesting a specific and saturable process. Detailed analysis of binding isotherms indicated a single class of fn binding sites on thrombin stimulated platelets with $Kd = 3.0 \pm 0.1 \times 10^{-7}$ M and a maximum of 100,000 sites/platelet. Fn binding to platelets was triggered at similar thrombin doses as those inducing platelet secretion. Thus, doses of thrombin which trigger secretion induce the expression of saturable binding sites for plasma fn on human platelets. The Kd of 3×10^{-7} M suggests that at plasma fn concentrations, significant quantities of this molecule are bound to thrombin stimulated cells *in vivo* and may modulate the interactions of thrombin stimulated platelets with each other and with connective tissue surfaces.

344 PURIFICATION OF MEMBRANE GLYCOPROTEINS THAT MEDIATE THE INTERACTION OF HUMAN PLATELETS
Lisa K. Jennings and David R. Phillips, St. Jude Children's Research Hospital and The University of Tennessee Center for the Health Sciences, Memphis, TN 38101
Human platelet glycoproteins IIb and III are integral membrane components which have been identified as aggregation sites during thrombin-induced platelet aggregation. Glycoproteins IIb and III were solubilized from human platelet membranes in Triton X-100 and purified by gel filtration. Glycorpoteins IIb and III co-eluted using gel filtration or DEAE-cellulose chromatography in an apparent 1:1 ratio suggesting that the two glycoproteins exist in an equimolar complex. In support of this view, immunoprecipitation studies with antiglycoprotein IIb, demonstrated to be specific for glycoprotein IIb by the immuno-overlay technique, showed that the antibody precipitated both glycoproteins IIb and III from Triton-solubilized platelet membranes and purified material. Also, crosslinking of glycoproteins IIb and III was accomplished at low concentrations of DSP [dithiobis (succinimidyl propionate)] which did not crosslink ovalbumin and albumin under identical experimental conditions. Individual glycoproteins were purified to homogeneity in the presence of 2 M urea. The relationship of these two glycoproteins and their role in platelet aggregation will be discussed.

345 PLATELET PROTEIN ORGANIZATION: ANALYSIS OF NEAREST-NEIGHBOR RELATIONSHIPS.
G.E. Davies and J. Palek, St. Elizabeth's Hospital, Tufts University, Boston, MA 02135
Specific protein associations within and between the platelet membrane and cytoskeleton are believed to play an essential role in platelet function. We have examined nearest-neighbor relationships of platelet proteins by cross-linking of washed, intact platelets with diamide or dithiobis(succinimidyl propionate) (DTSP). Cross-linked complexes were separated by polyacrylamide gel electrophoresis in the absence of reducing agent and their composition determined after reductive cleavage and analysis in a second dimensional gel. Platelets were cross-linked before and after incubation with tetracaine-dimethylsulfoxide, to fully suppress filopodia extension, or thrombin-EDTA, to fully activate platelets without causing aggregation. Cross-linked species produced by diamide include (a) a high molecular weight complex containing cytoskeletal proteins and a 230,000 dalton protein, and (b) cytoskeletal protein homopolymers, such as myosin heavy chain dimer and actin oligomers. DTSP forms similar complexes and also homopolymers of membrane glycoproteins IIb and III and a high molecular weight complex containing these glycoproteins. Cross-linking of tetracaine-treated platelets forms the high molecular weight complex, and activation of platelets before cross-linking results in increased formation of myosin dimers and the high molecular weight complex. We conclude that resting platelets may contain a partially assembled cytoskeleton and that activation is associated with further cytoskeletal protein assembly and with reorganization of membrane glycoproteins IIb and III.

346 THE BLOOD PLATELET AND THE FIBRONECTIN, FVIII-VWF AND FIBRINOGEN ANTIGENS,
Judith Lahav and Richard O. Hynes, Massachusetts Institute of Technology, Cambridge, MA 02139

The enzyme-linked immunosorbent assay (ELISA) was modified for the study of protein-protein interaction as well as binding of proteins to platelets. As few as 5.10^3 bound platelets could be determined quantitatively and proteins bound from solutions as dilute as 0.1 μg/ml could be detected. Using this method the interaction of the plasma proteins fibronectin, FVIII-VWF and fibrinogen with one another, with components of the basement membrane and with the blood platelet can be studied. We have prepared antibody against carefully washed human platelets. This antiserum recognizes fibronectin, FVIII-VWF, and fibrinogen as well as platelet surfaces. However, specific antisera to these three proteins fail to bind to the surface of gel-filtered, inactivated platelets. When gel-filtered platelets adhere to plastic in the absence of plasma proteins, they spread. Such platelets do react with antibodies to fibronectin, FVIII-VWF, and fibrinogen. These results suggest that these three plasma proteins are found inside platelets but not on their surfaces prior to activation and that they become exposed upon spreading. These findings confirm and extend recent suggestions concerning the associations of fibronectin, FVIII-VWF and fibrinogen with human platelets.

347 PARTIAL STRUCTURAL HOMOLOGY AND MEMBRANE ORIENTATION OF PLATELET GLYCOPROTEINS IIb AND III. Dolores M. Peterson and Sai-Hung Yu, University of Texas Medical School, Houston, Texas 77030.
Platelet surface membrane glycoproteins IIb and III are thought to mediate platelet aggregation and are deficient in platelets of patients with Glanzmann's thrombasthenia, an inherited bleeding disorder characterized by decreased platelet aggregation. Structural analysis of IIb and III has been lacking because of failure of purification methods. We separated platelet membrane proteins by two-dimensional gel electrophoresis (O'Farrell JBC 250: 4007, 1975) and found IIb (MW 123,000) and III (MW 105,000) were focused at nearly the same isoelectric point. Tryptic peptide maps of IIb and III were prepared by iodination of NP-40-solubilized plasma membranes from normal platelets followed by purification by two-dimensional gel electrophoresis before trypsinization. The number of tryptic iodopeptides detected was 33 in III and 29 in IIb, and about 65% of the iodopeptides were present in both glycoproteins as shown by coelectrophoresis of the two tryptic digests. Analysis of the surface exposure of glycoproteins IIb and III in normal platelets by lactoperoxidase-catalyzed radioiodination of intact platelets and subsequent peptide mapping of IIb and III showed that almost all radioiodopeptides that were labeled in solution were also labeled on the surface of intact platelets indicating a mostly external orientation of both IIb and III. Partial homology of IIb and III and the deficiency of both in an hereditary disorder may indicate that both are the modified products of a common precursor peptide. External orientation of these glycoproteins implies that IIb and III function as receptors or recognition molecules rather than as transport enzymes.

348 SURFACE AND STRUCTURAL EVENTS AFTER PLATELET STIMULATION, Adrian R.L. Gear and Virginia M. Haver, University of Virginia, Charlottesville, Virginia, 22908.
Platelets challenged by agents such as ADP exhibit a number of surface and internal changes. Disc shape may be lost and surface projections rapidly form before aggregation begins. Understanding the biochemical and structural basis for these changes is an important goal. Our research has been directed to two areas: first, in the involvement of membrane glycoproteins; and second, in the kinetics and extent of morphological events. Glycoprotein function has been investigated by examining surface-labeling efficiency before and after reversible aggregation. It was found that less radioactivity was incorporated when platelets were labelled after aggregation, suggesting loss of membrane material. However, there was no detectable loss when pre-labelled platelets were tested. This result indicates that aggregation may alter glycoprotein topology or cause clustering, without significant degradation. Heterogeneous labelling among platelet populations of differing aggregability was also observed, and should be taken into account when interpreting the data. The second area of research has employed the resistive-particle counter with quenched-flow techniques to measure the kinetics and extent of platelet volume changes before aggregation. After ADP challenge, particle volume begins to increase by 0.3 seconds and is nearly complete by 2 to 3 seconds. The volume increase may be as much as 20 percent. Scanning-electron microscopy has been used to visualize platelet shape after drug and temperature treatment and suggests that the rapid increases in volume induced by ADP may not be artifacts of particle shape. (Supported by USPHS, AM-20727 and AM-22125).

Growth Factors Involved in Wound Healing and Atherogenesis

349 PLATELET DERIVED GROWTH FACTOR: PRELIMINARY CHARACTERIZATION, Thomas F. Deuel, Jung San Huang, Richard T. Proffitt, Donald Chang, Barbara Kennedy, Washington University School of Medicine, St. Louis, MO 63110
The platelet derived growth factor is a potent mitogen felt to be important in wound healing and in the pathogenesis of atherosclerosis. Because of the small quantities found in platelets, purification and characterization have been difficult.

We have purified the human platelet derived growth factor to near homogeneity. Activity has been localized in two proteins of M.W. $\simeq$ 28,000 and $\simeq$ 31,000, respectively (SDS PAGE). Analysis of each protein showed near identity in amino acid composition. Each protein is rich in basic amino acid residues. After reduction, each protein shows in SDS PAGE a common peptide of $\simeq$ 14,000 M.W., which, along with the amino acid analysis data, suggests that the $\simeq$ 28,000 M.W. species is a proteolytic product of the 31,000 M.W. species. The $\simeq$ 28,000 M.W. protein has an additional peptide of $\simeq$ 15,000 M.W. and the $\simeq$ 31,000 M.W. protein has an additional peptide of $\simeq$ 17,000 M.W. The isoelectric point of the combined proteins in 4 mM urea is 10.3. A strong mitogenic response in 3T3 cells is stimulated by levels of purified platelet derived growth factor $<$ 1 ng/ml.

Our results thus provide a preliminary characterization of the platelet derived growth factor and form the basis for subsequent structure/function studies of this potent platelet derived mitogenic protein and how it relates to normal and abnormal cell growth.

350 CHARACTERIZATION OF A FIBROBLAST GROWTH FACTOR DERIVED FROM A PERMANENT HUMAN T-LYMPHOBLAST CELL LINE, Jerome E. Groopman, Aldons J. Lusis and David W. Golde, Department of Medicine, UCLA School of Medicine, Los Angeles, CA 90024

The human T-lymphoblast cell line (Mo) derived from a patient with a T-cell variant of hairy-cell leukemia elaborates a number of growth-promoting lymphokines. Among these we have identified a fibroblast growth factor. This activity is elaborated constitutively under serum-free conditions and released into the medium. Higher activity levels can be induced by mitogens such as phytohemagglutinin. Since the factor is necessary but not sufficient for induction of DNA synthesis in density-arrested BALB/C 3T3 fibroblasts it appears to be a "competence" factor, analogous to the platelet-derived growth factor and bovine fibroblast growth factor which permit entry of target cells into S phase. The T-cell fibroblast growth factor has an apparent molecular weight of about 38,000 under native conditions while under dissociating conditions (6 M urea) the major active component exhibits a molecular weight of about 13,000. The Mo fibroblast growth factor is heat stable to 100°C for 10 minutes and binds to concanavalin A, suggesting that it is a heat-stable glycoprotein. A number of mesenchymal cell strains respond with increased proliferation upon exposure to the Mo fibroblast growth factor under serum-free conditions; these include the murine 3T3 cells, WI-38, human lung fibroblasts, and the trisomic foreskin fibroblast, CCL54. A fibroblast growth factor elaborated by human T lymphocytes may be important in the pathogenesis of such diverse disorders as scleroderma, idiopathic pulmonary fibrosis, and nodular fibrosis in Hodgkin's disease.

351 FATE OF CELL-BOUND EPIDERMAL GROWTH FACTOR, Harry T. Haigler, Dept. Physiology and Biophysics, University of California, Irvine, California 92717.

As a first step toward understanding the molecular mechanism by which epidermal growth (EGF) stimulates cell growth, we have studied the metabolic fate of the cell-bound hormone. Using fluorescent, ferritin and ^{125}I derivatives of the hormone, the following pathway has clearly been defined in a number of cultured fibroblastic and epithelioid cell lines. EGF binds to its specific receptor on the plasma membrane which is initially diffusely distributed. The hormone induces a rapid clustering of the ligand:receptor complex in regions of the membrane that are coated on their cytoplasmic face with the protein clathrin. The clusters are then specifically internalized into endocytic vesicles that eventually fuse with lysosomes. In the lysosomes the EGF (probably along with its receptor) is degraded.

When cultured Swiss 3T3 cells are simultaneously labeled with ferritin labeled EGF and gold labeled α_2-macroglobulin (prepared by Robert Dickson, NIH), the two ligands cluster in the same coated pits and were internalized into the same endocytic vesicles. Although the morphological pathway of internalization of these two ligands is identical, the molecular mechanisms involved appear to be different. Compounds that inhibit the cellular enzyme transglutaminase (e.g. methylamine, diazooxonorvaline, and bacitracin) inhibit α_2-macroglobulin uptake. These compounds were without effect on the cellular uptake of EGF. Differential inhibition of the internalization of these two ligands by transglutaminase inhibitors suggests that molecular mechanisms exist that are capable of selectively regulating the uptake of specific ligand: receptor complexes. The possibility that the coated pit pathway of internalization of polypeptide growth factors serves a regulatory function in growth control will be discussed.

The Cytoskeleton of Abnormal Red Blood Cells

352 IN VIVO CARBOXYMETHYLATION OF MEMBRANE PROTEINS IN SICKLE CELLS, Gloria A. Green, Gurjit S. Sethi and Vijay K. Kalra, University of Southern California, Los Angeles, CA 90033.

Erythrocytes obtained from patients homozygous for the sickle cell gene were fractionated into subpopulations of reversibly sickled cells (RSCs) and irreversibly sickle cell-rich (ISCs) fractions by density gradient centrifugation. Incubation of normal (AA) and sickle cell (SS) fractions with tritiated methionine resulted in the incorporation of ^{3}H-methyl groups into membrane protein. The relative quantities of alkali labile ^{3}H-methyl group incorporated into normal (AA) ghosts protein was 84.9% ± 3.6 of the total counts incorporated compared to 71.2% ± 4.6 and 55.6% ± 10.2 for RSCs and ISCs, respectively. Alkali labile ^{3}H-methyl incorporation was inhibited by cycloleucine, a known inhibitor of SAM-synthetase. Specific membrane proteins identified as substrates for protein methylase I PAS-1 and PAS-2 Bands were methylated by this procedure. Data indicate that carboxymethylation of PAS-1 and PAS-2 is diminished in RSC and ISC compared to normal (AA) cells. AA cells incorporated 15.5% of the total radioactivity into PAS-1 compared to 10.6% and 5.6% for RSCs and ISCs, respectively. Radioactive methyl group incorporation into PAS-2 was 10.8% for normal cells compared to 4.56 and 5.8% for RSCs and ISCs, respectively. Protein methylase I activity quantified by the addition of C^{14}-SAM and exogenous protein substrate (r-globulin) was similar for normal (AA), RSCs and ISCs. However, protein methylase I activity levels measured with C^{14}-SAM and endogenous protein substrates indicated that ISCs incorporated significantly less C^{14}-methyl groups into ghost protein compared to normal (AA) and RSCs. (Supported by NIH 1F32-HL06113.)

353 INFLUENCE OF PHOSPHOLIPASE-INDUCED MEMBRANE LIPID ALTERATIONS ON RED CELL MORPHOLOGY AND FUNCTION. Bertram Lubin+, William Mentzer* and Danny Chiu+. +Bruce Lyon Mem. Res. Lab., Children's Hospital Medical Center, Oakland, CA. * Univ. of Calif., San Francisco, CA.

To investigate possible relationships between phospholipid (PL) organization, membrane proteins and cellular abnormalities, we studied the effects of two phospholipase treatments on normal and abnormal RBC's. Phospholipase A_2 (PLA_2) obtained from bee venom and sphingomyelinase (SMC) from S. aureus were incubated with intact erythrocytes. PLA_2 hydrolyzed glycero-PL located in the outer leaflet of the lipid bilayer into lysolipids and induced echinocytic transformation. SMC hydrolyzed 80% of sphingomyelin into choline phosphate and ceramide and resulted in stomatocytic transformation. In contrast to normal RBC's, neither spectrin-deficient mouse RBC's, heat denatured normal RBC, or pyropoikilocytes underwent echinocytic transformation following PLA_2 treatment. In addition to abnormal shape transformation, both PLA_2 and SMC-treated normal RBC's exhibited an increase in cation permeability, cell volume, osmotic fragility, susceptibility to lipid peroxidation, and decreased filterability. Since the transport of non-electrolytes, such as thioglycerylethylmaleimide, which pass through lipid domains, has been shown to be normal in phospholipase-treated RBC's, the altered cation permeability we observed may be mediated through secondary defects in protein arrangement. Taken together, our data suggest that interrelationships between membrane lipids and proteins have an effect on cation permeability, red cell shape, and deformability. Furthermore, our data also indicate that phospholipases may be useful tools to investigate normal and abnormal lipid-protein interactions in red cell membranes. Funded by National Foundation Grant 6-49 and NIH Fellowship HL 05694.

354 A MOLECULAR DEFECT IN HEMOLYTIC ELLIPTOCYTOSIS, Peter Agre, Wellcome Research Laboratories, Research Triangle Park, N.C. 27709, and Vann Bennett, Johns Hopkins University School of Medicine, Baltimore, Md. 21205.

There is a group of disorders of erythrocytes characterized by abnormal shape and fragility. It has been postulated, but not proven, that these disorders are due to defects in the cell membrane or cytoskeleton. Two unrelated families have been referred to us by Dr. E. Orringer, U.N.C. and David Chui, McMaster U. They appear to have the hemolytic variant of elliptocytosis. Scanning E.M.'s showed microcytosis and marked poikilocytosis. SDS PAGE failed to show abnormalities of the major membrane proteins.

We used the techniques developed to analyze erythrocyte membrane protein interactions on inside-out vesicles with ^{125}I-labelled spectrin or ankyrin. The interactions between spectrin and ankyrin, and between ankyrin and the cell membrane (presumably band 3) were analyzed. The patients' vesicles appeared to bind spectrin (purified from a normal individual) in a normal manner. Likewise, the patients' spectrin appeared to bind to control vesicles normally. The patients' ankyrin bound normally to control (ankyrin-stripped) vesicles. However, the ankyrin-stripped vesicles prepared from our patients did not bind ankyrin normally. The high affinity ankyrin binding sites were reduced by 30-50%, however, the low affinity binding appeared normal. The ankyrin-binding fragment of band 3 was enzymatically cleaved from these vesicles but did not appear different from normal in solution. Also, differential heating experiments failed to show any peculiar thermolability of the patients' spectrin, ankyrin, or vesicles. This suggests that the primary structural defect may be a reduced number of high affinity membrane binding sites for ankyrin.

355 APPROACHES TO THE STUDY OF MOLECULAR VARIATIONS OF SPECTRIN, William J. Knowles and Vincent T. Marchesi, Yale University School of Medicine, New Haven, Ct. 06510.

Spectrin, a peripheral protein of the erythrocyte membrane, is believed to play a prominent role in the maintenance of red cell cytoarchitecture. Spectrin dimer consists of two unique polypeptide subunits (α and β) which are, in turn, composed of several unique chemical domains (PNAS 77:5673). These domains can be isolated from the intact subunits by limited tryptic digestion. In order to investigate the molecular variations of spectrin occuring between different species and within the human population, we have subjected a large number of spectrin samples to limited tryptic digestion or chemical cleavage and analyzed the peptide domains by high resolution 2-dimensional electrophoresis (isofocusing-SDS-polyacrylamide gels). The sensitivity and reproducibility of this technique has allowed us to detect both similar and dissimilar domains that exist between human spectrins and spectrins from closely related primates. A spectrin variant has also been found in the human population. This variation occurs in a single α-chain domain and can be detected both in the heterozygous and homozygous forms. The above approach is currently being used to investigate conformational and structural alterations in human spectrin isolated from abnormal red cells.

356 CYTOSKELETAL ALTERATIONS IN HEREDITARY ELLIPTOCYTOSIS, Thomas J. Mueller and Martin Morrison, Dept. Biochemistry, St. Jude Children's Research Hospital, Memphis, TN 38101
The core or "shell" of the human erythrocyte cytoskeleton is a complex composed primarily of spectrin, actin and band 4.1 (M.P. Sheetz (1979) BBA, 557: 122). Band 4.1, which on discontinuous SDS-acrylamide gel electrophoresis is resolved into two components, bands 4.1a, b, appears to play a key role in the structural organization of the cytoskeletal core and in the attachment of the core to the bilayer aspect of the membrane. Band 4.1 binds to spectrin and enhances the interaction of spectrin and actin (C.M. Cohen et al. (1980) Cell, 21: 875). Extraction of stroma with pH 11.5 NaOH removes all of the peripheral proteins except band 4.1, demonstrating that band 4.1 also interacts directly with the membrane. We have detected a patient with hereditary elliptocytosis whose membranes are nearly completely devoid of bands 4.1a, b. Extraction of the membranes of this patient with Triton X-100 solubilizes 50-60% of the spectrin, whereas Triton solubilizes little or no spectrin from the membranes of normal cells. This suggests that there is decreased interaction among the cytoskeletal core proteins due to the band 4.1 deficiency. The Triton-insoluble residue of this patient is also devoid of glycoconnectin (PAS 2) which, in normal cells, connects the cytoskeletal core with the bilayer domain, perhaps via band 4.1 (T.J. Mueller & M. Morrison (1980) JCB, 87: 202a). Thus, the absence of bands 4.1a, b appears to result in both decreased protein:protein interactions within the cytoskeletal core, as well as decreased association of the core with the bilayer domain. These data provide strong support for the premise that the cytoskeletal core is involved in the maintenance of red cell morphology and also represents an apparent example of a cytoskeletal protein alteration resulting in altered cellular morphology.

357 SELF-ASSOCIATION OF SPECTRIN IN ABNORMAL RED BLOOD CELLS. Shih-Chun Liu, Jiri Palek, Joseph Prchal, and Richard P. Castleberry. St. Elizabeth's Hospital, Tufts University Boston, MA 02135 and University of Alabama Medical School, Birmingham, AL 35294.
Spectrin tetramers are the predominant membrane skeletal component in normal red cells. They are formed by head-to-head association of heterodimers and are presumably linked into a two-dimensional network by actin oligomers and band 4.1. We have examined the alteration of self-association of spectrin in four hereditary disorders characterized by abnormal red cell shapes; i.e., hereditary elliptocytosis (HE), hereditary pyropoikilocytosis (HPP), hereditary spherocytosis (HS) and irreversible sickle cells (ISC). The oligomeric state of spectrin extracted at 0°C (0.1 mM $NaPO_4$, pH 8.0) from red cell ghosts were analyzed by non-denaturing gel electrophoresis. Spectrin from five (out of eleven) individuals with mild HE (>30% elliptocytes) and four HPP patients was in part extracted in dimeric form (10-30%). This was accompanied by a defective reassociation of spectrin dimers into tetramers in solution (e.g. $(K_a)_{Normal}=8x10^5$ M^{-1}, $(K_a)_{HPP}=1x10^5$ M^{-1}). HE and HPP membrane skeletons prepared by extracting ghosts with Triton X-100 exhibited an increased susceptibility to fragmentation by mechanical shaking. Normal, HS and ISC red cell contained mostly spectrin tetramers and had mechanically stable membrane skeletons. We conclude that in HPP and some HE membranes, but not in HS and ISC membranes, spectrin is defective in self-association into tetramers, presumably due to a primary sequence defect. This may be responsible for the diminished stability of HPP and HE membrane skeletons.

358 THE MOLECULAR ALTERATION IN THE CYTOSKELETON OF HEREDITARY SPHEROCYTES, Steven R. Goodman, Joseph J. Kesselring, Scott A. Weidner and Elaine M. Eyster, The Milton S. Hershey Medical Center, Hershey, PA 17033
The structural instability of red cells from Hereditary spherocytic (HS) patients suggest that the primary molecular alteration in this disorder resides in the spectrin cytoskeleton. The protein composition of HS red cell ghosts, spectrin depleted inverted vesicles, and triton shells was quantitatively normal as judged by SDS-PAGE. The binding of ^{32}P-labelled spectrin α-β heterodimers to spectrin depleted inverted vesicles (physiological ionic strength, 4°C) indicated a K_D of 19.6 nm ± 2.4 (mean ± S.E.) and a maximal binding capacity of 95 ± 8.4 µg spectrin/mg membrane protein for 7 HS patients, and 19.4 nm ± 2.1 and 114 µg/mg ± 9.9 for 7 normal (N) controls. Therefore the binding of spectrin to the syndeins (Band 2.1-2.6), its high affinity membrane binding site is unaltered in HS. The binding of ^{32}P-labelled spectrin heterodimers from HS and N patients to ^{125}I-band 4.1 (N) at physiological ionic strength (4°C, 2h) was assayed by rate zonal sedimentation. Spectrin heterodimers (N) bind to purified Band 4.1 (N) as previously reported (Tyler et al, 1979); this binding is eliminated if heat denatured spectrin (N) is substituted in the assay. However, spectrin heterodimers (HS) did not bind to Band 4.1 (N). One dimensional tryptic peptide analysis indicated no difference between HS and N ^{125}I-Band 4.1, but tryptic peptide analysis of HS and N ^{32}P-spectrin β chain indicated an alteration in HS spectrin. We conclude that the alteration in the cytoskeleton of HS erythrocytes is a defect in the terminal region of the spectrin β chain which is the attachment site for Band 4.1. (NIH Grant #HL26059 awarded to S.R.G.)

359 MOBILITY OF CON A RECEPTORS IN SPECTRIN DEFICIENT ERYTHROBLASTS, Joyce B. Geiduschek, University of California at San Diego, La Jolla, CA 92093

Expulsion of the mammalian erythroblast nucleus is concomitant with asymmetric redistribution of membrane proteins: increased density of receptors for concanavalin A (Con A) in the plasma membrane surrounding the emerging nucleoplast (1) and complete segregation of spectrin to the membrane of the incipient reticulocyte (2).The erythrocyte membrane of the common house mouse with hereditary spherocytosis (sph/sph) is spectrin-deficient (3). Immunofluorescence microscopy of bone marrow from these mice (generously provided by Dr. S. E. Bernstein) shows the same spectrin deficit on the erythroblast membrane. The existing trace of spectrin segregates normally, indicating that the observed deficiency in the spherocytic erythrocyte is not due to defective enucleation. Increasing spectrin concentration is associated with decreasing lateral mobility of lectin receptors in the developing erythroblast: Con A receptors can be made to cap in the early erythroblast and patch at a later stage but show no mobility (at the light microscope level) in the prospective reticulocyte of the enucleating erythroblast (2). Experiments with sph/sph erythroblasts show patching and clearing of Con A receptors in the plasma membrane of this spectrin-deficient prospective reticulocyte. Thus, comparison of the capping-patching properties of normal and sph/sph cells provides further evidence that spectrin plays a role in the immobilization of surface components.

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Membrane Changes of Normal and Abnormal Leukocytes

360 ALTERED EXPRESSION OF HISTOCOMPATABILITY ANTIGENS ON 'B' LARGE CELL LYMPHOMAS, Bruce A. Woda and Henry Rappaport, City of Hope National Medical Center, Duarte, Ca 91010.

With few exceptions, mature lymphoid cells express surface histocompatability antigens. In the course of our studies on the expression of HLA-A,B,C (HLA), HLA-DR (DR) and beta-2 microglobulin (B_2m) by normal and neoplastic lymphoid cells, we found 3 diffuse "histiocytic" (large non-cleaved) lymphomas which expressed monoclonal surface immunoglobulin and DR antigens but did not express HLA or B_2m as assessed by indirect immunofluorescence. Cells from 5 other diffuse large cell lymphomas (3 B cell, 2 T cell) had detectable HLA and B_2m. Cells from normal peripheral blood or tonsil could not be found with this phenotype (immunoglobulin positive, HLA and B_2m negative) suggesting that this cell does not have a normal counterpart. It is not likely that 1) HLA and B_2m were masked by autoantibodies, sialic acid residues or trypsin sensitive glycoproteins or 2) that altered membrane fluidity prevented the detection of HLA or B_2m. We cannot exclude the possibility that HLA and B_2m were present at a low level, below the threshold of detection or that antigenic variation prevented their detection. Whether these proteins were not synthesized or, if synthesized were not inserted into the plasma membrane, awaits further study. Supported by NIH grants 1-R01-CA 26422-01 and 1-T32-CA-09308.

361 PARTIAL CHARACTERIZATION OF CELL SURFACE PROTEASE ACTIVITY ON BONE MARROW CELLS FROM THE RAT, HAMSTER, MOUSE AND GUINEA PIG. David A. Hart, Cheryl Hendrix, R. Jerrold Fulton and Witold Cieplak. University of Texas HSC at Dallas, Dallas, TX. 75235.

A number of biological processes are controlled by proteolytic mechanisms. These include the humoral proteolytic cascades as well as cellular processes. Cell surface proteases have been postulated to play a role in cellular functions such as 1) shedding of plasma membrane components into the extracellular space; 2) regulation of receptor-ligand interactions such as hormone-receptor interactions; and 3) regulation of cell proliferation. The bone marrow (BM) environment contains several subpopulations of cells which are actively engaged in proliferation and differentiation and therefore if proteases are involved in such processes, the BM cells (BMC) should express unique proteolytic activities. The proteases associated with intact BMC (unfractionated as well as subpopulations) were analyzed using a ^{125}I-caseinolytic assay. Cell associated proteases have been detected with BMC from rats, hamsters, mice and guinea pigs. The majority of the proteases do not appear to be secreted by the cells. In contrast to previous results with thymocytes and lymph node cells, BMC from the different species expressed dramatically different levels of neutral protease activity. Also in contrast to previous results with other cells, the protease inhibitor profile of the proteases expressed on BMC showed species specific patterns. Cell fractionation studies have revealed that the protease activity expressed by subpopulations of cells are both quantitatively and qualitatively unique. These results raise the possibility that specific proteases could serve as markers for subpopulations of BMC and that interference with the activity of these proteases could modify the proliferation and differentiation of these cells. (Supported by NCI Grant CA-24444)

362 RECEPTOR BINDING AND INTERNALIZATION OF IMMOBILIZED TRANSCOBALAMIN II BY MURINE LEUKEMIA L1210 CELLS, Donald W. Jacobsen, Kiyoshi Takahaski and Medhi Tavassoli, Scripps Clinic and Research Foundation, La Jolla, CA 92037

Membrane transport of vitamin B_{12} (cyanocobalamin; CN-Cbl) into mammalian cells is mediated by the serum protein transcobalamin II (TCII). The latter forms a complex with the vitamin (CN-Cbl + TCII $\rightleftharpoons$CN-Cbl-TCII; $K_a = 3 \times 10^{11} M^{-1}$) and other circulating cobalamins, namely methyl-, aqua- and adenosylcobalamin. Cultured murine leukemia L1210 cells bind [^{57}Co]CN-Cbl—TCII to membrane receptors in a rapid, temperature-independent step and internalize the complex by a slow, temperature-dependent process. In an attempt to identify and characterize the receptors involved, TCII has been immobilized to macro- and minibead supports. The macrobeads (70 μ), prepared by reacting aminopropylcobalamin (AP-Cbl) with CNBr-activated Sephacryl (S) to give S—AP-Cbl (1 μmole Cbl/ml beads), readily adsorbed apo-TCII from serum and served as foci for adherence of L1210 cells (ca. 150 cells/bead) (Jacobsen et al., Blood 55, 160 (1980)). Scanning electron micrographs (SEM) revealed intimate and extensive contact between cellular microvilli and the bead surface. Cells did not adhere to beads that had been previously photolyzed with visible light, a procedure that releases TCII-Cbl. When the purified complex was coupled to latex particles (0.35 μ) and incubated with L1210 cells at 4°, the Cbl-TCII-minibeads were found (via SEM) attached predominately to microvilli. Incubation of the cells at 37° resulted in the internalization of the minibeads as visualized by transmission electron microscopy. Endocytosis appeared to occur in clathrin-coated pits and vesicles. Minibeads derivatized with myoglobin or the non-TCII Cbl-binding protein from chicken serum did not adhere to L1210 cells. Supported by NIH grants AM-25510, AM-25406, CA-6522, CA-16600; ACS grant CH-31.

363 AVIAN SARCOMA VIRUS-INDUCED NEOPLASIA: THE ROLE OF MACROPHAGES IN TUMOR INITIATION AND GROWTH, Mark A. Wainberg and Richard G. Margolese, Jewish General Hospital, Montreal, Quebec.

We have previously shown (Infection and Immun. 22: 328, 1978) that injection of immunostimulants (e.g. BCG) into chicken wing webs, 1 week prior to inoculation of avian sarcoma virus (ASV) into the same site, leads to a dramatic enhancement of ASV-induced tumor growth. It is known that BCG attracts substantial numbers of macrophage-like cells into the inoculation site, and we postulated that macrophages might play an important role in tumor initiation. To resolve this issue, we have cultured tumor cells from developing ASV-induced tumors and studied them for various macrophage-like characteristics. Although variation was observed among individual tumors, approximately 10-20% of cells studied were actively phagocytic and were positive for each of presence of avian immunoglobulin Fc receptors and non-specific esterases. In the case of tumors induced following BCG inoculation, these percentages were increased by approximately 50%. Immunofluorescence studies on the Fc receptor-positive population, separated by centrifugation of rosetting cells through Ficoll-Hypaque, revealed them to be virus-infected. In addition, these macrophage-like cells were oncogenic upon injection into previously uninoculated hosts, and gave rise to tumors from which fully-transforming ASV could be isolated. These results indicate that macrophages are active targets for ASV infection in vivo and play an important role in ASV-induced tumor growth.

364 REGULATION OF β-ADRENERGIC RECEPTORS IN FRIEND LEUKEMIC CELLS, Michel Guyaux, Henri Schmitt and Raphaël Kram, Department of Molecular Biology, University of Brussels, Belgium.

We have recently described an induction of β-adrenergic receptors in Friend erythroleukemic cells (FLC) exposed for 24 h. to either butyrate, DMSO or hexamethylene bisacetamide. As in Hela cells and rat fetal hepatocytes, the induction of β receptors by this group of chemical inducers of differentiation was correlated with an increased response of adenylate cyclase to catecholamines. We report here studies on the relationship between the early induction of β receptors and the subsequent erythroid differentiation of FLC. The tumor promoter TPA and dexamethasone, which inhibit hemoglobin synthesis in FLC, did not significantly prevent the induction of β receptors by 2 mM butyrate. Furthermore, we were able to block the early induction of β receptors without affecting the subsequent differentiation using two new pharmacological agents from Janssen Pharmaceutica (Beerse, Belgium) : cinnarizine and its difluoroderivative, flunarizine. These drugs abolish the contraction of vascular smooth muscle caused by potassium depolarization or norepinephrine and we initially used them as potential inhibitors of calcium fluxes during the induction of differentiation in FLC. At 10^{-5}M, both drugs neither exert cytotoxicity nor inhibit cell proliferation but were equally potent in inhibiting the induction of β receptors by chemical inducers, in addition to causing a decrease in the number of β receptors on untreated FLC. Moreover, these drugs depressed the activation of adenylate cyclase by catecholamines in untreated as well as in butyrate-treated FLC. Under similar conditions, the basal activity of adenylate cyclase and its stimulation by prostaglandin E_1 were not altered by either cinnarizine or flunarizine.

365 PHAGOCYTOSIS OF BACTERIA BY MACROPHAGES: CHANGING THE CARBOHYDRATE OF LIPOPOLYSACCHARIDE ALTERS INTERACTION WITH COMPLEMENT AND MACROPHAGES, C.-J. Liang-Takasaki*, P. H. Makela**, and L. Leive*, *National Institutes of Health, Bethesda, MD 20205, U.S.A. and **Central Public Health Laboratory, Helsinki, Finland
The pathogenicity of Salmonella for mice was shown previously to be influenced by the polysaccharide of lipopolysaccharide (LPS). Thus, when S. typhimurium containing abequose, mannose, rhamnose, and galactose in the side chain of its LPS is transduced with genes that replace the abequose with tyvelose its pathogenicity is reduced 10-fold, and when the above sequence is replaced with a mannose polymer, pathogenicity is reduced 100-fold. This effect was shown not to involve antibody and was postulated to involve phagocytosis. In the present work we show that uptake of the above bacterial strains by the mouse macrophage cell line J774 is inversely proportional to pathogenicity: the most pathogenic is taken up slowest, that of intermediate pathogenicity at an intermediate rate, and the least pathogenic, fastest. The affinity of the three types of bacteria for the macrophages (apparent K_m) varies over a 50-fold range while the ability to ingest the bacteria once they have interacted with the cell (apparent V_{max}) is identical for all three. The uptake is dependent on a heat-labile (56°, 30 min) component in serum. We conclude that differences in LPS polysaccharide affect phagocytosis by macrophages in vitro, possibly explaining differences in pathogenicity in vivo. We hypothesize that the different polysaccharides activate complement to different extents resulting in altered ingestion via the known macrophage C3b receptor. Experiments to test this hypothesis will be presented.

366 FUNCTIONAL DIFFERENTIATION OF TWO MONOCYTIC LEUKEMIC CELL LINES BY INDUCING AGENTS, H.C. Kim, Y.M. Cho and P. Saidi, CMDNJ-Rutgers Medical School, Green Brook, NJ 08812
The leukemic cells accumulate in-vivo because of their inability to mature to functional non-dividing end cells. We tested the efficacy of various differentiation-induction agents on two human monocytic leukemic cell lines we established in our laboratory. The cell line, EJ-1, which was derived from a patient with chronic myelogenous leukemia in blastic crisis, grows in suspension culture with doubling time of 40 hours. The cell line, HB-1, which was originated from a patient with acute monocytic leukemia, has doubling time of 52 hours. Both of these cell lines have monocytic characteristics as determined by positive stains for α-naphthyl acetate esterase (non-specific esterase), negative for AS-D chloracetate esterase stains, presence of bactericidal and phagocytic activity, and strong membrane Ia antigen expression by OK-Ia monoclonal antibody. In order to test the ability of inducing agent on these leukemic cell lines, cells were incubated with DMSO (1.5% V/V), phorbol ester (TPA, 1.6×10^{-8}M), retinoic acid (10^{-5}M) or lithium carbonate (10mM) for 6 days. We measured the inhibition of cell growth by cell count and ^{3}HTdR uptake, and induction of cellular non-specific esterase enzyme activity by spectrophotometric assay using α-naphthyl acetate as a measure of cell maturation. Retinoic acid and lithium carbonate were effective in both EJ-1 and HB-1 cell lines in induction of non-specific esterase to 50-150% of untreated cells and inhibition of cell proliferation by 60% of untreated cells. However, DMSO and TPA had induction capacity only to EJ-1 cell line without any effect of HB-1 cell line. This finding suggests that various inducing agents are capable of inducing maturation on leukemic cell lines through different mechanism. These cell lines may permit the study of defects in maturation of leukemic cells.

367 A TISSUE SPECIFIC GROWTH INHIBITING FACTOR IN LUNG TISSUE, Raymond G. Hall and Helen G. Seibert, Loma Linda University, Loma Linda, CA 92350
An ultrafiltration fraction of bovine lung tissue contains a factor that specifically prevents lung cells of established cell lines from entering the S phase when the extract is added to the culture medium. Non-homologous cell lines are not affected by extract treatment. Primary lamb lung cells are also inhibited by the extract factor. Cytofluorograph measurements show that this effect is specific for the GI phase of the cell cycle.

We also noticed that many of the cells of homologous extract treated cultures appeared larger. To measure cell volumes, cultures were treated with an EDTA-trypsin in solution until they were free from the culture dish and spherical in shape. The cells were then placed on a slide with a grid of known dimensions and photographed. From the photographs, cell diameters were determined and cell volumes calculated. The cell volume of extract treated homologous cells was slightly more than double that of control cultures, whereas non-homologous extract treated cultures showed no significant difference in cell volumes from control cultures.

368 MEDIATORS OF HUMAN PROGRANULOCYTIC LEUKEMIA (HL-60) CELL GROWTH IN VITRO. James K. Brennan, Camille N. Abboud, and Marshall A. Lichtman. University of Rochester School of Medicine, Rochester, New York 14642.
Human cell populations were studied for the ability to elaborate colony stimulating activity (CSA) for normal neutrophil-monocyte progenitors (CFU-NM) and for HL-60 cells. Media conditioned (CM) by macrophages and the macrophage-like cell lines GCT and RC-4 stimulated both CFU-NM and HL-60 whereas CM from a variant of GCT (GCT-NA) and other cell lines of macrophage (SUDHL-1, U-937), myeloid (K-562, GM-1) T-lymphoid (MOLT-4F, RPMI-8402) and B-lymphoid (P1R, RPMI-1788) origin did not stimulate CFU-NM or HL-60. Activity in GCT-CM for CFU-NM and HL-60 co-chromatographed from Ultrogel AcA54 with an apparent M_r of 30,000. In addition, CM from HL-60 cells (R.C. Gallo passages 9,23,40,76) stimulated the clonal growth of HL-60 cells. The active moiety in HL-60 CM was trypsin-sensitive and eluted from Ultrogel AcA54 with an M_r of $\sim$ 13,000. Cell lines derived from embryo fibroblasts (WI-38), carcinoma cells (KB), lymphoblastic leukemia cells (NALM-16) and myelogenous leukemia cells (ML-1) also elaborated a low M_r stimulator of HL-60 growth. Neither HL-60 CM nor these CMs stimulated or inhibited CFU-NM growth. The growth responsiveness of HL-60 cells to CSA and the low M_r activity increased during passage but HL-60 cells did not mature in response to these mediators. Thus, HL-60 cells grow not only in response to a normal mediator but also to one or more low M_r factors which do not appear to stimulate normal progenitors.

369 RESTORATION OF GLUCAGON RESPONSIVENESS IN TRANSFORMED MDCK CELLS BY PROSTAGLANDIN E_1. Michael C. Lin, Shay-Whey M. Koh and Douglas D. Dykman, NIH, Bethesda, MD 20205.
Presence of a hormone-responsive adenylate cyclase system is a highly differentiated characteristic of certain eukaryotic cells; our objective is to understand the regulation of synthesis and assembly of such system. Glucagon stimulation of adenylate cyclase of the MDCK kidney cells leads to increases in intracellular cyclic AMP. This response is selectively lost when these cells are virally transformed (Harvey MSV); the transformed cells remain responsive to β-adrenergic hormones, vasopressin and prostaglandin E_1, suggesting that each type of hormone response is regulated uniquely. Studies of hormone binding using (^{125}I)-glucagon demonstrate that disappearance of glucagon receptors is responsible for the loss of hormone response. Sensitivity towards glucagon and the presence of glucagon receptors are partially restored when the transformed MDCK cells are cultured for five days in a chemically defined medium containing prostaglandin E_1. While prostaglandin $F_{2\alpha}$ is without effect, a phosphodiesterase inhibitor, 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone, mimics the effects of prostaglandin E_1 in restoring glucagon sensitivity, as does sodium butyrate, a differentiation inducer. The parental MDCK cells produce prostaglandins of the E and F series; in contrast, synthesis of these compounds was reduced to less 5% of control values in the transformed cells. We suggest that some phase of synthesis and/or assembly of glucagon receptors is controlled by endogenously produced prostaglandin in MDCK cells; loss of glucagon receptors and hormone responsiveness in the transformed cells occurs as a consequence of the inability of these cells to synthesize this prostaglandin.

370 MYELOID COLONY-STIMULATING ACTIVITY ELUTED FROM THE CELL SURFACES OF HUMAN MYELOID LEUKEMIC CELL LINES, Francis Ruscetti and Robert Gallo, N.C.I., Bethesda, MD 20205
Previous experiments from our laboratory have shown that HL-60 cells, developed from a patient with promyelocytic leukemia, have a leukemic derivation, distinct myeloid features, a clear commitment to myeloid differentiation, and capacity for terminal differentiation _in vitro_ when induced by certain agents. Growth of HL-60 cells initially was dependent on conditioned medium from a human embryonic lung culture. The subsequent independent growth of HL-60 cells could be a result of production of an endogenous growth factor. Numerous attempts to find colony-stimulating activity (CSA), factors necessary for growth and differentiation of normal marrow cells _in vitro_, or related growth-promoting factors in the conditioned media of the HL-60 cells were unsuccessful. Cell surface bound proteins were eluted from HL-60 cells with 0.1 M glycine buffer, pH 2.5. The HL-60 cells were continuously grown in serum-free media containing insulin and transferrin (Breitman _et al._, _Exp. Cell Res._ 126:494, 1980) to eliminate concentration of serum proteins on the cell surface. After dialysis at pH 7.4, these eluates from HL-60 cells contain CSA activity when assayed on the nonadherent fraction of fresh human bone marrow. Different eluates prepared from 10^8 HL-60 cells/ml stimulated from 10-65 colonies per 10^5 marrow cells/0.1 ml of eluate. Eluates from several other human leukemic cell lines not of myeloid origin did not have CSA activity. Three newly developed suspension cell lines that spontaneously release CSA (unpublished data) did not have detectable CSA in their eluates. However, KG-1, another cell line with clear myeloid characteristics, did have cell surface bound CSA. These results suggest that the independent growth of HL-60 cells may be due to the ability of the cells to produce their own growth-promoting factors.

371 INCREASED HORMONE SENSITIVITY FOLLOWING IN VITRO TRANSFORMATION OF HEMOPOIETIC CELLS. W. D. Hankins, LTVG, National Cancer Institute, Bethesda, MD. 20205

The polycythemia- and anemia-inducing strains of Friend virus, FVP and FVA respectively, produced qualitatively different erythroid transforming effects in vitro. While both strains induced erythroid bursts without added erythropoietin (EPO), the FVA-induced bursts were significantly underhemoglobinized compared to those induced by FVP. The time course of appearance and disappearance of erythroid bursts was the same with either virus (maximum on day 5 post-infection). Addition of EPO at t=0 increased the efficiency of transformation of both variants and brought about complete hemoglobinization of the FVA bursts. EPO added to FVA treated cells as late as 4 days subsequent to injection still led to increased hemoglobin synthesis on day 5. Time lapse photography, ^{59}Fe incorporation, and EPO dose-response data indicated that, compared to uninfected cells, the FVA-treated cells exhibited increased sensitivity to the hormone. This finding suggests that acute leukemia viruses may transform hemapoietic cells by increasing the sensitivity of these cells for a naturally occurring growth regulator. In such cases, the increased growth and differentiation of selected precursor cells would reflect a "stimulation" rather than a "block" of a specific differentiation pathway.

372 FRACTIONATION OF GCT CELL ERYTHROID ENHANCING ACTIVITY (EEA) AND COLONY STIMULATING ACTIVITY (CSA) USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) AND HYDROPHOBIC CHROMATOGRAPHY. C.N. Abboud, G.N. Abraham, J.K. Brennan and M.A. Lichtman, U. of Rochester School of Medicine, Rochester, NY 14642.

Serum-free conditioned medium from macrophage-like cell line GCT was concentrated 200-fold by ultrafiltration and applied to a TSK-3000 SW HPLC column. CSA and EEA rich fractions were then applied to a phenyl-Sepharose column in a 1M ammonium sulfate buffer. CSA and EEA were eluted first by a decreasing salt gradient followed by a step elution in a salt free buffer with 50% ethylene glycol. The less hydrophobic pool I was enriched for EEA and Eo-CSA. Eo-CSA stimulates eosinophil colonies at 14 days. The hydrophobic pool II contained most of the CSA which gives rise to neutrophil-monocyte colonies at 7 days (NM-CSA). The differences in specific types of activities were retained when a dose-response titration was performed on these pools. Pool I contained detectable Eo-CSA as well as erythroid enhancing activity even after 1/1024 dilution. Pool II at a 1/32 dilution had NM-CSA but no detectable EEA or Eo-CSA. Preliminary experiments also showed that reverse-phase HPLC could be adapted to the fractionation of GCT cell derived EEA and CSA to obtain pools with differing hydrophobic characteristics. Thus, by combining HPLC and hydrophobic chromatography one can achieve partial separation of these chemical mediators of human marrow progenitor cell growth.

373 ERYTHROID-POTENTIATING ACTIVITY: A GROWTH FACTOR FOR HUMAN K-562 LEUKEMIA CELLS, Ch. Gauwerky, A.J. Lusis and D.W. Golde, Department of Medicine, UCLA School of Medicine, Los Angeles, CA 90024

Our laboratory has established a human T-lymphoblast cell line (Mo) that produces factors stimulating the proliferation of hematopoietic cells. These include a colony-stimulating factor for normal granulocytes and macrophages, and a factor with erythroid-potentiating activity (EPA) that enhances the proliferation of normal erythroid progenitors in vitro. EPA is an acidic glycoprotein of 45,000 daltons with remarkable heat stability. We have found that fractions of conditioned medium (CM) containing EPA have a growth-promoting effect on human K-562 erythroleukemia cells and murine Friend erythroleukemia cells in vitro as detected in a serum-free clonogenic assay. EPA has not been purified to homogeneity but observations suggest that the factor elaborated by Mo cells that stimulates erythroleukemia cell growth is EPA. The erythroleukemia growth factor co-purifies with EPA on gel filtration chromatography, isoelectric focusing and ion exchange chromatography. In addition, the activities show similar heat stability properties. Other sources of EPA such as CM from peripheral blood leukocytes, CM from the human monocyte-like cell line CGT, and partially purified preparations of regulatory protein from urine of anemic patients also stimulated human K-562 cell proliferation. Murine sources of EPA known as "burst-promoting activity" and "burst-feeder activity" stimulated Friend leukemia cells, but were inactive on K-562 cells, confirming the species specificity observed with respect to normal hematopoietic progenitor cells. The availability of homogeneous populations of erythroleukemia cells responsive to factors related to EPA will facilitate the investigation of the action of these factors on target cells.

374 EVIDENCE FOR A SHARED METHOTREXATE-METHIONINE MEMBRANE TRANSPORT PROTEIN(S) IN HUMAN LYMPHOCYTES, Kevin J. Scanlon, Michele E. Pallai and Samuel Waxman, Mount Sinai School of Medicine, New York, NY 10029.
We have reported that folate deficiency or the addition of methotrexate (MTX) can irreversibly damage (>50%) methionine uptake in stimulated but not resting lymphocytes. This inhibition of methionine transport occurs independent of the other known "A", "L" or "ASC" amino acid transport systems. Conversely, methionine partially blocks the cellular uptake of MTX. These data suggest that methionine which is intimately involved in folate metabolism, is influenced at the membrane level by folate and anti-folates. Since it is known that there is a small component of MTX (5-10% of 10μM) that binds irreversibly to the surface of cell membranes, studies were undertaken to determine if this binding could be inhibited by methionine. Labelled MTX (10μM) was preincubated for 2 minutes with stimulated lymphocytes in the presence and absence of unlabelled amino acids (500μM). Only methionine abolished the acid precipitable MTX labelled proteins. Analysis of these labelled protein(s) revealed a single band of radioactivity (MW-68,000) on PAGE in 10% SDS. These data further support the existence of a specific methionine membrane transport protein influenced by folates and anti-folates. Studies are currently under investigation to characterize the expression of this protein(s) in normal and leukemic lymphocytes. The implication of these findings will be discussed in the use of anti-folates in cancer chemotherapy.
This work was supported by NIH Grant AM 16690-5.

375 DIFFERENCES IN MEMBRANE FLUORESCENCE POLARIZATION AND CHOLESTEROL/PHOSPHOLIPID CONTENT IN NORMAL AND LEUKEMIC LYMPHOID CELLS. Leonard Liebes, Edward Pelle, and Robert Silber, New York University School of Medicine, New York, NY 10016.
Studies of cholesterol/phospholipid (C/PL) content of whole cells revealed distinct differences between lymphocytes and monocytes from normal donors and mononuclear cells from patients with chronic lymphocytic leukemia (CLL) and hairy cell leukemia (HCL). When membrane preparations were compared, differences in C/PL ratios between normal lymphocytes, monocytes and CLL lymphocytes were no longer apparent. HCL mononuclear cells had a C/PL ratio of 0.67 $\pm$ 0.10 compared with a mean of 0.56 $\pm$ 0.08 for the other cell types. Steady state fluorescence polarization anisotropy (P) studies using 1,6-diphenyl 1-3-5 hexatriene (DPH) showed HCL cells to have a higher range of membrane polarization (P = 0.305 $\pm$ 0.019 at 25°C). Membranes from normal monocytes and lymphocytes were indistinguishable from each other (P = 0.302 $\pm$ 0.009 and 0.302 $\pm$ 0.009 at 25°C). CLL lymphocyte membranes were found to have lower levels of DPH fluorescence polarization (P = 0.253 $\pm$ 0.022 at 25°C). The similarity in C/PL ratios for normal and CLL lymphocyte membranes and the observed lower levels of DPH polarization for CLL membranes suggest that other membrane factors other than cholesterol content may contribute to the increased fluidity in CLL membranes. Extreme differences in membrane fluorescence polarization between HCL and CLL cells (ΔP = 0.052) suggest that increased membrane fluidity as assessed by DPH fluorescence polarization is not an absolute measure of malignant change.

376 DIFFERENTIATION ANTIGENS ON HUMAN MYELOMONOCYTIC CELLS. Bice Perussia, Giovanni Rovera and Giorgio Trinchieri, Wistar Institute, Philadelphia, Pa. 19104
Antigens present on human myelomonocytic cells at different stages of maturation have been analysed by Monoclonal antibodies. Two antibodies (B9.8.1 and B13.4.1) detect antigens present on granulocytes and monocytes, and have been used to study the process of myelomonocytic differentiation. The surface antigens recognized are not present on immature myeloid elements or on nonmyeloid cells but can be detected on the cells as they differentiate to myelocytes and metamyelocytes, respectively. HL60 promielocytic leukemia cells, were induced to differentiate into mature myeloid elements by treatment with retinoic acid or with dimethyl sulfoxide. 70-90% of the differentiated cells expressed both surface antigens. Cell sorting studies on these treated HL60 cells indicated that myelocytes and metamyelocytes were the more immature cells expressing such markers. Expression of the two surface antigens was also observed when the HL60 cells were induced to differentiate into monocyte/macrophage cells by TPA treatment. These findings indicate that human leukemic cells, induced to differentiate *in vitro* by treatment with specific chemical agents, express lineage-specific differentiation antigens with the same patterns as those observed on normal bone marrow myeloid cells at the corresponding stage of differentiation.

377 TUMOR CELL KILLING BY PHORBOL ESTER-DIFFERENTIATED HUMAN LEUKEMIA CELLS. J. Brice Weinberg, VA Medical Center and Duke University Medical Center, Durham, NC 27705
Monocytes and macrophages of murine or human origin, after appropriate *in vivo* or *in vitro* manipulation, are cytostatic and cytocidal for tumor cells *in vitro*. Previous studies have shown that the phorbol ester 12-0-tetradecanoyl-phorbol-13-acetate (TPA) causes cells of the stable human promyelocytic cell line HL60 to differentiate to cells which resemble macrophages. The purpose of this study was to determine if TPA-differentiated HL60 cells were cytotoxic for tumor cells. After three days in suspension cultures in hydrophobic vessels with or without TPA, HL60 cells were washed and assayed for tumor cytotoxicity by quantifying release of ^{3}HTdR or ^{51}Cr from prelabeled target cells, or by visual target cell counting to determine "net cytotoxicity." Undifferentiated HL60 cells were not cytotoxic for tumor cells. However, after a three day coculture with $\geq$10-25 ng/ml TPA, the macrophage-like cells were cytotoxic and cytocidal for various targets including HeLa, HEp2, HSB, transformed WI38, and undifferentiated HL60, as well as the mouse lines 3T12 and L cell. For example, untreated with 100 ng/ml TPA caused release of 24.8 $\pm$ 1.2%. Neither supernatants nor lysates of TPA-treated HL60 cells mediated the tumor cytotoxicity. The toxicity was relatively specific for transformed cells (e.g., TPA-treated HL60 cell net cytotoxicity of 91.8 $\pm$ 0.2% for HeLa cells vs 18.5 $\pm$ 1.2% for normal fibroblasts). Thus, these macrophage-like cells are selectively cytotoxic for tumor cells (including the parent undifferentiated HL60 cells). Agents that induce this desirable differentiation to nondividing antitumor effector cells may be useful in the experimental treatment of leukemia.

378 MEROCYANINE 540: WHAT SURFACE FEATURE DOES THIS FLUORESCENT PROBE RECOGNIZE ON LEUKEMIA CELLS? Robert A. Schlegel*, Neal Hermanowicz*, Karen Kozarsky†, and Patrick Williamson†, *The Pennsylvania State University, University Park, PA 16802 and †Amherst College, Amherst, MA 01002
The surfaces of leukemia cells and immature hematopoietic cells exhibit patchy fluorescence when stained in the presence of serum with merocyanine 540 (MC540), an impermeant fluorescent membrane probe. In the presence of serum, this dye also stains artificial lipid vesicles which are in a fluid state, but not ones in a gel state. We have therefore proposed that MC540 detects discrete disordered regions of natural membranes. If this is the case, the absence of staining of normal erythroid or lymphoid cells in the presence of serum suggests that the plasma membranes of these cells are relatively ordered. In accordance with this hypothesis, comparison of fluorescence polarization measurements of MC540 in artificial lipid vesicles and in normal erythrocytes indicates that the plasma membrane of erythrocytes is in a gel-like state.

Erythrocytes treated with tetrathionate, an agent which abolishes lipid bilayer asymmetry, stain with MC540 in the presence of serum. Based on this finding we suggest that only the outer leaflet of the plasma membranes of untreated erythrocytes is ordered.

379 GROWTH INHIBITION and INDUCTION OF FUNCTIONS in HUMAN MYELOID TUMOR CELL LINES, Peter Ralph, Sloan-Kettering Institute, Rye, NY 10580
Our studies with murine macrophage tumor lines show that immunomodulators inducing phagocytosis, antibody-dependent cellular cytotoxicity (ADCC), activated killing of tumors, and other differentiated functions also inhibit their growth in culture (J Immunol 121,300,1978; Cancer Res 38,1414,1979). The following experiments were undertaken to test the concept of terminal differentiation in human granulocyte (KG-1)/monocyte (U937, Rc2a) culture lines. Lines showed moderate lysis of mouse preB lymphoma 18-8 in a 22 hr radiolabel release assay, which was stimulated by phorbol myristate (PMA), LPS, lymphokine (LK, sup. of PHA-stimulated blood cells) or antibody. Lines preincubated 3 days with PMA showed increased Fc and C receptors, latex phagocytosis and spontaneous or activated killing. Preincubation with LK also stimulated ADCC in U937 and Rc2a. Function stimulation was correlated with growth inhibition or death of the myeloid lines. LPS stimulated phagocytosis and surface receptors but not tumor toxicity. In contrast, ADCC in murine lines more mature than the human ones was greatly enhanced by LPS, PMA, LK and nonlymphoid sources of CSF. The latter suggests that it is CSF-like molecules in LK inducing terminal differentiation in the human lines.

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380 MEMBRANE FUNCTION AS A MEASURE OF NORMALCY, Paul K. Horan, K. Duguid, M.J. Petruzzi, M. Summers and A. Waggoner,+ Universtiy of Rochester Medical Center, Dept. of Pathology, 601 Elmwood Avenue, Rochester, NY 14642 and +Amherst College, Amherst, Mass 01002
Current trends in hematology are strongly pointed at identifying cell subsets using marker analysis. Such analysis as the identification of T-6 vs T-10 antigen expression is being used to identify differentiation steps in cellular maturation pathways. While these are important first steps it must be remembered that the more important questions are: 1) what is the functional role of this subset and 2) CAN this cell function. This presentation is directed to the demonstration of methods of measuring functionality of membranes.
Using flow cytometry single cell analysis of membrane function is evaluated by monitoring the kinetic changes in membrane potential of leukocyte subpopulations (PMN, Ly, Mϕ). The cyanine dye Di-O-C_7(3) was added to Percoll purified subpopulations at Time = 0. Fluorescence intensity per cell was measured by the cell sorter until equilibrium staining was achieved. At that point varying concentrations of KCl were added to the cells and kinetic changes in fluorescence intensity (and membrane responsiveness) were recorded. After the cells reached a second equilibrium they were exposed to the ionophore Valinomycin resulting in hyperpolarizing the membrane and a corresponding change in fluorescence.
This analysis is achieved for resting G_O leukocytes and stimulated leukocytes. For PMN's the stimulant is the addition of bacteria, for macrophages the stimulant is polystyrene spheres, and for lymphocytes the stimulant is mitogen. Data demonstrating the changes of membrane potential will be presented.

Regulatory Functions of Calcium

381 PURIFICATION OF A PROTEIN KINASE FROM MOLONEY MURINE LEUKEMIA VIRUS. William S. Kloetzer and Ralph B. Arlinghaus, The University of Texas System Cancer Center M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77030
Protein kinase activity from the Clone 1 strain of Mo-MuLV was purified using cation exchange and hydrophobic-interaction chromatography. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) revealed a protein of about 8000 mol. wt. that is unique to the enzymatically-active fractions. The virus for this study was obtained from infected NIH/3T3 cells that were labeled with ^{3}H-leucine. Purified virus (20mg) was solubilized using NP40 and high salt, dialyzed, and then applied to a phosphocellulose column. Using a linear gradient of increasing KCl concentration, fractions were assayed for kinase activity as follows: 30 min incubation at 23°C in 10mM Tris HCl (pH8), 0.1% NP40, 5mM MgCl, 50μg α-casein, 10μCi[γ-^{32}P]ATP (1000-3000Ci/mmol) in a volume of 100μl. The enzymatically-active fractions were then applied to a ω-amino butyl agarose column and eluted with a gradient of increasing KCl concentration. All ^{3}H-containing protein peaks were precipitated with trichloroacetic acid and subjected to SDS-PAGE. The fractions containing protein kinase activity displayed only 2 major bands; a viral structural protein that comigrated with p15 and a unique band of about 8000 daltons not present in any other column fractions. Whether this 8000 mol. wt. polypeptide is viral or host coded remains to be determined.

382 LIMITED PROTEOLYSIS AS A PROBE OF THE CONFORMATION OF CALMODULIN IN SOLUTION, Mary D. Oldewurtel, Claude B. Klee, National Institutes of Health, Bethesda, Md.20205

Calmodulin undergoes a Ca^{2+}-dependent conformational transition which affects its susceptibility to proteolysis (Walsh, et al. J. Biol. Chem. 252 (1977) 7440-7443). Limited proteolysis with trypsin and quantitation of the resultant peptides by high pressure liquid chromatography were used to probe the structure of Ca^{2+}-free calmodulin and to identify the regions of the protein affected by the Ca^{2+}-dependent transition. As previously reported, in the presence of EGTA, trypsin cleaves preferentially at Arg 106 and Arg 90 located in the postulated α-helical portions of the third Ca^{2+}-binding domain. The peptide bond at Arg 126 in the α-helix arm of the fourth Ca^{2+}-binding domain is resistant to trypsin. However, the NH_2-terminal fragments (residues 1-106 and 1-90) are also rapidly cleaved at Arg 37 located in the α-helical portion of the first Ca^{2+} domain leaving the second Ca^{2+} domain intact. In contrast, the connecting peptide (residues 76 to 84) is easily cleaved by trypsin either in the presence or absence of Ca^{2+}. Thus, the increased α-helix content, which accompanies the binding of Ca^{2+}, reflects stabilization of the α-helical portions of domains I and III which are probably unfolded in the Ca^{2+}-free protein as opposed to domains II and IV which appear to be highly structured both in the presence and absence of Ca^{2+}. These results are in good agreement with the conformation of calmodulin predicted from the amino acid sequence by the method of Chou and Fasman (Ann. Rev. Biochem. 47 (1978) 251-276.

383 AN INCREASE IN INTRACELLULAR Ca^{2+} IS REQUIRED FOR MURINE ERYTHROLEUKEMIA CELL DIFFERENTIATION, Lewis Cantley, Dept. of Biochemistry & Molecular Biology, Harvard University, Cambridge, MA 02138; Robert Levenson and David Housman, Dept. of Biology and Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, MA 02139.
The role of ion transport in commitment of murine erythroleukemia (MEL) cells to terminal differentiation has been investigated. Studies with amiloride (an inhibitor of passive Na^+ transport) have shown that an increase in the rate of Ca^{2+} influx is essential for dimethyl sulfoxide (DMSO) induction of MEL cell commitment (Levenson, R., Housman, D., and Cantley, L., 1980, Proc. Natl. Acad. Sci. USA 77, 5948). The Ca^{2+} influx alone however is not sufficient to induce differentiation since the Ca^{2+} ionophore, A23187, does not induce differentiation of MEL cells in the absence of DMSO. A study of the time course of commitment to terminal differentiation shows that no significant commitment occurs during the first 12-15 hours of exposure to DMSO and then the fraction of cells committed increases linearly with time for the next 40 hours. When A23187 is added along with DMSO at time zero, linear commitment occurs from time zero with no lag. These results indicate that the 12 to 15 hour lag time after addition of inducer is the time required for DMSO to alter Ca^{2+} transport properties of the cells. This slowest step in commitment to terminal differentiation can be circumvented by making cells selectively permeable to Ca^{2+}.

384 PHOSPHORYLATION OF THE RECEPTOR FOR IMMUNOGLOBULIN E ON BASOPHILS, Clare Fewtrell, Andrew Goetze and Henry Metzger, NIAMDD, NIH, Bethesda, Md. 20205.
Mast cells and basophils have high affinity receptors for immunoglobulin E (IgE) on their surface membranes. Aggregation of these receptors to form dimers or larger receptor clusters results in Ca^{2+}-dependent exocytosis. We have studied the state of phosphorylation of the receptor for IgE in order to learn more about its biochemistry and the molecular mechanism by which it triggers secretion. These studies employed a rat basophilic leukaemia (RBL) cell line in which the receptor for IgE has been well characterized. The receptor is composed of two sub-units, a 55K α chain which binds IgE and a β chain with a M_r in the region of 35K. The two chains interact non-covalently in a 1:1 stoichiometry and this association can be stabilized using covalent cross-linking reagents. Short-term (~ 2hr) exposure of RBL cells to $^{32}P_i$ results in the labelling of a protein which is indistinguishable from the β chain of the receptor for IgE. Thus, specific immune precipitation of IgE:receptor complexes using anti-IgE antibodies yields a single phosphorylated protein M_r ~ 35K. This species disappears after exposure to the cleavable cross-linking reagent dimethyl dithiobispropionimidate. Instead a phosphorylated species with a M_r of about 90K is seen which is consistent with the 55K + 35K (α + β) complex of the receptor for IgE. Subsequent reduction leads to the recovery of the 35K phosphoprotein which we conclude is the β chain of the receptor for IgE. The α chain of the receptor does not appear to be phosphorylated. Incorporation into the β chain was observed even when the cells were exposed to $^{32}P_i$ after the receptors had bound IgE. This suggests that phosphorylation occurs after the receptor has been inserted into the plasma membrane. We have so far been unable to observe any changes in the state of phosphorylation of either chain of the receptor during IgE-mediated triggering of RBL cells.

385 ROLE OF CALCIUM IN VOLUME REGULATION BY PERIPHERAL BLOOD LYMPHOCYTES, Sergio Grinstein, Ann DuPre, Roy Cheung, Michael Dosch, Erwin Gelfand and Aser Rothstein, Research Institute, The Hospital for Sick Children, Toronto, Canada
Peripheral blood lymphocytes (PBL) first swell when suspended in hyptonic media, but then return to near normal volume within minutes. The shrinking phase is associated with K^+ loss. It can be prevented or reversed by abolishing or reversing the transmembrane K^+ gradient. Ca^{++} is known to regulate K^+ permeability in several types of cells. We analyzed the possible role of Ca^{++} in volume regulation by PBL. Increasing the cytoplasmic [Ca^{++}] with A23187 in cells suspended in Ca^{++}-containing, K^+-free media induced shrinking. Conversely, Ca^{++} plus ionophore induced K^+ uptake and cell swelling in K^+-rich media. These observations suggest that an increase in internal Ca^{++} results in an increased K-permeability across the plasma membrane of PBL. When micromolar concentrations of quinine, an inhibitor of Ca^{++} -induced K^+ fluxes in other cells, are added during osmotic challenge, the shrinking response is prevented. Phenothiazine drugs are known to inhibit several calmodulin-activated systems. One of these drugs, trifluoperazine (10μM), abolished the shrinking phase of osmotically swollen PBL. It is suggested that an elevation in cytoplasmic [Ca^{++}] triggered by cellular swelling increases the plasma membrane permeability to K^+. The ensuing K^+ efflux causes cell shrinkage. The effects of Ca^{++} may be mediated by calmodulin or a calmodulin-like activator.
(This study was supported by grants from the Medical Research Council and National Cancer Institute (Canada).

386 ROLE OF PHOSPHATIDYLINOSITOL TURNOVER IN THE ELEVATION OF CYTOSOL Ca^{2+} IN PLATELETS AND SALIVARY GLANDS. John N. Fain, Irene Litosch, and J. Adolfo Garcia-Sainz, Brown University, Providence, Rhode Island 02912.

Hormones or other stimuli which elevate intracellular Ca^{2+} invariably increase the turnover of phosphatidylinositol. We have suggested (Life Sciences 26, 1183-1194) that the alpha-2 adrenoceptors mediate effects of catecholamines secondary to inhibition of adenylate cyclase while alpha-1 receptors mediate effects on elevation of intracellular Ca^{2+} and phosphatidylinositol turnover. In platelets the aggregation due to epinephrine is mediated through alpha-2 receptors as is the increased de novo synthesis of phosphatidylinositol. Platelets do not have alpha-1 catecholamine receptors or show any alpha-1 effects on phosphatidylinositol turnover. Thrombin on the other hand appears to elevate intracellular Ca^{2+} and increases phosphatidylinositol degradation. However, the elevation in intracellular Ca^{2+} apparently further activates phospholipase C. In blowfly salivary glands serotonin increases Ca^{2+} influx via separate alpha receptors which appear to be different from those involved in adenylate cyclase activation. Serotonin activates phosphatidylinositol breakdown in salivary glands via a mechanism independent of changes in intracellular Ca^{2+}. The phospholipase C which hydrolyzes phosphatidylinositol is active in the absence of Ca^{2+}. The direct addition of serotonin to cell-free homogenates of blowfly salivary glands increases the breakdown of phosphatidylinositol. These data support the hypothesis of Michell that phosphatidylinositol breakdown is involved in the entry of Ca^{2+} into salivary glands and possibly platelets.

387 CALMODULIN REGULATION OF CALCIUM FLUXES IN HUMAN PLATELET MICROSOMAL FRACTIONS, Gilbert C. White, II, University of North Carolina, Chapel Hill, N.C. 27514

Cytoplasmic calcium levels in platelets appear to be regulated in part by calcium fluxes across a sarcoplasmic reticulum-like fraction called the dense tubular system. Factors controlling calcium fluxes across this fraction remain unclear. In the present study, we have examined the potential role of a calcium-dependent regulator of membrane (Ca^{2+}/Mg^{2+})-ATPase, calmodulin (CaM), in calcium fluxes across a 1,400-40,000 x g particulate fraction from sonicated washed platelets. Accumulation of calcium was dependent on ATP as an energy source, was inhibited by preincubation of the fraction with trypsin, but was not affected by amrinone or vanadate. The fraction contained up to 200 units CaM/mg protein (4 pg/mg). The further addition of CaM in doses between 0.05-4.8 pg/mg had no effect on calcium accumulation. Similarly, an antisera to CaM had no effect on calcium accumulation by this fraction. Two inhibitors of CaM, trifluoperazine (TFP) and $ZnCl_2$, were tested for their effect on calcium accumulation. TFP produced a dose-dependent inhibition of the rate and extent of calcium accumulation at concentrations between 0.1-200 uM (I_{50} 15 uM). $ZnCl_2$ also produced inhibition at concentrations between 0.01-1 mM (I_{50} 0.19 mM). Neither compound effected the efflux of calcium from preloaded membrane fractions indicating that their effect was on the uptake of calcium rather than on calcium release. These results suggest that CaM may play an important role in regulating calcium fluxes in platelet subcellular fractions and may therefore modulate cytoplasmic levels of calcium in the intact cell.

388 REGULATION OF CALCIUM TRANSPORT BY HUMAN LYMPHOCYTE PLASMA MEMBRANES. Andrew H. Lichtman, George B. Segel, and Marshall A. Lichtman, Univ. of Rochester, N.Y. 14642.

We have found that human lymphocytes stimulated to enter the cell cycle by lectins have an increase in Ca exchange without a net increase in cell Ca. An outward Ca pump responding to a heightened leak after lectin treatment could explain these results. Therefore, we have studied the Ca transport properties of lymphocyte plasma membranes. Inverted plasma membrane vesicles were prepared, which had a 30 fold enrichment of 5-nucleotidase. Ca accumulation by these vesicles was linear for 20 minutes in the presence of Mg, ATP and oxalate. Ca transport into vesicles did not occur if Mg or ATP was omitted, or if ATP was replaced by ADP, AMP, GTP, UTP, ITP, TTP or CTP. The Vmax for Ca transport was 2 pmol Ca/µg protein/min and the Kms for Ca and ATP were 1.0 and 150 µmol/L respectively. A23187 (1 µmol/L) completely inhibited net Ca uptake and caused the release of previously accumulated Ca. Cyanide, oligomycin, ouabain, and varying Na/K concentrations had no effect on Ca uptake. A Ca-activated Mg-ATPase was present in the same vesicles and had a Vmax of 25 pmol Pi/µg protein/min and Kms for Ca and ATP of 0.6 and 100 µmol/L respectively. The ATPase activity was not altered by the presence of potassium oxalate or A23187. Both the Ca-ATPase and the Ca transport activity of EGTA-"stripped" lymphocyte plasma membranes were increased 2-fold by a cytoplasmic component prepared by gel filtration and ion exchange-chromatography of lympholysates. These studies have characterized kinetic features of a Ca stimulated transport and energy transduction system in the lymphocyte plasma membrane, which can be modified by a cytoplasmic activator (calmodulin). This system is presumably responsible for the prevention of increased calcium in hyperpermeable, lectin-treated lymphocytes.

Differentiation and Function and Hematopoietic Cell Surfaces

389 IS THE ACTIVATION OF LYMPHOCYTE NA,K-ATPASE BY EGTA THE RESULT OF CA OR ZN CHELATION? M.A. Lichtman, G.B. Segel, W. Simon and A.H. Lichtman, U. of Rochester, N.Y. 14642.
A chelating agent such as EGTA is required to fully activate the Na,K-ATPase of lymphocyte plasma membrane vesicles. In the absence of EGTA, ionized Ca as measured by a calcium electrode was 2 μM and the Na,K-ATPase was 25 to 30% of maximum. Twenty-five μM EGTA reduced ionized Ca to 0.6 μM and resulted in maximal Na,K-ATPase activity. Several lines of evidence established that Ca was not the cation inhibiting the ATPase activity. First, histidine, a potent chelator of most divalent cations bound by EGTA except for Ca, activated the Na,K-ATPase fully. Free Ca was not reduced during histidine activation. Second, addition of Ca to either the EGTA or the histidine activated Na,K-ATPase did not inhibit its activity until a free Ca was reached that was six times that present in the poorly-activated system prior to addition of EGTA or histidine. These data suggested that EGTA activated the Na,K-ATPase because of chelation of a cation other than Ca. Zn (2 μM) was present in the ATPase assay system. When Zn was added to the Na,K-ATPase partially activated by 50 μM histidine, inhibition of the ATPase was observed. At 35 μM of added Zn, the calculated ionized Zn concentration was 2 μM, similar to the initial Zn value; and, the inhibition of the ATPase was similar to that in the absence of chelator. Studies with varying concentrations of histidine and zinc produced results predicted by the hypothesis that zinc was inhibitory to the enzyme activity. These data show that chelation of very low concentrations of divalent cations enhance greatly the catalytic rate of the lymphocyte Na,K-ATPase and that Zn is the principal cation that modulates the plasma membrane Na,K-ATPase under the conditions of these studies.

390 THE FUNCTION OF FACTOR V IN PROTHROMBIN CONVERSION, K.G. Mann, M.E. Nesheim, P.B. Tracy, L.S. Hibbard and J.W. Bloom, Hematology Research, Mayo Clinic, Rochester, MN 55901
Factor V is a rod-like single chain protein with a molecular weight of 330,000. It possesses three binding sites for calcium one of which has $K_d < 10^{-8}$M. Factor V is best described by the term "procofactor". Treatment with thrombin results in a number of discrete cleavages in the molecule, and the generation of the active cofactor. Factor Va possesses approximately 400 times the cofactor activity of factor V. Both factor V and factor Va bind to phosphatidylcholine-phosphatidylserine vesicles and to unactivated platelets. We have examined the participation of factor V and factor Va, as well as the other components of the prothrombinase complex in the conversion of prothrombin using the probe DAPA. These studies suggest that factor Va functions in part by partitioning factor Xa to the surface of the phospholipid vesicle or to the platelet membrane surface. The rate of prothrombin conversion contained is independent of whether the vesicle or platelet is used as a lipid equivalent.

Regulatory Role of Prostaglandins and other Arachidonate Metabolites

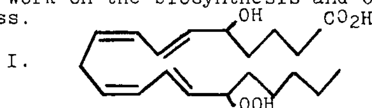
391 ARACHIDONATE METABOLISM BY HUMAN NEUTROPHILS, Moseley Waite, Christopher E. Walsh, Michael J. Thomas, Lawrence R. DeChatelet and Robert L. Wykle, Bowman Gray School of Medicine, Winston-Salem, NC 27103
Human neutrophils were prelabeled with [^{14}C] stearate (C18:0) and [^{3}H] arachidonate (C20:4) in order to determine what metabolites of C20:4 can be produced from membrane derived C20:4 and the mechanism of release of C20:4 from the neutrophil membrane upon challenge. We found that challenge by opsonized zymosan and Ca^{2+} ionophores caused the release of up to 20% of the [^{3}H] C20:4 but no [^{14}C] C18:0 from cellular lipid. Approximately 70% of the [^{3}H] C20:4 was derived from phosphatidyl inositol (PI) and 30% came from phosphatidyl choline (PC). No [^{14}C] lysolipid (from phospholipase A_2) nor [^{14}C] monoglyceride (from phospholipase C plus lipase) was recovered. When exogenous C20:4 was added at the time of stimulation, an increased incorporation of C20:4 was found. We interpret these results to mean that the ionophore stimulates deacylation via a phospholipase A_2 with a concomitant reacylation by acyl CoA.

The C20:4 metabolites formed were a mixture of bioactive HETEs as well as leukotriene. Most of the released C20:4 remained cell associated whereas half of the HETE and nearly all the leukotriene was released from the cell. One-third of the HETE but no leukotriene was reincorporated into cellular lipid, primarily phospholipid.

392 STIMULATION OF ERYTHROID BURST COLONY FORMATION BY PROSTACYCLIN, Peter P. Dukes, Willye B. Powell and Andrew Ma, Depts. of Peds., and Biochem., USC School of Med. and Childrens Hospital of Los Angeles, CA 90054
Prostaglandins have been reported to influence erythropoiesis in vivo and in vitro. It was therefore of interest to study the effect of prostacyclin (PGI_2) and its metabolite 6 keto-$PGF_{1\alpha}$ on clonal culture of erythroid progenitor cells. For this purpose mononuclear cells were isolated from the blood of normal volunteers by the Ficoll-Hypaque procedure. The cells were cultured in a methylcellulose containing medium, human urinary erythropoietin (1.3 U/ml) served as the stimulus for erythroid burst forming unit (BFU-E) derived colony formation. BFU-E were scored after 10 days incubation. The effects of the presence in this system of PGI_2, 6 keto-$PGF_{1\alpha}$ and PGE_2 were compared. Doses employed ranged from $10^{-4}M$ to $10^{-8}M$. PGI_2 was found to be stimulatory at concentrations from $10^{-4}M$ to $10^{-6}M$, with an optimum at $10^{-5}M$ ($103 \pm 39\%$ increase over control, mean $\pm$ SE of 5 experiments). PGE_2 on the other hand totally inhibited colony formation at $10^{-4}M$, it stimulated at lower concentrations with an optimum at $10^{-7}M$ ($264 \pm 103\%$, 3 experiments). The effect observed with PGI_2 may have been due to its principal metabolite 6 keto-$PGF_{1\alpha}$ since this compound at $10^{-5}M$ concentration was also stimulatory ($155 \pm 84\%$, 3 experiments). These data suggest that in hematopoietic tissues, locally produced PGI_2 and/or its metabolite could have a role in the modulation of BFU-E expression. (Supported in part by NIH grants AM-26500 and HL 10880).

393 REGULATION OF SKELETAL MUSCLE MITOCHONDRIAL PROTEIN SYNTHESIS BY INSULIN, Diana S. Beattie, Joyce Roberson and Ronald W. Rinehart, Mt. Sinai School of Medicine, New York, New YOrk 10029
Reinvestigation of amino acid incorporation into protein by isolated skeletal muscle mitochondria confirmed the previous report from our laboratory that diabetes mellitus caused a 50 to 60% decrease in the rate of protein synthesis over the entire time course of incubation, when either ATP phosphoenolpyruvate kinase or ADP-glutamate were used to provide energy. Treatment of diabetic animals with insulin restored the rate of skeletal muscle mitochondrial protein synthesis in vitro to control levels. Mitochondrial protein synthesis was also decreased by a two day fast which is known to lower plasma insulin levels. The products of protein synthesis by skeletal muscle mitochondria isolated from control and diabetic rats were identical suggesting that synthesis of all proteins in the mitochondria is decreased in the absence of insulin. Recent studies with aurintricarboxylic acid, an inhibitor of chain initiation, have suggested that skeletal muscle mitochondria from diabetics are unable to initiate protein synthesis at a rate comparable to controls. The possible phosphorylation of mitochondrial proteins as a control mechanism for mitochondrial protein synthesisat the initiation step is currently under investigation.
(Supported by NIH grant, AM 20953).

394 NOVEL HYDROXY-HYDROPEROXY ARACHIDONIC ACID METABOLITE FROM RAT MONOCYTES, Richard L. Maas, Alan R. Brash, and John A. Oates, Vanderbilt University School of Medicine, Nashville TN 37232
Lipoxygenase metabolites of arachidonic acid may be important in regulating leukocyte activity in inflammation and other immune processes. The lipoxygenase pathway of arachidonate metabolism was studied in thioglycolate-induced rat peritoneal mononuclear cells, stimulated with calcium ionophore A23187. Substantial quantities of a new lipoxygenase product, identified as I, 5-hydroxy-15-hydroperoxy-6,8,11,13-eicosatetraenoic acid, were isolated. No other monohydroperoxy metabolites (HPETEs) of arachidonic acid were isolated. The structure of I was determined from ultraviolet spectroscopy, gas-chromatography-mass spectrometry, and comparison via HPLC to reference material (characterized by ^1H-NMR) produced by the action of soybean lipoxygenase on chemically synthesized ($\pm$)-5-HETE. Other lipoxygenase metabolites identified include leukotrienes (LT) B,C,D, and E, two trihydroxy metabolites of LTB, and 5,12, and 15-HETEs. In contrast to LTB, compound I was inactive in leukocyte migration and enzyme release assays. Further work on the biosynthesis and on additional transformations of I is in progress.

I. 

395 SPECIFICITY AND REGULATION OF AGONIST-MEDIATED PROSTACYCLIN SYNTHESIS IN CULTURED HUMAN VASCULAR CELLS, Nancy Lewis Baenziger, Frances J. Fogerty, and Lila E. Mertz, Washington University School of Medicine, St. Louis, MO 63110.
We have previously shown that histamine triggers the production of PGI_2 by cultured primary human venous endothelial cells via an H1-receptor-mediated mechanism. We have now assessed the cell specificity of this interaction in primary human umbilical vein smooth muscle cells (3 donors) and primary to 6th passage human abdominal aorta smooth muscle cells (3 donors, 12 cultures). They produced 0.3-1.9 ng $PGI_2/10^5$ cells in response to arachidonic acid or mechanical agitation, but did not synthesize PGI_2 in response to histamine. In endothelial cells thrombin yielded an additive response with histamine when both were added simultaneously in either saturating or subsaturating concentrations. Cultures of $1\text{-}2 \times 10^5$ cells produced 5.4±1.5 ng PGI_2 with 1 U/ml thrombin, 5.0±1.5 ng PGI_2 with 5.5 μM histamine, and 9.0±1.7 with both, or 86% of the sum of the 2 agonists alone (n=8). Sequential addition of the agonists 15 seconds apart yielded the same amount of PGI_2 as that produced by the first agonist alone (n=3). PGI_2 synthesis in response to histamine was decreased 79% at 20 min to 3 hr following an initial histamine stimulus, and subsequently returned to its original value within 6-9 hours. The presence of serum was required for the return; response to histamine remained low in serum-free medium with 0.25-10 μM arachidonic acid in fat-free albumin. Thus, histamine-mediated PGI_2 synthesis is an endothelial cell-specific function with several possible levels of regulation.

Monoclonal Antibodies

396 ENUMERATION OF LEUKEMIC CELLS IN HUMAN BONE MARROW AFTER TREATMENT WITH MONOCLONAL ANTIBODIES TO CELL SURFACE DETERMINANTS, Karel A. Dicke, Marilyn D. Lafferty and Christopher L. Reading, Departments of Developmental Therapeutics and Tumor Biology, The University of Texas System Cancer Center, M.D. Anderson Hospital and Tumor Institute, Houston, Texas 77030
The use of autologous remission marrow for repopulation of the hemopoietic system after high dose cytoreductive therapy for the treatment of relapsed acute leukemia is limited by the presence of leukemic cells in the aspirated marrow cell suspension. Antibodies to determinants on the surface of acute lymphocytic leukemia and acute myelogenous leukemia cells have been developed in order to treat the marrow to remove the leukemic cell population. The specificity of the antibodies are being tested in a leukemic cell colony assay in culture in order to see whether the clonogenic leukemic cell population reacts with the antibody. At the same time, tests are being performed to study the effect of those antibodies on the various subsets of normal hemopoietic cells inducing the hemopoietic precursor cells such as the CFU-C and the BFU-E.

397 REACTIVITY OF MELANOMA AND LUNG CARCINOMA DERIVED MONOCLONAL ANTIBODIES WITH HUMAN LEUKEMIC AND NORMAL BONE MARROW CELLS, Marilyn D. Lafferty and Karel A. Dicke, Department of Developmental Therapeutics, The University of Texas System Cancer Center, M.D. Anderson Hospital and Tumor Institute, Houston, Texas 77030
The search for monoclonal antibodies with specificities to leukemic antigenic determinants has involved antibody production with immunogens of leukemic cells. It is also possible that antigenic determinants found on other neoplastic cell types may be differentially expressed on leukemic cell populations and/or normal hemopoietic precursor cells. To test this hypothesis, five "melanoma" and six "lung carcinoma" monoclonal antibodies were tested for reactivity with populations of acute myelogenous, acute myelomonocytic, acute lymphocytic and blast crisis leukemic cells using an enzyme-linked immunosorbant assay. The selected antibodies showed differential binding patterns with various neoplastic and normal cell types. Thirty different cell populations were used as targets; the binding patterns from each antibody varied from reactivity with three of thirty and twenty-two of thirty cell samples. Each antibody reacted with 20-100% of the leukemic cell populations, but no set pattern of reactivity within each leukemic classification was evident. The antibodies also showed varied binding patterns with fractions of normal bone marrow and with CFU-C. This data suggests that the blast cells found in each classically defined leukemic type exhibit different antigenic properties.

398 MONOCLONAL ANTIBODIES TO K562 CELLS INHIBIT GROWTH OF BONE MARROW PROGENITORS IN VITRO
Neal Young and Sheam Pey Hwang-Chen, National Heart, Lung, and Blood Institute, Bethesda, Maryland 20205
K562 cells, leukemic blasts which express erythroid properties on induction, may share antigenic determinants with bone marrow progenitor cells like the BFU-E and CFU-C. Spleen cells from mice immunized with K562 cells were fused with SP-2 myeloma cells, and hybridoma clones which produced antibody to K562 cells identified by radioimmunoassay with ^{125}I-protein A. A small number of clones produced antibody which bound to K562 cells but not to peripheral blood lymphocytes; these antibodies were tested for inhibition of bone marrow colony growth. Normal bone marrow cells were briefly incubated with antibody in the presence of autologous serum as a complement source, washed, and cultured in methylcellulose with growth factors to promote either erythroid or myeloid colony growth. 6 of 12 monoclonal antibodies assayed completely inhibited growth of both BFU-E and CFU-C derived colonies and 5 inhibited growth of CFU-C colonies only. CFU-E colonies were unaffected. The 6 antibodies which abolished colony proliferation showed no binding to bone marrow or peripheral blood lymphocytes by C'-mediated cytotoxicity and immunofluorescence. No inhibitory antibody bound in radioimmunoassay to a panel of human cells including blood B and T cells, granulocytes, adult and fetal erythrocytes, and cultured B and T cell lines. Their profound effect on hematopoietic bone marrow cells, strong binding to an erythroid blast-like cell, and failure to bind to a significant fraction of bone marrow cells or mature blood elements make anti-K562 cell immunoglobulins good candidates for anti-stem cell antibodies.

399 MONOCLONAL ANTIBODIES DISCRIMINATE MURINE BONE MARROW CELL TYPES M.R. Loken, D.S. Dessner, G.E. Van Zant, E. Goldwasser. LaRabida-University of Chicago, Chicago, Ill. 60649.
Three hybridomas secreting monoclonal antibodies directed against murine leukocytes have been produced. Spleen cells from rats immunized and boosted with a pre-B cell line and then mouse neonatal liver were fused with the cell line SP2/0-Agl4. Using quantative immunofluorescence on a Fluorescence Activated Cell Sorter (FACS) the reactivity of these antibodies to normal tissue was determined. One monoclonal antibody, DNL1.9, reacted with lymphocytes of the B cell lineage including all plaque forming cells, normal B lymphocytes, pre-B cells, and a population of "null" cells in bone marrow. This antibody did not react with thymocytes, mature T cells or prothymocytes. The "null" cell population identified by this antibody in the bone marrow may be the very early precursors of B cells. The second monoclonal antibody, DNL3.7, reacted with 95% of all cells in the bone marrow including lymphoid, myeloid, and erythroid cells. This antibody did not bind to lymphocytes in the thymus or lymph node. The third antibody, DNL4.4, identified myeloid but not erythroid or lymphoid cells in the bone marrow. These monoclonal antibodies were used to determine the cell surface antigenic phenotype of CFU-S, CFU-C, and BFU-E. DNL1.9 and DNL4.4 did not bind to any of the stem cells assayed. Preliminary studies have indicated that DNL3.7 also may not bind to these hematopoietic cells. From these data the expression of three cell surface antigens can be correlated with differentiation stages of hematopoietic cells.

400 MONOCLONAL ANTIBODIES TO SURFACE ANTIGENS ON ACUTE MYELOBLASTIC LEUKEMIA CELLS,
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Monoclonal antibodies that detect surface antigens on acute lymphoblastic leukemia cells have improved the diagnosis and classification of that disorder. To determine if a similar approach would be of value in the study of acute myeloblastic leukemia (AML), a series of murine monoclonal antibodies have been generated to human AML cells. Of 300 clones initially screened, 80 were found to be reactive with the AML cells used for initial immunization. Twelve of these 80 antisera were unreactive with an autologous B cell line and were selected for further study. These antisera were tested for reactivity with normal myeloid cells, monocytes, lymphocytes, AML, ALL, CML, CLL patient cells, and various cell lines. All of the monoclonal antibodies so far developed react with certain normal myeloid cells, as well as with a variable proportion of AML patients. One antiserum, G7, has reacted with 44/45 patients with AML, 0/10 ALL, and 0/10 CLL. It is also positive with peripheral blood granulocytes and weakly positive with monocytes. Two other antisera, G3 and K8, react preferentially with normal monocytes and cells of most acute monoblastic leukemia patients. The use of these monoclonal antibodies to study myeloid differentiation will be discussed.

401 DIFFERENTIATION ANTIGENS IDENTIFIED BY MONOCLONAL ANTIBODIES

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A panel of immune reagents reacting with individual antigens expresssed on the cell surface of mouse hemopoietic cells has been obtained. Cell lines secreting antibodies were selected by immunofluorescence on live bone marrow cells. The restricted cellular specificity of the antibodies was confirmed by radioactive binding assays on several tissues and a panel of tumor cell lines. The expression of each antigen was quantitatively measured by absorption analysis and antibody binding at saturation. Sigle cell fluorescence assays were performed to further characterize the cellular distribution of the antigens recognized. Immunoprecipitation of detergent solubilized membrane preparations radiolabeled by lactoperoxidase catalyzed iodination allowed the identification of three proteins with molecular weights of 210, 180, and 105 kilodaltons. One antibody consistently coprecipitated the 180 and 105 components which were further investigated. The two components could be precipitated from bone marrow, thymus, spleen, peritoneal polymorphonuclear and macrophage cells and gave the same pattern under both reducing and non reducing conditions. Both proteins are biosynthetically labeled with ^{35}S-methionine and ^{3}H-fucose and have isoelectric points between 6.5 and 6.8. The tryptic peptide mapping of ^{125}I labeled molecules did not show any major shared peptide between them. The sequential antigenic expression during the maturation and differentiation of cells of the myeloid series is being investigated with some of the probes available.